Improving Individual Identification of Wolves (*Canis lupus*) using the Fundamental Frequency and Amplitude of their Howls: A New Survey Method

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Abstract

Many bioacoustic studies have been able to identify individual mammals from variations in the fundamental frequency (F_0) of their vocalizations. Other characteristics of vocalization which encode individuality, such as amplitude, are less frequently used because of problems with background noise and recording fidelity over distance. In this thesis, I investigate whether the inclusion of amplitude variables improves the accuracy of individual howl identification in captive Eastern grey wolves (*Canis lupus lycaon*). I also explore whether the use of a bespoke code to extract the howl features, combined with histogram-derived principal component analysis (PCA) values, can improve current individual wolf howl identification accuracies. From a total of 89 solo howls from six captive individuals, where distances between wolf and observer were short, I achieved 95.5% (+9.0% improvement) individual identification accuracy of captive wolves using discriminant function analysis (DFA) to classify simple scalar variables of F_0 and normalized amplitudes. Moreover, this accuracy was increased to 100% when using histogram-derived PCA values of F_0 and amplitudes of the first harmonic. When this method was extended to wild Eastern wolf howls, a similar result was achieved of 100% for solo howls and 97.4% for chorus howls from 119 wolves using histogram derived PCA values. This was a new result for wild Eastern grey wolves. Individuality in howls was then tested in 10 other subspecies. The results showed that all wolf subspecies tested showed individuality in the F_0 and amplitude changes of their howls and could be identified with 74.0% to 100% accuracy. Finally, the use of artificial neural networks (ANNs) to survey howls using novel data was assessed. The ANNs achieved higher accuracy than DFA, where DFA did not achieve 100%, and were capable of attributing novel howls to known wolves. Therefore howls could be used as a survey method *in situ*.

‘Only a mountain

has lived long enough

to listen objectively

to the howl of a wolf’

Aldo Leopold (1949)

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Introduction

1.1 Answering the Call of the Wild: Using Acoustics in Wildlife Monitoring

1.1.1 Introducing bioacoustics

Bioacoustics is the science of animal sound and relies on audible communication between individuals and groups. It is increasingly being used to monitor species presence, numbers and behaviour without having to witness individuals (Catchpole *et al.* 2008). However, using animal vocalisations is not new, and has been used by ornithologists to identify species for millennia (Eichholz 1962). Today, with the development of digital sound recordings and software designed to extract and identify the key sound variables (Bradbury and Vehrencamp 1998), the application of bioacoustics has become greatly enhanced. For example, since Nikol'skii (1984) suggested using audio libraries to study vocalisations, in particular bird song, bioacoustics has moved so far forward that birds can now be identified and monitored at an individual, breeding pair and population level (Walcott *et al.* 2006; Mager *et al.* 2007b).

Bioacoustics can provide an excellent alternative to visual surveys because perception of sound is not dependent on line of sight or high visibility of the study species. Sound analysis methods which have been used for over thirty years on birds, bats and cetaceans are now being used for a more diverse range of species including grey mouse lemurs

(*Microcebus murinus*) (Leliveld *et al.* 2011), Arctic foxes (*Alopex lagopus*) (Frommolt *et al.* 2003) and bullfrogs (*Rana catesbeiana*) (Bee and Gerhardt 2002). The findings of these studies link vocalisations to advertisements of variables from body size and condition to group size and regional accent (learnt) or geographic-associated signature (inherited) (Wright 1996; Abgrall *et al.* 2003; Mathevon *et al.* 2003; Benson-Amram *et al.* 2011; Charlton and Reby 2011; Yu *et al.* 2011; Briefer and McElligott 2011a; Hall *et al.* 2013; Balint *et al.* 2013). Moreover, extensive studies of bat vocalisations have identified them to species level, with a particular application for separating cryptic species by vocalisation (Adams *et al.* 2010b). Bat calls have also been used to track individuals (Fenton *et al.* 2004), separate individuals belonging to different groups (Boughman and Wilkinson 1998), identify associations with their home-range's roost site (Jameson and Hare 2009), and even track long-term maternal effects (Jones and Ransome 1993). Over time, it is hoped that these findings will be applied to many other taxa including insects and anurans (Ganchev and Potamitis 2007; Bencsik *et al.* 2011; Han *et al.* 2011).

1.1.2 Animal communication

Communication occurs when one animal sends a signal which is received by another. These signals vary hugely from species to species and may consist of scent, sound, touch or visual signals such as colouration, bioluminescence or body language. However, true communication requires information to be encoded and transmitted by the sender for decoding by the receiver, although this message does not need to be consciously encoded For example, a seal pup will instinctively vocalise without knowing that it is communicating to its mother (Collins *et al.* 2006). Vocalisations have typically evolved to communicate information of advantage to the sender, rather than the receiver, with animals advertising factors such as size and health (Bradbury and Vehrencamp 1998). Nevertheless, this information may be intercepted by unintended observers. For example, a deer scent-marking its territory may be detected by both other deer (intended recipients) and wolves (*Canis lupus*) (unintended recipients), which may lead to predation on the scent-marking deer (Mech 1970). In addition, scientists may intercept these signals with more benign motives for tracking animals by their calls (Joslin 1967).

Animals communicate in a variety of ways but most studies have focused on long-range vocalisations such as howls, pant calls, songs and bellows rather than short-range vocalisations such as grunts, growls and whimpers (Bradbury and Vehrencamp 1998). The first class of these vocalisations tend to be territorial advertisements or calls to advertise presence to other members of a group or kin e.g. seal pups calling to their mothers (Collins *et al.* 2006). The second class of calls are typically used to communicate immediate responses to other individuals' behaviours, such as a warning growl of a wolf in response to an attempt to steal food (Mech 1970). These calls do not necessarily need to communicate individual identity nor mood or warning, although the growls can be like territorial calls in terms of threatening and advertising possession of a resource (Mech 1970).

1.1.3 Bioacoustics – from first recognising birds to today

Identifying animals by the sounds they make is an ancient science. Pliny the Elder wrote the first documented book on ornithology as part of the Historia Naturalis Book X in 77 AD, describing the habits and biology of a variety of species with details varying from perfectly accurate to perfectly ludicrous (Eichholz 1962). For example, the common nightingale (*Luscinia megarhynchos*) is described as singing continuously for fifteen days, with every bird learning song from its elders then presenting a unique song itself (Eichholz 1962). This is the first published scientific description of animal communication and the part about learning song is true (Kiefer *et al.* 2010). Since Pliny the Elder, thousands of books and papers have been devoted to the subject of bird song and other forms of animal communication. Some of this has filtered through to the public consciousness well enough that ornithology is a popular pastime and most people can at least identify a few basic animal sounds (e.g., the songs of whales or the howls of wolves) despite perhaps never having heard more than a recording of the noise in passing. With more acclimatisation, this knowledge becomes more precise; for example, dog owners can distinguish between their dog's barks and those of others (Molnar *et al.* 2006). Wolf keepers can also do this with howls (V. Allison-Hughes, *pers. comm.*).

Over the past fifty years, analyses of bird song, bat echolocation squeaks and whale song have shifted from obscure hobby interests to vital survey techniques. Advances in recording technology mean that computerised sound analysis can now decode information in the calls which was previously ignored. For example, it has become possible to identify species, family groups, sex, age and individuals by vocalisation variables alone (Bradbury and Vehrencamp 1998) in a vast range of species (see Table 1.1). These advances have been rapid; whale song is now considered to be so familiar and well known that recordings of it are sold as relaxation aids (Bradbury and Vehrencamp 1998). However, when American military researchers first recorded the sound in the 1950s as part of an acoustic experiment off the coast of Hawaii, it was not identified as the long and complex song of humpback whales (*Megaptera novaeangelia*) until 1967 (Payne and McVay 1971). Since then, whale song has been shown to encode species (Baumgartner *et al.* 2008), kinship, sex and maternal inheritance patterns as well as possible ecotype of killer whales (*Orcinus orca*) (Riesch *et al.* 2006; Miller *et al.* 2007; Deecke *et al.* 2010).

1.1.4 Bioacoustics for surveying populations and species distributions

The science of bioacoustics has developed to enable the vocalisations of different species to be utilised in monitoring populations and in exploring the relationship between the animal and its individual call (Bradbury and Vehrencamp 1998). However, application *in situ* is often limited by the accuracy of identification, whether to species, group or individual, so improving this accuracy is vital before surveys that can reliably identify individuals in the wild using vocalisations alone can be undertaken. As acoustic monitoring systems become more advanced (Blumstein *et al.* 2011), recording vocalisations *in situ* has become easier and cheaper, and surveys relying on their analysis is now possible and affordable.

Recording and counting vocalisations of species emitted either spontaneously or in response to playbacks is increasingly being used to count and monitor populations for a number of reasons including conservation and wildlife management. Although sperm whale (*Physeter macrocephalus*) populations have been estimated using underwater acoustic methods since 1982 (Watkins and Moore 1982) and bird studies have a similar longevity (Brown and Smith 1976), more recently these techniques have been applied to an increasing number of taxa including birds (Cheng *et al.* 2012), bats (Rodhouse *et al.* 2011), cetaceans (Whitehead 2009) and canids (Darden *et al.* 2003). Bioacoustics surveys have even been shown to outperform visual surveys leopard seal (*Hydruga leptonyx*) vocalisations can successfully be used to survey the numbers of seals present in an area with greater accuracy i.e. fewer false absences, than visual surveys (Rogers *et al.* 2013), although modelling this for more species requires similar comparisons of visual and acoustic surveys *in situ*.

Differences in vocalisations between even closely-related species are now being exploited for tracking purposes. The simplest form of this assigns species identity to a vocalisation, such as knowing that the howl is from a wolf rather than a coyote, but far more is possible. Oswald *et al.* (2007) used the whistles of nine Delphinid species to correctly classify them with 80% accuracy. These whistles can now be used to show the presence or absence of a species in a known area and thus track them over the enormous ranges of oceanic mammals as well as to separate the calls of closely related species to establish their ranges (Oswald *et al.* 2007). The delphinids benefit from knowing the species of the caller because they can moderate their behaviour accordingly, e.g. long-finned pilot whales (*Globicephala melas*) increase their group size in response to killer whales calls (Cure *et al.* 2012), a clear effect of the presence of one species affecting the behaviour of another. Regional-associated signatures have been used to separate such diverse species as Ryuku scops owls (*Otus elegans*) (Takagi 2013), two species of pika (*Ochotona princeps* and *O. collaris*) (Conner 1982; Trefry and Hik 2010), and Weddell seals (*Leptonychotes weddellii*) (Pahl *et al.* 1997). Geographic and population associated signatures can be used to separate populations but these signatures need to be used with caution as they may change if the animals migrate to a new territory and alter their call to match the locals' call, as in common loons (*Gavia immer*) (Walcott *et al.* 2006).

1.1.5 The Physics of and Physical Characteristics Expressed in Vocalisations

The way that vocalisations are formed vary between taxa, and familiar examples include dogs barking, birds singing and bats emitting high pitched chirps, all produced by different mechanisms. The most commonly used component to describe these vocalisations is the fundamental frequency (F_0) which is the pitch of the vocalisation expressed in Hertz (Hz) (Bradbury and Vehrencamp 1998). On musical instruments, this would be called the note played. However, as is clear from the dissimilarity in sound from one musical instrument to another, a single note played on different instruments sounds dissimilar while still using the same F_0 (Bradbury and Vehrencamp 1998). Source filter theory was originally developed in studies of human vocalisations and describes how differences between animals' sound production mechanisms affect the sound produced (Taylor and Reby 2010). In mammals, the 'source-filter' theory states that vocal signals result from a two-stage production with sound starting in the larynx, referred to as the source, then travelling out through the supralaryngeal vocal tract, referred to as the filter (Taylor and Reby 2010). The qualities of these two physical characteristics therefore control and constrain the sounds produced when air is expelled from the lungs and are often related to the physical mass and size of the animal vocalising (Riede and Fitch 1999; Taylor and Reby 2010). The vocal folds of the larynx, often called the vocal chords in humans, consist of three layers: epithelium, muscle and vocal ligament and along with the spacing between them form the glottis where vocal sounds are produced (Janik and Slater 1997; Bradbury and Vehrencamp 1998). Fundamental frequency is determined by the rate of opening and closing of this glottis, called the glottal wave (Taylor and Reby 2010). Lower fundamental frequency results from longer and heavier folds which vibrate more slowly than smaller vocal folds (Bradbury and Vehrencamp 1998). The sound is then modulated through the filter of the supralaryngeal vocal tract, which consists of all the air cavities between the larynx and the opening of the mouth and or nostrils (Taylor and Reby

2010). The shapes of these cavities and their constriction or relaxation affect the overall sound produced by shaping the resonant frequencies (Taylor and Reby 2010). These resonant frequencies form spectral peaks called formants and these formants may directly reflect the length of vocal tract in many non-human mammals and denote body size in some species including the dog (*Canis familiaris*) (Riede and Fitch 1999). The physical differences between vocal tracts alter the sounds produced and constrain what can be produced and therefore may encode information about the individual vocalising (Riede and Fitch 1999; Taylor and Reby 2010). For a fuller review of sound production and the effects of differences in source and filter see Taylor and Reby (2010).

Animal vocalisations can encode this information in a variety of ways as well as the fundamental frequency including the amplitude, number of harmonics, duration, abrupt shifts in frequency and repetition of the call (Bradbury and Vehrencamp 1998). Vocalisations can be distinguished from one another by quantifying and subsequently analysing different components of the sound which make up the vocalisation (Bradbury and Vehrencamp 1998). Fundamental frequency is described using variables such as its mean, maximum, minimum and range. Changes in F_0 over time, which include the coefficient of modulation and the coefficient of variation (Theberge and Falls 1967; White *et al.* 1970; Bradbury and Vehrencamp 1998), are typically used for identification, sometimes with its harmonics (Theberge and Falls 1967). Harmonics are always an integer multiple of the $F_{0.}$ If the F_0 is f the harmonics have the frequencies $2f$, $3f$, $4f$ (Bradbury and Vehrencamp 1998). These harmonics are the overtones which complete the sound. The F_0 forms the pitch of the sound, which is heard when the animal vocalises, while the number of harmonics forms the timbre of the voice. Changes in the F_0 are typically the focus of most identification studies because it is relatively robust to distance from receiver, and easy to track (Bradbury and Vehrencamp 1998).

Absolutes of, and changes in, amplitude, which measure vocalisation volume (Bradbury and Vehrencamp 1998), are used far less often because of difficulties in reliably recording it at distance, but in a small number of studies it has been found useful to identify individuals (Yin and McCowan 2004; Charrier and Harcourt 2006; Charlton *et al.* 2009; Briefer and McElligott 2011b; Pitcher *et al.* 2012). In these studies, amplitude changes were found to include individual vocal signature and to potentially encode information on both the temporal change of the amplitude and its absolute values (Yin and McCowan 2004; Charrier and Harcourt 2006; Charlton *et al.* 2009; Briefer and McElligott 2011b; Pitcher *et al.* 2012). The changes in F_0 and amplitude have quantifiable differences in the various components of vocalisations which can therefore be used to decipher a large amount of information from an animal, such as species (Thinh *et al.* 2011), individual identity (Yin and McCowan 2004; Vannoni and McElligott 2007) and kinship (Hoffmann *et al.* 2012), and can also be used to describe the physical characteristics of the vocalising animal (Briefer and McElligott 2011a).

Physical characteristics of individuals affect vocalisations as differences in the length and shape of the vocal tract, mouth, palate and tongue, hormonal state, quality of physical condition, body size and lung capacity can all affect noise production (Bradbury and Vehrencamp 1998). For example, smaller animals tend to have higher frequency vocalisations both between and within species; smaller bodied juveniles vocalise at a higher pitch than adults (Bradbury and Vehrencamp 1998). Where the physical characteristics have a direct effect on vocalisations, bioacoustics can be used to indicate the body size, age, sex and condition of animals at a distance (Growcott *et al.* 2011). The evolutionary advantages for this are obvious: avoiding predation and attracting mates (Davies and Krebs 1997).

Advertising size through vocalisations has been noted in a diverse collection of species including dogs (*Canis domesticus*) (Taylor *et al.* 2010), common loons (Mager *et al.* 2007b), goats (*Caprica hircus*) (Briefer and McElligott 2011a), rock hyraxes (*Procavia cavensis*) (Koren and Geffen 2009) and lions (*Leo panthera*) (Pfefferle *et al.* 2007). Dogs can use growls to assess the size of an unseen dog (Farago *et al.* 2010), although this can be an unreliable signal depending on whether the intent conveyed is playfulness or aggression (Balint *et al.* 2013). Age can also be monitored using vocalisations, with individuals of known ages used to calibrate the variables for individual species e.g. meerkats (*Suricata suricatta*) (Hollen and Manser 2006). Typically, higher frequency contact calls are emitted by younger animals, with a few exceptions such as the alarm call anti-predator deception of ground squirrels (*Spermophilus suslicus* and *S. fulvus*) (Matrosova *et al.* 2007). Advertisements of size and age can also be exploited during species surveys: Rogers *et al.* (2013) described vocalisation differences between individual leopard seals at different ages, allowing populations to be separated into age classes (sub-adult and adult) as part of the survey. However, each species may have different acoustic indicators of the actual age of the animal beyond what is directly affected by body size.

Most studies have found that vocalisations can be used to assign individuals reliably to a gender including in dogs (Chulkina *et al.* 2006). However, sex differentiation is similarly complicated by the effect of body size on vocalisations. For example, the sex-specific roar characteristics of lions may be due to the large dimorphism between the sexes, with males up to 50% larger than females (Pfefferle *et al.* 2007). Nevertheless, of the reviewed literature, 100% of sexually dimorphic and 93.3% of non-sexually dimorphic species encoded sex in their vocalisations (see Table 7.1, Appendix 1). Only black-legged kittiwake (*Rissa tridactyla*) and big brown bats (*Eptesicus fuscus*) were excluded as they have contradictory studies (see Table 7.1, Appendix 1). Therefore, whether a species is sexually dimorphic or not, the vocalisation is likely to advertise the sex of the caller which can be used as a survey tool when assessing wild populations (Mager *et al.* 2007b; Charlton *et al.* 2012). Although the applications of this have not yet been fully explored, bioacoustics surveys could provide a means of establishing the sex ratios of populations, providing insights into the behavioural ecology of species.

Body condition and health can also be advertised in vocalisations and have been found in species such as dogs (Taylor *et al.* 2010), red deer (*Cervus elaphus*) (Reby and McComb 2003), fallow deer (*Dama dama*) (Vannoni and McElligott 2009), bison (*Bison bison*) (Wyman *et al.* 2008), common loons (Mager *et al.* 2007b) and brown skuas (*Catharacta antarctica lonnbergi*) (Janicke *et al.* 2007), where anatomical constraints enforce an honest signal in males. The advantages of an honest signal of condition are to attract mates and to reduce competition between unevenly matched rivals (Dawkins and Guilford 1991). While body size and condition are often advertised honestly, dishonest signalling is also seen in the natural world. Dishonest signalling can benefit the caller if, for instance, it avoids predation by sounding larger than it is (Matrosova *et al.* 2007). This is the case for ground squirrels where the pups have lower calls than would usually be true for their body size (Matrosova *et al.* 2007). For acoustic surveys, it is necessary to establish whether a signal is honest or dishonest before using it as a criterion for assessment as for male green frogs (*Rana clamitans*) where the fundamental frequency can be manipulated to dishonestly signal larger body size, but higher rate of vocalisation cannot similarly be faked (Bee *et al.* 2000).

1.1.6 Evolving vocalisations: speciation, geographic-associated signatures and environmental effects

Exploring whether animals have fixed geographic-associated signatures would provide a window into the history of both mobile individuals and movement between populations. For example, socially learnt regional accents are found in many non-human species such as songbirds (Wright 1996; Mendes *et al.* 2011), suggesting that populations could diverge into subspecies, with this evolution reflected in their changing vocalisations (Thinh *et al.* 2011). Speciation often depends on reproductive isolation through geographic separation; therefore identifying the geographic variation in vocalisations within a species can provide historical information on the separation not readily available from other non-genetic methods (Conner 1982). Studies of cetaceans have moved from simple species separation to complex assessments of how and when species arose and are still diverging (Riesch and Deecke 2011; Filatova *et al.* 2012; Murray *et al.* 2012). The bioacoustics of terrestrial mammals show that there may be a similar structure of vocalisation reflecting species identity across taxa as in aquatic mammals. This is exemplified in the American pika where the most distant populations show the greatest vocal divergence, and so the interconnectedness of the populations can be inferred from geographic-associated signatures in their vocalisation (Conner 1982). Furthermore, American and collared pika (*O. collaris*) vocalisations show geographic differences which probably reflect genetic divergence and thus illuminate the evolutionary history of the two species (Trefry and Hik 2010).

1.1.7 Social affiliation displayed in Vocalisations

Kinship and social affiliation have already been shown in the vocalisations of species as varied as rhesus monkeys (*Macaca mulatta*) (Rendall *et al.* 1996), killer whales (Deecke *et al.* 2010) and wild house mice (*Mus musculus musculus*) (Hoffmann *et al.* 2012). Payne *et al.* (2003) attributed elephant (*Loxodonta africana*) calls to individuals and family groups as the basis of an acoustic monitoring scheme that is one of the first implemented for terrestrial mammals. Where kinship groups are separated, knowing which individuals are calling as well as their relatedness allows more accurate monitoring of ranges and behaviour than knowing kinship or identity in isolation (Payne *et al.* 2003).

With increasing sensitivity to differences between individuals and groups, recent studies of vocalisations have shown that animal communication may encode far more information than previously believed. For example, goats (*Capra hircus*) (Briefer and McElligott 2011b; Briefer *et al.* 2012), sheep (*Ovis aries*) (Sebe *et al.* 2010) and wild house mice (Galaverni *et al.* 2012) show the ability to recognise their kin by their calls often, but not exclusively, between mother and offspring. The advantages of this are clear: to avoid parental investment in non-kin juveniles and to avoid within-kin aggression. Additionally, kinship advertisements between individuals may mediate territorial behaviours and reduce potential conflicts. Furthermore, Italian wolves (*C. lupus italicus*) are known to display their pack’s signature in their howls (Zaccaroni *et al.* 2012). What is not known is how this pack signature is controlled, by social learning or genetic mechanisms, or whether it is stable over time and with changes in the pack composition.

1.1.8 Individual identification from vocalisations

One of the most effective uses of bioacoustics is the ability to define individuality without obtaining sightings of obvious physical traits or analyses of genetic markers (Thompson *et al.* 2010a). This is especially useful in species which do not show obvious differences between individuals, or are particularly cryptic. Individual identification can be used to optimise surveys of populations by allowing capture-mark-recapture instead of presence/absence surveys by matching vocalisations to individuals (Tripp and Otter 2006), and for many species this could substantially improve knowledge of territory size and range as well as showing movement of individuals within ranges (Thompson *et al.* 2010a).

Individual identity in vocal signatures has been shown in many studies (Table 1.1). The studies typically used fundamental frequency (F_0) variables, with descriptive variables established for individuals using analysis of variance (ANOVA), or similar analysis, into differences between mean values. When these mean values were shown to exhibit greater between-individual than within-individual differences, they were used to relate vocalisations back to the originating individual (via a classification scheme) and the level of accuracy achieved was recorded. Discriminant Function Analysis (DFA) was the most frequent form of classification analysis, with 84% (n = 52) of studies reviewed using DFA to identify individuals from their calls. DFA is used to analyse a variety of variables, both temporal and spectral F_0 and amplitude modulation, extracted from sonograms of the recorded sounds. The lowest identification accuracy achieved was 29% in Weddell seals (Collins *et al.* 2006) and the highest combined accuracy was 99% in the swift fox (*Vulpes velox*) (Darden *et al.* 2003). This difference in accuracy is sometimes a function of the age of the study with earlier studies involving less developed techniques, thus exhibiting lower accuracies (e.g. Bee and Gerhard (2001) vs. Bee (2004) for the bullfrog *Rana catesbiana*). Additionally, dogs have been shown to distinguish between strange and familiar dogs’ barks and to respond accordingly (Molnar *et al.* 2009).

Table 1.1 Individual identification studies and their analysis methods

Order	Common Name	Latin Name	Analysis Method	Accuracy	Reference
Accipitriformes	Bald eagle	*Haliaeetus leucocephalus*	DFA	83 -100%	(Eakle *et al.* 1989)
Amphibia	Armobatid	*Allobates femoralis*	DFA	64.9%	(Gasser *et al.* 2009)
Amphibia	Bullfrog	*Rana catesbiana*	DFA	52-100%	(Bee and Gerhardt 2001)
Amphibia	Bullfrog	*Rana catesbiana*	PCA, DFA	75.5%	(Bee 2004)
Anseriformes	White-faced whistling duck	*Dendrocygna viduata*	DFA	93-99%	(Volodin *et al.* 2005)
Artiodactyla	Fallow deer	*Dama dama*	DFA	36.6-53.6%	(Vannoni and McElligott 2007)
Artiodactyla	Fallow deer, males only	*Dama dama*	Neural network	87.9%	(Reby *et al.* 1998)
Artiodactyla	Goitred gazelle (juveniles & adolescents)	*Gazella subgutturosa*	DFA	52.1-64.4%	(Lapshina *et al.* 2012)
Caprimulgiformes	Marbled frogmouth	*Podargus ocellatus*	DFA		(Jones and Smith 1997)
Carnivora	African wild dog	*Lycaon pictus*	DFA	67.0%	(Hartwig 2005)
Carnivora	Asiatic wild dog	*Cuon alpinus*	DFA	44.7-96.7%	(Volodina *et al.* 2006)

Carnivora	Barking fox	*Alopex lagopus*	DFA	90%	(Frommolt *et al.* 2003)
Carnivora	Coyote	*Canis latrans*	DFA	69-83%	(Mitchell *et al.* 2006)
Carnivora	Dog, domestic	*Canis familiaris*	DFA	40%	(Yin and McCowan 2004)
Carnivora	Dog, domestic	*Canis familiaris*	DFA	63.5%	(Chulkina *et al.* 2006)
Carnivora	Eastern wolf	*Canis lupus lycaon*	DFA	86.5%	(Tooze *et al.* 1990)
Carnivora	Iberian wolf	*Canis lupus signatus*	DFA	84.7%	(Palacios *et al.* 2007)
Carnivora	Meerkat	*Suricata suricatta*	Multi-nomial regression	90%	(Schibler and Manser 2007)
Carnivora	Swift fox	*Vulpes velox*	DFA	99%	(Darden *et al.* 2003)
Cervidae	Red deer	*Cervus elaphus*	Homo-morphic analysis & hidden Markov models	93.5%	(Reby *et al.* 2006)
Cetacea	Amazonian manatee	*Trichechus inunguis*	DFA	Not given	(Sousa-Lima *et al.* 2002)
Cetacea	Bottlenose dolphin	*Tursiops truncatus*	DFA	75.7%	(Lopez-Rivas and Bazua-Duran 2010)
Chiroptera	African large-eared long-tailed bat	*Otomops martiensseni*	DFA	70%	(Fenton *et al.* 2004)
Chiroptera	Bechstein's bat	*Myotis bechsteinii*	DFA not reliable results	N/A	(Siemers and Kerth 2006)
Chiroptera	Big brown bat	*Eptesicus fuscus*	DFA	63%	(Kazial *et al.* 2001)

Chiroptera	Big brown bats (juveniles only)	*Eptesicus fuscus*	DFA	79%	(Camaclang *et al.* 2006)
Chiroptera	Cuban Evening bat	*Nycticeius cubanus*	DFA	Not given	(Mora *et al.* 2005)
Chiroptera	Little brown bat	*Myotis lucifugus*	DFA, Univariate, Multivariate	66-89%	(Melendez and Feng 2010)
Coraciiformes	Rufous-headed hornbill	*Aceros waldeni*	DFA	89%	(Policht *et al.* 2009)
Coraciiformes	Visayan hornbill	*Penelopides panini panini*	DFA	90%	(Policht *et al.* 2009)
Cuculiformes	Pheasant coucal	*Centropus phasianinus*	Not proved	N/A	(Maurer *et al.* 2008)
Gruiformes	Corncrake	*Crex crex*	DFA	80-100%	(Peake *et al.* 1998)
Gruiformes	Siberian crane	*Grus leucogeranus*	DFA	97.3%	(Bragina and Beme 2010)
Hyracoidea	Rock hyrax	*Procavia capensis*	DFA	93.3%	(Koren and Geffen 2011)
Marsupialia	Koala	*Phascolarctos cinereus*	DFA	87.7%	(Charlton *et al.* 2011a)
Mustelidae	California sea otter	*Enhydra lutris nereis*	DFA	80% mothers, 75% juveniles	(Mcshane *et al.* 1995)
Mustelidae	Leopard seal	*Hydruga leptonyx*	Markov process	83%	(Rogers and Cato 2002)
Mustelidae	Northern elephant seal	*Mirounga angustirostris*	PCA	54-64%	(Insley 1992)

Mustelidae	Northern fur seal	*Callorhinus ursinus*	PCA	79-82%	(Insley 1992)
Mustelidae	Stellar sea lion	*Eumetopias jubatus*	Back propagation network	71%	(Campbell *et al.* 2002)
Mustelidae	Weddell seal	*Leptonchotes weddellii*	DFA	29-52%	(Collins *et al.* 2006)
Passeriformes	Brownish-flanked bush warbler	*Cettia fortipes*	DFA	90-99%	(Xia *et al.* 2010)
Passeriformes	Eastern wood-pewee	*Contopus virens*	PCA, DFA	95.0-97.%	(Clark and Leung 2011)
Passeriformes	Passerines	*Order: Passeriformes*	Feature extraction methods; neural network architecture	69.3-97.1%	(Fox *et al.* 2008)
Passeriformes	South-western willow flycatcher	*Empidonax traillii extimus*	DFA; Artificial neural network	86%; 81%	(Fernandez-Juricic *et al.* 2009)
Passeriformes	Spotted antbird	*Hylophylax naevioides*	DFA	>70%	(Bard *et al.* 2002)
Primates	Agile gibbons	*Hylobates agilis agilis*	PCA, DFA	Not given	(Oyakawa *et al.* 2007)
Primates	Barbary macaque	*Macaca sylvanus*	DFA	80.5-96%	(Hammerschmidt and Todt 1995)
Primates	Orangutan	*Pongo pygmaeus wurmbii*	DFA	21-100%	(Delgado 2007)
Primates	Red-bellied lemur	*Eulemur rubriventer*	DFA	80.5%	(Gamba *et al.* 2012)

Primates	Red-capped mangabey	*Cercocebus torquatus*	PCA / MANOVA		(Bouchet *et al*. 2012)
Primates	Spider monkey	*Ateles geoffroyi*	DFA	50%	(Chapman and Weary 1990)
Psittaciformes	Green rumped parrotlets	*Forpus passerines*	DFA	39-55%	(Berg *et al*. 2011)
Rodentia	Belding's ground squirrel	*Spermophilus beldingi*	DFA & Fixed effect linear regression	45-100%	(McCowan and Hooper 2002)
Rodentia	European ground squirrel	*Spermophilus citellus*	DFA	98%	(Schneiderova and Policht 2010)
Rodentia	House mouse	*Mus musculus musculus*	Stepwise DFA	63.9-69.2%	(Hoffmann *et al*. 2012)
Rodentia	Taurus ground squirrel	*Spermophilus taurensis*	DFA	94%	(Schneiderova and Policht 2010)
Strigiformes	African wood owl	*Strix woodfordii*	DFA	80.9-100%	(Delport *et al*. 2002)
Strigiformes	European eagle owl	*Bubo bubo*	DFA	89-98%	(Grava *et al*. 2008)
Strigiformes	Great grey owl	*Strix nebulosa*	DFA	71.4-92.8%	(Rognan *et al*. 2009)
Strigiformes	Queen Charlotte saw-whet owl	*Aegolius acadicus brooksi*	DFA	69-75%	(Holschuh and Otter 2005)
Strigiformes	Western screech owl	*Megascops kennicottii*	DFA	92.3%	(Tripp and Otter 2006)

1.1.9 Advancing the technology and applications

Population studies of cryptic species typically rely on either *in situ* camera trapping or post-hoc monitoring involving the collection of genetic material, scat, etc. from known or suspected territories (for a review of existing techniques, see Long 2009). However, bioacoustics tracking does not require invasive techniques of capturing and is akin to camera trapping in terms of implementation and post-hoc monitoring techniques. Visual analysis tools can be used to identify individual leopards from their spots (Miththapala *et al.* 1989) or polar bears from their whisker patterns (Anderson *et al.* 2007) from photographs, enabling fine scale surveys and the tracing of life histories through remote cameras (Miththapala *et al.* 1989; Anderson *et al.* 2007). The technical ability to monitor acoustics is nowhere near as well-developed as that for camera trapping, although an exception to this is cetacean studies where techniques are advancing rapidly with complex systems comparable to those of camera trapping (Klinck *et al.* 2012). It is hoped that bioacoustics will become to be viewed as a similarly useful tool to camera trapping which could potentially even be extended to silent species such as fish where populations and species could be monitored via echograms (Petitgas *et al.* 2003).

Technological advances in software programming have not kept pace with the expanding number of species known to show individuality, with many analyses still relying on manual programming choices instead of a single automatic programme for the entire process. However, the sound analysis of bat vocalisations is common enough for programmes such as Anabat (O'Farrell and Gannon 1999) and Anascheme (Adams *et al.* 2010b) to have been developed to automate the analysis of echolocation calls and assign vocalisations to species and region with a high degree of accuracy (>50% for species, up to 99% for region)

(Adams *et al.* 2010b). Being able to assign any vocalisation to species and region allows an excellent and fast method of characterising populations and monitoring them over time. Showing how different populations relate to one another, when used in conjunction with the known geography of the regions where the samples were collected, can show how they may have evolved distinctive calls and how geographical features may affect the development of the calls (Ziegler *et al.* 2011; Ey and Fischer 2009; Irwin *et al.* 2008).

Acoustic recordings of hundreds of species have been collected in vast audio libraries such as the Macaulay Sound Archive (USA), British Library Sound Archive (UK), Borror Laboratory (USA) and the Tierstimmen Archiv of the Museum für Naturkunde (Germany). These recordings have been collected by professional individuals and organisations such as the British Broadcasting Corporation (BBC) and also amateur enthusiasts. The iBats Program, a bat call database which uses volunteers to record and submit bat echolocations to an international database for analysis to yield information on species distribution (Walters *et al.* 2012) forms an excellent model for future species monitoring systems as it utilises simple methods which can be implemented by citizen scientists. For instance, in addition to bird watchers recording visually identified species at specific sites, they could record their calls. This would give scientists a database of species distribution and, with individual recognition becoming increasingly used, could also be used to track movements of individuals between populations or over time.

1.2 A Brief Introduction to the Wolf: Biology, Ecology and Social Structure

1.2.1 Brief Overview

The grey wolf is the largest and was historically the most widely distributed canid in the world (Macdonald and Sillero-Zubiri 2004). However, due to persecution and habitat fragmentation, wolves are now less widely distributed than the red fox (*V. vulpes*) (Macdonald and Sillero-Zubiri 2004). Wolves inhabit a wide range of habitats across North America, North Africa, Europe and Asia (Mech 1970; Rueness *et al.* 2011). Their preferred prey is wild ungulates, although they will take livestock opportunistically when wild prey is scarce (Meriggi and Lovari 1996; Meriggi *et al.* 2011; Milanesi *et al.* 2012). They hunt in cooperative packs that range in size from 2-46 individuals, averaging 4 adults (Macdonald and Sillero-Zubiri 2004; Smith and Ferguson 2005). Persecution of wolves is still widespread but in countries with strong conservation lobbies, wolf numbers are beginning to recover, with some countries now recognising their important role in the ecosystem (Gula 2008; Liberg *et al.* 2012; Sandom *et al.* 2012).

1.2.2 Grey Wolf Taxonomy – Debate and Dissent

Grey wolf taxonomy is the subject of constant debate and revision (Wayne and Hedrick 2011) which causes problems for those attempting to conserve or study the species (Leonard and Wayne 2008; Mech 2009). There is a continually revised debate about how many subspecies exist but it is generally agreed that the previously recognised 24 subspecies of North America should be revised down to 6 subspecies based on Nowak (2003), listed in Table 1.2, and that Eurasia should have 12, listed in Table 1.3. These subspecies range in morphology, territory size, hunting behaviour and prey choice, which can be independent of prey availability (Mech 1970; Macdonald and Sillero-Zubiri 2004; Rueness *et al.* 2011). Further revision of subspecies and species lines within the genus *Canis* is ongoing, with a new subspecies added as recently as 2011 (Rueness *et al.* 2011), and revision of how *C. lupus* is identified must be changed, with biological, behavioural and morphological data in conflict (Bozarth *et al.* 2011). Because of the difficulty of collecting data on the more remotely located subspecies, such as the Tibetan wolf (*C. l. chanco*), it is possible that the number of wolf species will be revised upwards to separate the most isolated wolves, as suggested by Sharma *et al.* (2004).

Table 1.2 Currently accepted wolf subspecies of North America (Nowak 2003)

Common Name	Latin Name
Arctic	*Canis lupus arctos*
Eastern	*Canis lupus lycaon*
Great Plains	*Canis lupus nubilus*
Mackenzie Valley	*Canis lupus occidentalis*
Mexican	*Canis lupus baileyi*
Red	*Canis lupus rufus*

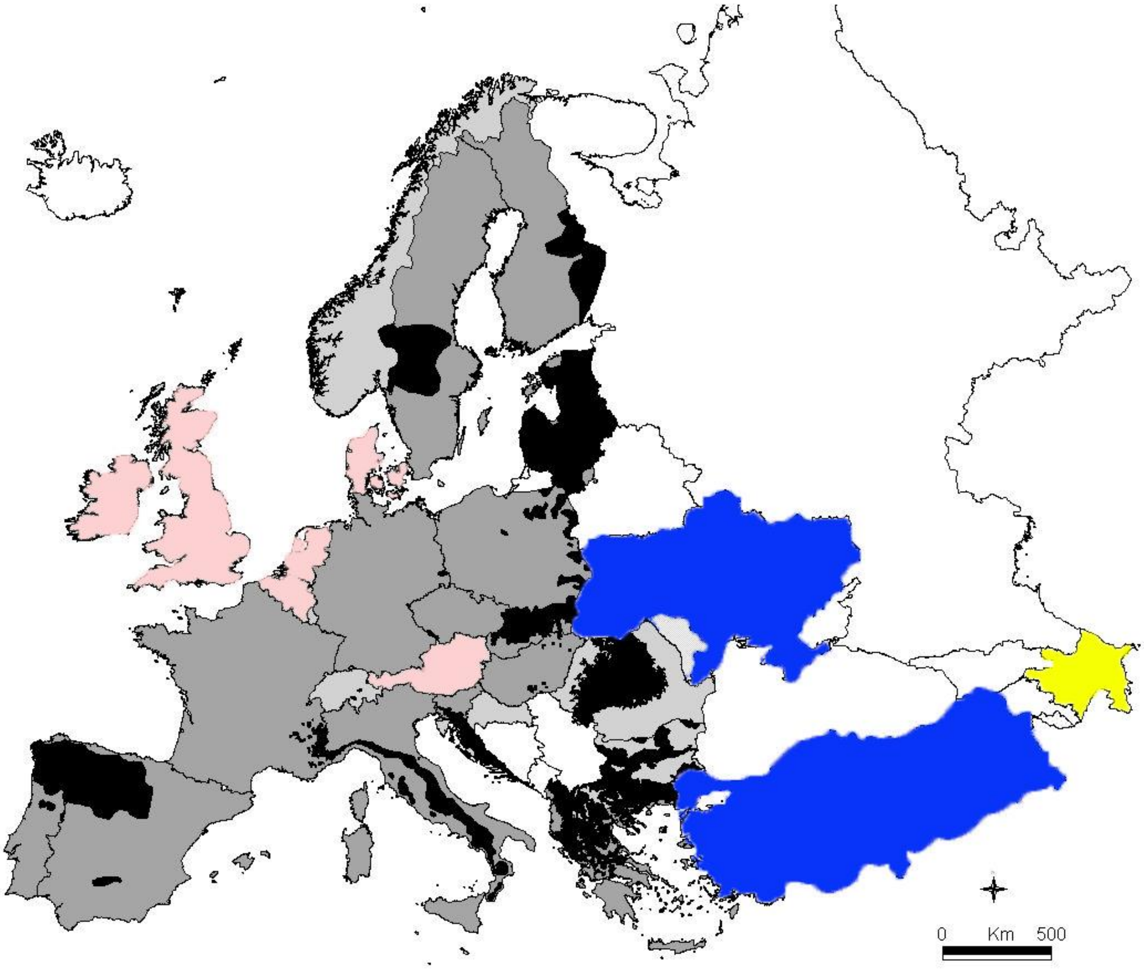
Table 1.3 Currently accepted wolf subspecies of Eurasia (Nowak 2003)

Common Name	Latin Name
Arabian	*Canis lupus arabs*
Caspian sea	*Canis lupus cubanensis*
Eurasian	*Canis lupus lupus*
North African / Golden Jackal	*Canis lupus lupaster* / *Canis aureus lupaster*
Himalayan	*Canis himalayensis*
Hokkaido	*Canis lupus hattai*
Honshu	*Canis lupus hodophilax*
Iberian	*Canis lupus signatus*
Indian	*Canis lupus pallipes*
Italian	*Canis lupus italicus*
Steppe	*Canis lupus campestris*
Tibetan	*Canis lupus chanco*
Tundra	*Canis lupus albus*

1.2.3 Distribution

The wolf's current status across the world is that of a slowly increasing population with large differences in hunting pressure, habitat suitability and prey availability, which are key factors defining the expansion and future range of the species (Salvatori and Linnell 2005). Their current distribution in Europe is shown in Figure 1.1 (Salvatori and Linnell 2005), although this should not be considered a fixed distribution. Wolves can travel up to 1, 000km and gene flow occurs across the north of Eurasia, with potentially interconnected populations across northern Russia, Finland and Norway (Wabakken *et al.* 2007; Aspi *et al.* 2009).

Grey wolves are found in 26 countries throughout Europe as well as North America, North Africa and parts of Asia (Boitani 2003; Rueness *et al.* 2011). The data informing analyses of the current North American and European population status of grey wolves varies hugely from country to country. While the American, Canadian, Italian, Polish, Swedish and Norwegian populations are relatively well documented, Eastern European wolves are less studied and more subject to hunting groups whose influence may inflate government figures (Salvatori and Linnell 2005; Busch 2007).



Current wolf distribution in Europe is indicated bythe black areas.

Legend

▒ Countries with recorded wolf presence, not included in the EU, that have ratified the Bern Convention.

▓ Countries with recorded wolf presence, included in the EU, that have ratified the Bern Convention.

🟦 Countries with recorded wolf presence, not included in the EU, that have ratified the Bern Convention, for which a map was not available.

🟨 Countries not included in the EU, that have ratified the Bern Convention, not included in the present report.

🟥 EU countries where wolf is absent.

⬜ Countries not included in the EU, and that have not ratified the Bern Convention

Figure 1.1 Current Grey Wolf Distribution in Europe from Salvatori & Linnell (2005) (adapted from black and white)

In North America, wolves have been well studied in terms of their biology and their role in an ecosystem (see Mech 1970, 1997; Ripple and Beschta 2003, 2005, 2009). These studies tend to focus on populations in easily observed habitats, such as the Arctic tundra (Mech 1995), and relatively closed systems, such as Isle Royale (Nelson *et al.* 2011), as the huge geographic distances that a wolf can travel in the search for a mate complicates tracking. One of the most visible subspecies, because of the excellent lines of sight over long distance of Arctic tundra, is the Arctic wolf but these have ranges which can exceed 6, 600km^2 and daily straight line distances of 41km or more (Mech and Cluff 2011). However, the information on North American subspecies is considerably better than that for Eurasian wolves due to the high public interest in the return of extirpated subspecies to their local wilderness (Smith and Ferguson 2005).

Asian wolf subspecies are the least studied of the genus. Their range is increasingly limited by conflicts with humans and they are now found in remote regions and uninhabited mountains, in developing countries without the money for wildlife research or protection, and are often still considered pests rather than balancers of prey populations, and thus ecosystems (Boitani 2003). The work that has been undertaken has focused on their genetics and distribution, and their relationship to domesticated dogs (Sharma *et al.* 2004). The Indian subcontinent holds two different basal clades of wolf-dogs, neither of which appears to have been involved in the domestication of wolves into dogs and whose position in the phylogeny is unclear (Sharma *et al.* 2004). Whether the Indian and Tibetan wolves are in fact grey wolf subspecies or species in their own right remains unclear and more work is needed to establish their position in the global distribution of subspecies and species (Sharma *et al.* 2004).

The gene flow between the populations of subspecies of wolves across Asia has not been established which complicates the population counts across the continent, although it has been shown that there are few geographic land barriers that can prevent wolves' dispersal, with individuals dispersing over mountain ranges as large as the Alps and Rockies and across the Mongolian steppes (Lucchini *et al.* 2002; Oakleaf *et al.* 2006; Marucco *et al.* 2009; Chen Jiu-Yi *et al.* 2011). There is much research to be done on their ecology, behaviour, distribution and use of habitat across these regions. Recent reliable estimates for Iraq, Iran, Afghanistan, Nepal and Bhutan are not currently available (Boitani 2003). A rough estimate, using all known estimates collated in Boitani (2003), puts the population of wolves on the Asian continent at approximately 100, 000 individuals but it is impossible to know how accurate this figure is, the distribution of subpopulations or the changes over time.

1.2.4 Biology, Reproduction and Pack Life

The basic social unit of the wolf pack is the mated pair, which is extended by their offspring which may stay with their parents for one to five years (Mech and Boitani 2003). Packs hold territories that vary in size from 33km^2 to 6, 664km^2; and pack size is dictated by prey availability and saturation of the habitat (Mech and Boitani 2003; Mech and Cluff 2011). Packs as large as 47 individuals including pups have been recorded but this is considered unusual, with 2 to 7 adults and resultant offspring more usual (Mech and Boitani 2003; Smith and Ferguson 2005).

Mating is elicited by the dominant female in the pack from a dominant male during oestrus, and gestation typically lasts 55-57 days (Mech and Boitani 2003; Packard 2003). Wolves birth their young into dens which can be simple scratched earth patches, caves or proper dug-ins, and there is evidence that wolf dens can be used for more than 700 years (Mech 1997; Mech and Boitani 2003). These dens are typically deep within the wolf's territory and pups stay in them until they are old enough to be moved to the rendezvous site at the age of six to eight weeks (Mech 1970). The rendezvous site is a place for them to play safely while their parents hunt, where the pups play and adults congregate (Theuerkauf *et al.* 2003). From the age of six weeks, the wolf pups can howl and will join in their parents' choruses (Harrington and Asa 2003). This howl is distinctive of their smaller body size, being higher in pitch than that of the adults (Harrington and Asa 2003).

The birth sex ratio is 50:50 in most cases, with no significant differences in survival between sexes (Mech 1970; Mech and Boitani 2003). Pup mortality is high with up to 70% dying before their first year, often during their dispersal from the natal pack at 10-12 months (Mech 1970). Anthropogenic effects cause up to 70% of wolf mortality through hunting, collisions with cars and collisions with trains but there are also cases of intraspecific (wolf on wolf) killing (Murray *et al.* 2010; Latham and Boutin 2011). In captivity, wolves may reach the age of 17 years but in the wild more than 9 is considered old and few are recorded over the age of 13 (Busch 2007). However, there is a great difficulty in assessing wolf age in the wild as there is a typical error rate of 1-3 years from tooth wear analysis (Gipson *et al.* 2000).

Both male and female pups typically disperse from their natal pack from the age of 10 months, unless a surplus of food is readily available which allows them to stay (Gese *et al.* 1996; Kojola *et al.* 2006). The dispersed offspring typically form their own packs with dispersers from other territories, rather than joining existing packs (Mech 1970). Genetic analyses are beginning to be used to identify the relationships between wolves within and between wild packs (Caniglia *et al.* 2012; Carroll *et al.* 2012).

Using evidence from faeces (scats), wolf pack life has been revised from the classic alpha model to a more flexible system where any wolf may change position from submissive omega to dominant alpha and back again over the course of a lifetime (Mech 1999). It is now understood that no wolf is born to be the dominant alpha or the omega ‘scape-wolf’ and that most so-called alpha behaviour relates instead to the parent wolves guiding and reprimanding their own offspring (Mech 1999). This accords with evolutionary principles as the younger wolves are usually closely related, either siblings or offspring, of the dominant pair, and the cooperative benefit of hunting together and raising pups follows kin selection theory (Hamilton 1964), with elder siblings helping younger to survive (Lehman *et al.* 1992).

Pack sociality is beneficial in terms of territory defence and predation (Mech 1970). There is increasing evidence that the availability of prey determines the size of the wolf pack, with packs living in areas where there is a large prey base more likely to have offspring that do not disperse after the first year (Mech 1999). As Mech (1999) noted that younger animals may not hunt well enough to help their parents for the first year of life, this may enable the yearlings to improve their own hunting skills. If conditions are particularly good, with prey availability high, offspring may even breed as part of the parental pack before risking the dangers of dispersal (Mech and Boitani 2003). The benefits to the parent wolves of non-dispersal by their yearlings may be found in avoiding the high mortality rate (70%) that accompanies the first year of dispersal and the yearlings becoming “baby-sitters” helping to raise the next year’s pups, which can improve their survival rate (Mech 1999). The cooperative benefits of working as a team in catching prey appear to be limited beyond the pair, as two wolves can successfully kill a bull moose (*Alces alces*), but the indirect benefits of continuing to provision and protect the pair’s older offspring when there is surplus food are clear (Mech and Boitani 2003). Some wolves do not reach sexual maturity until they are 5 years old and therefore may not be capable of breeding at once if they disperse at the youngest age of 10 months, meaning they will not lose reproductive time by remaining for some months with their parents beyond this age (Mech and Boitani 2003).

1.2.5 Habitat

Wolves exhibit a broad range of habitat choice including mountains, tundra, marshes, forests, farmland and deserts (Macdonald and Sillero-Zubiri 2004). Where prey is widely distributed and common, territory choice is decided by factors such as road density, avoiding human use of the land, and vegetation cover (Wabakken *et al.* 1984; Macdonald and Sillero-Zubiri 2004; Theuerkauf 2009).

What remains clear is that wolf habitat varies greatly in terms of geography, ecology and biodiversity, and suitability is mostly determined by prey availability rather than specific geographic characteristics (Mech 1970; Macdonald and Sillero-Zubiri 2004). While human density decreases preference, wolves living in already saturated areas will colonise land that brings them into conflict with humans and there are numerous anecdotal examples of wolves using human roads and tracks (Mech 1970; Smith and Ferguson 2005). Wolves also utilise human resources such as rubbish dumps and offal sites, suggesting that in future wolves may, like bears (*Ursa spp.*), be found at city outskirts, rooting through rubbish bins when wild ungulates are scarce (Meriggi and Lovari 1996). There is no current reliable model for predicting wolf habitat suitability or choice studies, although two studies use historical data that could prove to have predictive power (Karlsson *et al.* 2007; Jedrzejewski *et al.* 2008).

The wolf's very flexibility prevents accurate modelling and makes efforts to conserve suitable habitat difficult.

1.2.6 Wolves as Predators

Wolves can hunt as individuals or as co-operative teams in the form of the pack, although not all pack members may participate in every hunt (Mech 1970). They catch prey by running them down and biting at the haunches, throat and face; they do not, as long believed, hamstring their prey by snapping the tendons in the rear legs (Mech 1970). They attempt to pull down rather than run to exhaustion their prey, choosing injured animals preferentially (Mech 1970). Mech and Peterson (2003) estimate that a captive adult wolf requires 13 deer weighing 45kg per year to survive as a minimum; more is required for wild wolves and especially for breeding females due to the higher energetic costs associated with wider ranging activity and hunting activity. Calculating this in the wild is naturally difficult and so has not been done as yet.

Typical prey species of the grey wolf are moose (*Alces alces*), elk (*Cervus elaphus*), reindeer (*Rangifer tarandus*), white-tailed deer (*Odocoileus viginianus*) and other large ungulates including domestic species such as cows (*Bos primigenius*), sheep (*Ovis aries*) and horses (*Equus caballus*) (Mech 1970). They may also predate on beaver (*Castor canadensis*), mice (*Mus spp.*), Rocky Mountain goat (*Oreamnos americanus*), pronghorn (*Antilocapra americana*), arctic hare (*Lepus arcticus*), birds and wild boar (*Sus scrofa*) when available, but do not prefer domestic species to wild prey (Mech 1970; Meriggi and Lovari 1996).

Prey behaviour can affect sensitivity to wolf predation (Mech 1970). Estimates of hunting success on North American deer and elk suggest rates as low as 7-10% as wolves often "test" animals before committing to a long chase (Mech 1970; Mech and Boitani 2003; Mech and Peterson 2003). This "testing" essentially involves an individual being selected and a wolf running towards it; often, if it stands its ground, the individual will be left alone (Mech 1970). This testing relies on a degree of predation-avoidance behaviour not seen in animals which have lived in the absence of wolves, such as naive Scandinavian moose, which have not been exposed to hunting pressures for several generations (Sand *et al.* 2006). Wolf hunting success rate on naive moose was 45-64%, i.e. 35-57% higher than their success rate against wolf-habituated American moose (Sand *et al.* 2006). This shows that wolves do affect prey behaviour and use of habitat, although this is limited by the heterogeneity of the landscape (Kauffman *et al.* 2007; Theuerkauf and Rouys 2008). Prey animals such as deer preferentially use the best feeding sites in the absence of wolves, but 'landscapes of fear' created by wolves may affect entire ecosystems as they shift their prey from preferred areas to safer ones (Kauffman *et al.* 2010; McPhee *et al.* 2012). For ungulates, the single most important factor to counteract regulation by predators was spacing behaviour which corresponded to environmental heterogeneity (Skogland 1991). It may also be a way of wolves behaviourally mediating trophic cascades by creating areas where deer and moose choose not to feed due to predation risk (Kauffman *et al.* 2010).

Predator avoidance behaviour may also introduce interspecies commensalism in the presence of wolves. Ravens (*Corvus corax*) follow wolves and call to alert wolves to the presence of carcasses and American moose (*Alces alces*) use these calls to avoid predation by wolves (Berger 1999). However, this behaviour is quickly lost (within 10 generations) where wolves have gone extinct (Berger 1999). The ravens benefit when the wolves make a kill, producing scavenging meat for the birds (Stahler *et al.* 2002).

The relationship between birds and wolves is not well studied beyond ravens. What is known is that Berkut eagles (*Aquila chrysaetos*) prey on wolves (by smashing into their backs and paralysing them); some riparian songbirds benefit from wolves controlling ungulate numbers in Yellowstone through predation and that ravens benefit from wolves providing fresh carcasses in both Europe and America (Stahler *et al.* 2002; Ballard *et al.* 2003; Mech and Peterson 2003). Ravens employ a kleptoparasitic foraging strategy preferentially associating with Yellowstone wolves in winter when food is less available and are able to remove up to 37kg of flesh from a carcass per day (Stahler *et al.* 2002; Ballard *et al.* 2003; Mech and Peterson 2003). Ravens have an innate fear of novel food sources and by associating with wolves they know the provenance of their meal but they will not eat from experimentally placed meat (Stahler *et al.* 2002). They are attracted to howling wolves and are flexible enough to consider gunshots as similar indicators of scavenge meat (Harrington 1978; White 2005). The ravens' behaviour has a strange offset for the wolves as lone wolves are much less capable than packs of more than 10 of protecting their food, losing 66% of the kill to ravens compared to the pack's 10% (Ballard *et al.* 2003).

Wolves indirectly benefit a host of other species by providing scavenger-meat, including ravens, jays (*Garrulus glandarius*), wolverines (*Gulo gulo*) bald eagles (*Haliaeetus leucocephalus*), grizzly bears (*Ursa arctos spp.*) and even 57 species of beetles (Wilmers *et al.* 2003; Dijk *et al.* 2008). As apex predators, they are not frequently in direct conflict with these species. The greatest source of wolf mortality is not that from predatory grizzly bears or wolf-interspecies killing, but from humans (Morner *et al.* 2005; Gude *et al.* 2012).

1.2.7 Conflict with Humans and Future Conservation

The wolf has long been regarded as a figure of hate and distrust by many rural communities, with the fairytales of childhood warning of the danger of the wolf and a medieval identification of the wolf with the Christian Devil (Wallner 1998). This has led to the wolf being more widely reviled than other predatory species such as bears, despite many more cases of bear attacks recorded in both Europe and America (Breitenmoser 1998). Even positive stories of wolves such as Romulus and Remus, nursed by a she-wolf before going on to found Rome, and St Francis' taming of the Wolf of Gubbio, have undertones of violence – Romulus will kill Remus, the 'Wolf of Gubbio' begins by ravaging the town and devouring livestock before being tamed and, eventually, entombed and mourned (Anonymous 1973; Wallner 1998). Reported attacks of non-rabid, wild wolves on humans in the 20th century have not been borne out by independent researchers though historically there are some validated instances (Linnell *et al.* 2002). For a full review of the evidence of wolf attacks on humans, see Linnell *et al.* (2002). Factually unfounded it may be, but the fear of wolves has been a driving factor of their persecution for centuries.

This attitude of fear and negativity persists today and it has only been in the last 60 years that biologists have come to realise that while conflict may be inevitable, wolf extinction is not. In a now famous passage, Aldo Leopold (1949) describes his epiphany that wolf management should not equate to wolf extirpation:

“In those days we had never heard of passing up a chance to kill a wolf. In a second we were pumping lead into the pack, but with more excitement than accuracy; how to aim a steep downhill shot is always confusing. When our rifles were empty, the old wolf was down, and a pup was dragging a leg into impassable side-rocks.

“We reached the old wolf in time to watch a fierce green fire dying in her eyes. I realized then, and have known ever since, that there was something new to me in those eyes—something known only to her and to the mountain. I was young then, and full of trigger-itch; I thought that because fewer wolves meant more deer, that no wolves would mean hunters' paradise. But after seeing the green fire die, I sensed that neither the wolf nor the mountain agreed with such a view.”

(Leopold 1949)

Leopold's instinct was not a unique one. The wolf is extinct in many historic ranges and has only just begun to return to Norway and Sweden after centuries of persecution (Wabakken *et al.* 1984; Zimmermann *et al.* 2001; Ericsson and Heberlein 2003). The re-colonisation of habitats by wolves usually occurs when persecution is reduced or outlawed, with more tolerant or less populated countries such as Poland and Russia forming reserve populations from which wolves continually disperse to less tolerant neighbouring countries, like Belarus (Macdonald and Sillero-Zubiri 2004; Pilot *et al.* 2006). However, conflicts still occur and both legal and illegal hunting of wolves is a major cause of mortality and prevents normal dispersal and re-colonisation movements (Caniglia *et al.* 2010; Rogala *et al.* 2011; Gude *et al.* 2012). In Scandinavia, wolves are controlled by reindeer herders as a means of protecting their herds which limits their dispersal potential and population size (Tveraa *et al.* 2007). Across the world, conflict with humans is likely to define both where wolves will be able to re-colonise in future but also where they will be able to maintain viable, if fragmented, populations.

The future of the wolf as it re-colonises former territory may be seen in Finland. Where once the wolf had been completely extirpated by human effort, Finland now has a population of 185 wolves which are expanding their territory into the west and south where they have not been found for more than a hundred years (Kaartinen *et al.* 2005; Kaartinen *et al.* 2009). Between 1996 and 1999 there were around 100-120 wolves in Finland and 43 confirmed attacks on dogs by wolves, mostly on hunting dogs in forests where the wolves were hunting moose, and only one attack was on a dog in a house yard (Kojola and Kuittinen 2002). There have been no recorded attacks on humans, and wolves continue to avoid human dwellings and roads (Kaartinen *et al.* 2005). Further study showed that a single wolf pack was responsible for 71% of 21 confirmed attacks on domestic dogs and that the encounters appeared to be due to the wolves hunting the dogs instead of chance encounters (Kojola and Kuittinen 2002; Kojola *et al.* 2004). This suggests that future human-wolf conflict will be concentrated where wolves and hunters share ranges and prey choices rather than in villages or isolated farms. The historical fear of the wolf may reoccur where these conflicts occur, but is otherwise probably unjustified.

Reintroduction efforts are controversial, compensation and education schemes are not always successful, but more than fifteen years since the reintroduction of the wolf to Yellowstone it has been noted that if the abundance of Leopold’s habitat is to be restored, so must be wolves (Smith and Ferguson 2005; Hedrick and Fredrickson 2008; Milheiras and Hodge 2011; Sparkman *et al.* 2011). Tracking the movements of these wolves will be crucial to future conservation efforts as they begin to re-colonise former areas. Tracking wolves is a difficult, physical and time-intensive enterprise as wolves are shy and frequently inhabit remote and even hostile habitats (Mech 1970). Whether studying their behaviour, tracing their genetic flow across vast landscapes or monitoring their numbers for localised predator control or protecting livestock, knowing where wolves are and how they are using their territory will be essential for future work. Tools should include a combination of camera traps, radio collars, track and scat surveys and howl surveys.

1.2.8 Howling as a Tracking Method

Tracking wild carnivores is a difficult and expensive practice. The best data on individual movement comes from Global Positioning System (GPS) or radio telemetry collars, but the data gained from these are limited to the individuals and the animal must first be caught and sedated before it can be collared (Long 2008). Genetic analysis of scats can also identify individuals, but these cannot be collected without either scaring off the wolves from the area or waiting until they have left it. There is also the expense of deoxyribonucleic acid (DNA) analysis kits and personnel hours (Long 2008). Snow tracking is only possible when there is enough snow for tracks, either in winter or at high altitude, and is physically arduous, requiring long hours in the field (Ballard *et al.* 2003; Mech and Boitani 2006; Long 2008). There is a strong interest, therefore, in developing immediate methods of surveying populations without requiring expensive genetics or telemetry collars.

The cheapest method for surveying wolf pack distribution and abundance in the summer months when snow tracking is impossible is elicited howling, where a howl is played or howled by a researcher and responded to by wild individuals (Joslin 1967; Harrington and

Mech 1982). A wolf howl is a harmonic sound with a clear structure and a mean frequency in adults of 150-1000Hz and in juveniles of 200-1, 300Hz (Harrington and Asa 2003). Juvenile howls last just 3 seconds and adults up to 14 seconds, with a mean of 3-7 seconds (Harrington and Asa 2003). Pups emerge from the natal den at 3 weeks of age and will join the daily pack chorus from this time, but do not come into their adult voices until 6-7 months of age as their juvenile tone drops from 1, 100Hz at 2 weeks to around 350Hz (Harrington and Asa 2003). Single howls can be continued in bouts for up to 9 minutes, and chorus howls, those of more than two wolves together, last 30-120 seconds and may be repeated for up to 15 minutes (Harrington and Asa 2003). Chorus howls are distinguished using fundamental frequencies that differ by at least 15Hz from one another (Harrington and Asa 2003). Howl form and frequency characteristics have been found to differ consistently among individuals, allowing wolves to identify each other (Theberge and Falls 1967; Tooze *et al.* 1990).

Elicited howling is the method of counting wolves by induced responses to stimulated howling within pack territories during the summer (Harrington and Mech 1979). This method yields a rough survey of minimum wolf numbers from heard responses with a confidence interval of typically +/- 3 individuals per chorus (Harrington and Mech 1979)and is generally used for rough population estimates or for locating the rendezvous site where the pups are kept during the summer months (Harrington and Mech 1979). These rendezvous sites are areas with a high scat concentration and are often used to provide the data for diet studies and genetic studies of familial relationships (Mech 1970). More accurate winter estimates of adult, but not juvenile, wolves are typically made using snow tracking (Mech 1970). However, none of these methods yield a true census as there is no way of knowing whether every individual has been counted, so all survey methods can only yield minimum counts. Applied mathematics have begun to be used to separate howls within choruses in both wild and captive samples (Dugnol *et al.* 2007a; Dugnol *et al.* 2007b; Dugnol *et al.* 2008), although results have so far been limited to "noise" cleaning and have not established a clear method for wolf counting. More recently, Passilongo *et al.* (2012) achieved minimum counts using spectrographic counting.

Individual identity signatures have been recognised in wolves since 1967 (Theberge and Falls 1967). However, individual adult wolves were not identified by sound analysis of their howls until more recently, with 86.5% accuracy in captive Eastern wolves (*C.l. lycaon*) (Tooze *et al.* 1990), 84.7% accuracy in captive Iberian wolves (*C.l. signatus*) (Palacios *et al.* 2007) and 75.7% accuracy in wild Italian wolves (*C.l. lupus*) (Passilongo *et al.* 2012).

1.3 Rationale

Bioacoustics in the future can be expected to be as widespread and useful as camera traps are now for remote monitoring of populations *in situ*. Surveys of vocalisations are similarly easy to implement with low-maintenance methods of collection such as howl boxes (Ausband *et al.* 2011). Howl boxes are automated recording systems which play recorded wolf howls as elicitations to howl and then record any sounds for a set period afterwards, the advantage being that they are solar powered and can be left *in situ* for days (Ausband *et al.* 2011). It is likely that all species encode at least some information about themselves in their vocalisations, from individuality to gender and kinship, and that by describing these vocal differences bioacoustics can represent a tool for identification, monitoring and investigation of behaviour. This information is encoded in fundamental frequency, temporal variables and amplitude modulation. It may include information that is consciously amended with changing circumstance, as found when common loons change territories (Walcott *et al.* 2006) or information that is passed matrilineally and never lost, as found in killer whales (Miller and Bain 2000; Deecke *et al.* 2010). Research on bat vocalisations show potential ways forward for bioacoustics research in other species such as using automated identification programmes (Walters *et al.* 2012), exploring individuality and kinship on population levels (Yoshino *et al.* 2008) and monitoring the change of vocalisations over time and geographic space (Davidson and Wilkinson 2002). Just as humans can recognise voices automatically (Skaric 2008), such recognition is seen in other species that have been tested (Proops and McComb 2012) and it will not be surprising to see this level of recognition found in many more.

Mech (1970) considered the grey wolf to be very well studied in the wild, but codified that much remains to be learnt. Studies so far have highlighted the complexity of their social behaviour, their timidity of humans, their effects on their prey's behaviour and the cascade effect on their environment through their prey choices. They are apex predators with complex social lives and have direct effects on their prey's behaviour (Mech 1970). Wolves may act as top-down controls on their local habitat and their presence may indirectly influence willow trees (*Salix spp.*) (Creel and Christianson 2009), river banks (Beschta and Ripple 2008), song birds (Baril *et al.* 2011) and soil nutrients (Bump *et al.* 2009), and directly influence the behaviour of their prey (Kittle *et al.* 2008) and fellow predators such as coyotes (Arjo and Pletscher 1999).

However, in the absence of snow, reliable population counts are hard to obtain (Carlos Blanco and Cortes 2012; Duchamp *et al.* 2012). Howl surveys are undertaken to establish the presence of wolves and to identify rendezvous-sites, but have previously not been able to assess populations at the individual level (Joslin 1967). Developing new methods for tracking and surveying wolves is a priority for scientists interested in monitoring populations, exploring predator prey relationships and demonstrating the results of conservation efforts on population management (Carlos Blanco and Cortes 2012). The examination of wolf howls represents a survey method that can be improved by following advances in bioacoustics analyses for whales (Oleson *et al.* 2007) and corncrakes (Terry and McGregor 2002). The improvements can also be implemented with little financial investment compared to GPS and DNA tracking methods (Ausband *et al.* 2011). This study focuses on individuality present in the amplitude and fundamental frequency changes of wolf vocalisations and begins to indicate how wolf howls are complex, information-rich and important.

1.4 Aims

This thesis aims to establish whether the findings of individual identity expressed in vocalisations found for birds, bats and cetaceans can also be repeated for wolves with the intention of forming a basis for a future survey method. The usefulness and expediency of bioacoustics has become evident as the number of taxa studied has expanded. The identification of individual wolves via their howls have so far been limited to three subspecies and this thesis aims to improve the accuracy of the identification as well as showing whether these characteristics are expressed in other subspecies.

Therefore, this thesis aims to:

1. **Improve individual identification in captive Eastern grey wolves using the time course of howl amplitudes**

   This chapter will focus on testing whether the new method works in controlled *ex situ* circumstances, using a single subspecies with previous best result for accuracy with known individuals.

2. **Identify individual wild Eastern grey wolves using fundamental frequency and amplitude of howls**

   This chapter will extend the findings of Chapter 2 to wild wolves recorded in different conditions and circumstances on a range of equipment to demonstrate whether the sound feature extraction method works *in situ*.

3. **Demonstrate whether Artificial Neural Networks (ANNs) can classify howls to individual wolves as a novel survey method, using howls from Eastern wolves and ten other subspecies**

   This aims to show that unknown individuals can be successfully monitored by their howls alone using advanced statistical methods in order to prove that ANN models of howls could be used as a survey method *in situ* for both Eastern wolves and other grey wolves.

Improving individual identification in captive Eastern Grey Wolves (*Canis lupus* lycaon) using the time course of howl amplitudes

This chapter was published in Bioacoustics as Root-Gutteridge *et al.* (2014b).

2.1 Introduction

Bioacoustics studies are increasingly being used in population ecology because vocalisations have been found to be highly variable both within and between individuals (e.g. Tooze *et al.* 1990) and so can be used as a method of individual identification. This vocal individuality can be utilised for monitoring populations remotely over time and can thus be applied to conservation studies (for a review see Terry *et al.* (2005)). A large range of mammals have been found to show individual identity in their vocalisations including Eastern grey wolves (*Canis lupus lycaon*) (Theberge and Falls 1967), giant pandas (*Ailuropoda melanoleuca*) (Charlton *et al.* 2009) and red squirrels (*Tamiasciurus hudsonicus*) (Digweed *et al.* 2012). Such individuality is shown in variation in both the fundamental frequency (F_0) and duration of calls (Joslin 1967; Frommolt *et al.* 2003). A third key component of acoustic communication is amplitude variation within calls (Bradbury and Vehrencamp 1998), however most studies investigating individual recognition have ignored amplitude data, often because of the difficulty of *in situ* recordings (Frommolt *et al.* 2003) as amplitude attenuates (loses signal) over distance, particularly at higher frequencies (Bradbury and Vehrencamp

1998). Nevertheless, some studies have suggested that amplitude may carry as much individual information as fundamental frequency itself (Mcshane *et al.* 1995; Charrier and Harcourt 2006). Furthermore, the unequal attenuation of amplitudes between vocalisations can be compensated by measuring changes within amplitudes of individual vocalisations (i.e. normalised amplitude) rather than absolute amplitude data. For example, Charrier and Harcourt (2006) implemented normalised amplitude alongside fundamental frequency changes in Australian sea lions (*Neophoca cinerea*) and found a strong link between both amplitude and frequency modulations and individual identities. These parameters were used to predict strong individual recognition where the inclusion of amplitude data improved the accuracy of individual recognition over fundamental frequency alone (Charrier and Harcourt 2006). Similar findings have been shown in California sea otters (*Enhydra lutris nereis*) (Mcshane *et al.* 1995) and giant pandas (Charlton *et al.* 2009). Therefore, amplitudes may also be useful in improving individual identification accuracy in other mammal species.

Another source of error in many bioacoustics studies is the interference of background noise. Sound analysis programmes address this by using cross-correlation functions but not all achieve the removal of sound that is not harmonic, such as waves on a beach (Schrader and Hammerschmidt 1997). Praat (Boersma and Weenink 2005) is one such commonly applied vocal analysis software programme and has been used to extract acoustic features, such as frequency and amplitude, for analysis of individuality in mammal vocalisations (e.g. red lemurs (*Eulemur rubriventer*) (Gamba *et al.* 2012), spotted hyenas (*Crocuta crocuta*) (Benson-Amram *et al.* 2011), goats (Briefer and McElligott 2011b), and giant pandas (Charlton *et al.* 2009). However, Praat is not capable of tracking vocalisations precisely unless it is manually adjusted to get a good fit and may further require specially written code to extract all desired features (Briefer *et al.* 2012).

The examination of extracted acoustic features from vocalisations for individuality is typically ascertained using a Discriminant Function Analysis (DFA) (Tooze *et al.* 1990; Darden *et al.* 2003; Zsebok *et al.* 2012). This identifies a linear combination of independent variables that best discriminate groups, defined by the user (e.g. vocalisations from individual A), from each other. Simple scalar acoustic variables are singly-dimensioned values, describing a characteristic of the data, which are user-defined. For example, Palacios *et al.* (2007) identified mean fundamental frequency, maximum fundamental frequency, number of harmonics and frequency modulation as the most important discriminant variables in Iberian wolf (*Canis lupus signatus*) howls.

DFA can also be used to cross-validate the accuracy of individual identification using the selected best combination of variables by comparing predicted group membership (e.g. vocalisation belongs to individual A) with actual group membership. However, DFA requires the user to supply the 'group' to which any recordings belong, thus clustering together known vocalisations (e.g. where 'group' might refer to the same individual). Therefore, DFA is a 'supervised' classification technique, requiring the user to identify groups prior to the analysis.

When using simple scalar variables, the user chooses and computes specific scalars, using the time-course of the extracted parameters, such as the mean and standard deviation, maximum and minimum values, etc. (see Table 2.2). Although this method is robust and straightforward, it inherently carries the risk that 'some' important information is dismissed from the analysis. To remedy this, the analytical procedure for determining individuality can be refined further by using a Principal Component Analysis (PCA) to reduce the original scalar acoustic variable set to a smaller set of uncorrelated variables (principal components). The first principal component contains the largest variance in the data so accounts for as much of the variability in the data as possible. The principal component values, or "scores", can be plotted two or three-dimensionally to show a scatter and, where a scatter groups vocalisations from the same individual more closely than vocalisations from different individuals, identity is suggested (Pearson 1901; Tooze *et al.* 1990). The PCA values can be fed into a DFA to determine how accurately they can be used to identify individuals e.g. if the largest differences are indeed between individuals (Tooze *et al.* 1990). PCA is not supervised by the observer and does not describe the cause of the deviations in the data, it merely finds them (Pearson 1901).

Theberge and Falls (1967) were the first to suggest that Eastern wolves (*C.l. lycaon*) are able to discriminate between the howls of individuals and packs. Fundamental frequency variation has since been used to identify individuals in three subspecies of wolves; Eastern wolves (Tooze *et al.* 1990), Iberian wolves (Palacios *et al.* 2007) and Italian wolves (Passilongo *et al.* 2012). The accuracy of individual identification using DFA of simple scalar acoustic variables ranged from 75% (Passilongo *et al.* 2012) to 86.5% (Tooze *et al.* 1990), with the most accurate results achieved for captive wolves. However, individual vocalisation identity has also been found in other canid species and accuracy has been as high as 99% in swift foxes (*Vulpes velox*) (Darden *et al.* 2003). Nevertheless, no canid vocalisations have been tested for individuality using a combination of both fundamental frequency and amplitude data. Wolves are a good model species for such a study as their howls have evolved to be transmitted over long distances up to 10 km (Joslin 1967) for territory defence and to communicate individual identity to other pack members (Theberge and Falls 1967). With no visual or olfactory clues available over long ranges, wolf howls may have evolved to carry information about the identity of the individual, its pack and even its current state of arousal (Harrington and Asa 2003). One variable that is known to communicate individual identity in wolves is the fundamental frequency at the position of the maximum amplitude of the howl (Tooze *et al.* 1990). As the accuracy of individual vocalisation identity of wolf howls is currently 86.5% (Tooze *et al.* 1990), it is likely that wolf identity could be improved by adding amplitudes to the acoustic analysis.

2.2 Aims

The aims of this chapter are to:

1. Effectively prevent background noise from adding variation to an analysis by producing a bespoke code designed to extract the fundamental frequency features and amplitudes of the first four harmonics from wolf howls, and comparing this with features extracted using the commonly applied software Praat.

2. Improve the accuracy of individual wolf vocalisation identities by including the amplitudes of the first four harmonics of the howls, which are those with the lowest frequency and highest amplitudes.

3. Maximise the efficiency of the search for differences between individuals by adding a new statistical method of histogram-derived PCA values to increase the accuracy of individual identification.

2.3 Materials and methods

2.3.1 Source of wolf howls

Eighty-nine howls from six captive wolves (mean per wolf = 14.8, standard deviation: SD = ±20.1, Table 2.1) were captured on 12 recordings made at Wolf Park, Indiana, between 16th and 29th December 1997. Consecutive recordings of howls were used because Tooze *et al.* (1990) showed that individuals did not vary their calls enough to cause pseudo-replication when using consecutive calls. All howls were recorded on a single microphone set-up: a Marantz PMD-221 recorder and Audio Technica 835A microphone using no parabola; Master record number JT9701 on Analogue Cassette at an index of 1430 ms. These were digitised via Studer to A/D board via Akai cassette into Waveform Audio File Format (.wav). All recordings were made by the same observer (J. Tilley) standing next to the enclosure at a distance of no more than 20m from individual wolves (Monty Sloan of Wolf Park, pers. comm.). All howls were acquired from the Borror Laboratory of Bioacoustics, Ohio State University, with permission from the copyright holder.

Table 2.1 Number of wolf howls used per individual

Wolf ID	**Gender**	**Solo howls used**
Aurora	Female	54
NK	Male	5
Seneca	Male	5
Socrates	Male	4
Ursa	Female	2
Vega	Female	19
Total	**3 male, 3 female**	**89 howls**

2.3.2 Sound analysis

2.1.1.1 Standard analytical procedure using Praat and DFA

The free-access speech analysis programme Praat (Boersma and Weenink 2005) was used to extract both fundamental frequency and amplitude features from background noise. Praat is accepted method for identifying individuality from vocalisations and outputs fundamental frequency data by fitting points to spectrograms (Skaric 2008). The spectrogram time-step was set to 0.0468s (defined by bit rate of recordings), and harmonics were fitted to the fundamental frequency and exported as text files. The length of section was a compromise between recordings that were too long, which deteriorate the number of points one can extract along a specific howl, and those that were too short, which deteriorate the frequency assessment. Two howls were excluded from analysis because Praat could not isolate the howls from the background noise.

2.1.1.2 Bespoke Matlab code

Chebli & Bencsik (unpbl.) developed two codes in Matlab® (Mathworks Inc. 2005) for (i) semi-automatic extraction of the time course of both the amplitude and frequency of the vocalisation, and (ii) further assessment of the benefit of exploiting the amplitude data. Chebli, Bencsik and I discussed the aspects of the howl feature which could be extracted and the purpose of the code. The howl feature extraction code can be found in Appendix 2. I used the same 89 wolf howls to compare features extracted by Praat with those extracted by the bespoke Matlab-derived code.

Within each howl, the modulus Fourier spectrum of a short section (0.0468s) was calculated and stacked along time to obtain a ‘spectrogram’ (Figure 2.1).

a)

b)

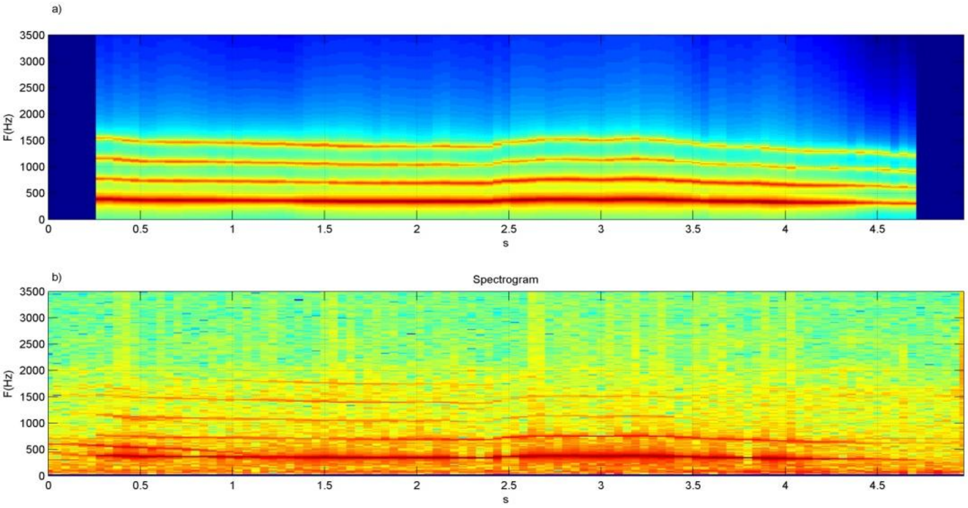


Figure 2.1 Processed (a) and raw (b) spectrograms from wolf howl 25082:2 extracted using the bespoke Matlab code. The colour codes the sound amplitude on a logarithmic (dB) scale. Note that the other howl present at 0 s to 1 s is successfully excluded.

Howl audio files showed sharp peaks at frequencies that were exact multiples of one another (see Figure 2.1). The best fitting model between the natural peak shape was a Lorentzian function, defined by

$$p(f) = A\frac{\gamma^2}{(f - F_o)^2 + \gamma^2},$$

where A was the peak's amplitude, F_0 the peak's frequency and γ the Lorentzian's half-width at half of its maximum. I found that fitting the value of γ resulted in spurious results (i.e. the fitted function was often mismatched to the experimental peak), whilst forcing its value to 30 Hz gave excellent match to the vast majority of the data. Note that the value of γ required updating if the frequency resolution (set to 1/46ms in this case) of the spectrogram was to be changed.

The full function fitted to any instantaneous spectrum, p(f), was the sum of four Lorentzian peaks forced to be exact multiple frequencies of each other, resulting in a five parameter fitting procedure:

$$P(F_o, A_1, A_2, A_3, A_4) = \frac{A_1\gamma^2}{(f - F_o)^2 + \gamma^2} + \frac{A_2\gamma^2}{(f - 2F_o)^2 + \gamma^2} + \frac{A_3\gamma^2}{(f - 3F_o)^2 + \gamma^2} + \frac{A_4\gamma^2}{(f - 4F_o)^2 + \gamma^2}$$

Each howl was extracted sequentially as the process was semi-automatic with the user required to define only the exact start and end of the howl within the recording, found by means of showing the user the full spectrogram and having the user define these parameters. The feature extraction then started exactly in the middle of these two user-chosen boundaries, as this was where the signal to noise ratio (SNR) was usually at its best. The user was prompted to check that the first fitted spectrum was correct. Next, the software extracted the rest of the feature fully automatically, moving frame by frame until the end of the howl, then going back to the middle and moving frame by frame to the start. By scanning the spectrogram in this way, and by automatically feeding starting values for the five parameters that were fitted in the immediate neighbour time-frame of that being examined, it was found that the fitting procedure was rendered faster and remarkably robust.

Some howls exhibited one or more discontinuities in the time course of the frequency, i.e. a large, abrupt change in frequency from one time frame to the next, which occasionally affected the feature extraction accuracy. This was tackled using the following strategy: for any particular instantaneous spectrum, an estimate of the frequency of the lowest peak was reliably obtained by identifying the maximum of the cross-correlation function between (i) the data and (ii) the five parameter function, P, in which the four amplitude values were set to those fitted in the immediate neighbour time-frame. Based on this estimate, the frequency and amplitudes of the four peaks with the lowest frequencies were successfully fitted until the entire spectrogram was analysed, thereby providing a dataset matrix of dimension N x 5, where N was the length of the howl divided by 46ms.

The code excluded background noise and harmonic sounds, such as bird song, by excluding any sound feature that was not a harmonic multiple of the F_0 of the vocalisation. This allowed lower quality recordings containing background noise to be used, excluding the noise from the output file.

Close agreement was found between raw and fitted data (Figure 2.1). The time course of the resulting five extracted parameters is shown individually in Figure 2.2. The histograms for the same howl are shown in Figure 2.3.

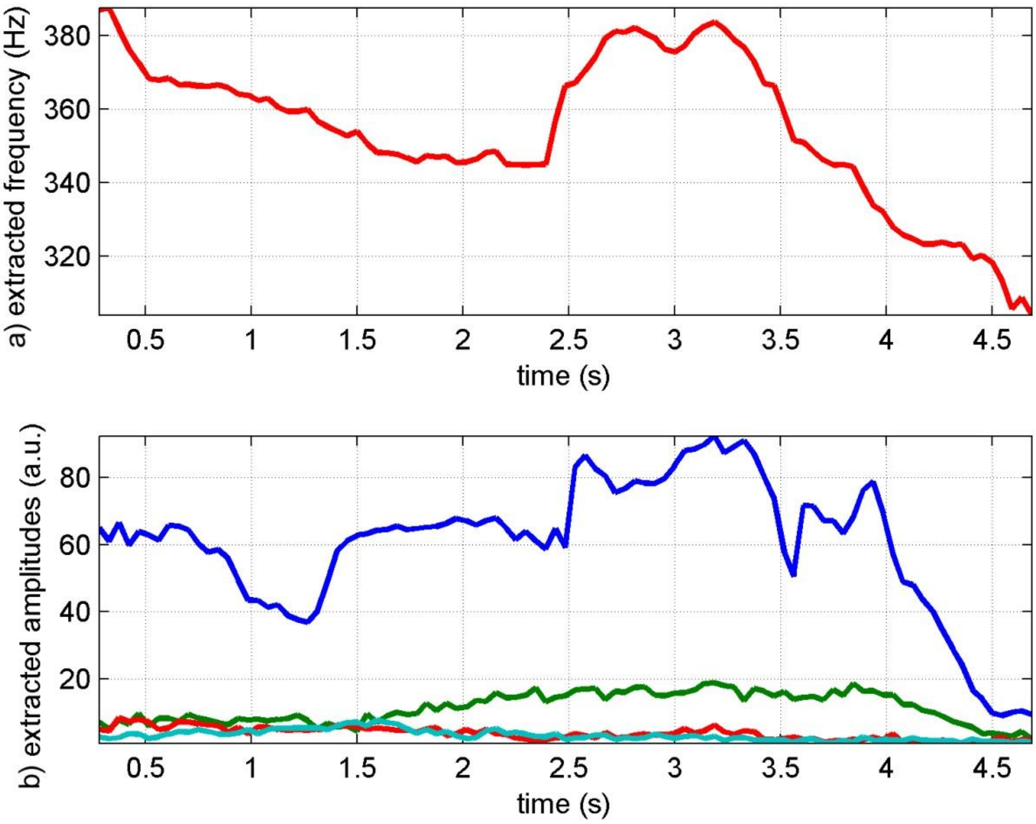


Figure 2.2 Time course of the fitted frequency (a) and amplitudes (b) for the howl shown in Figure 2.1; a) red represents F_0 changes over time; b) the four colours present the four different amplitudes of harmonics 1-4. Note the independence between the time courses of the four fitted amplitudes on the bottom plot, thereby justifying their individual extraction.

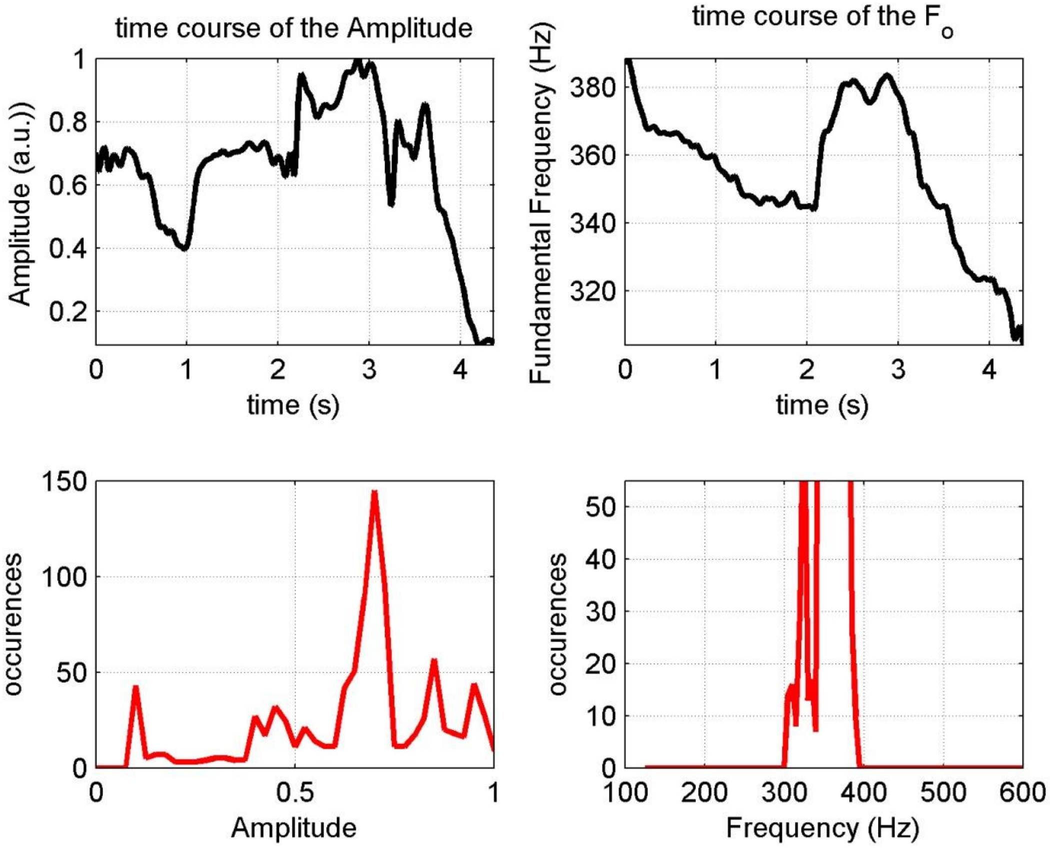


Figure 2.3. Time course (top graphs) and corresponding probability histograms (bottom graphs) for the amplitude (left graphs) and the frequency (right graphs) of the same howl as shown in Figure 2.1 and Figure 2.2. In the histograms, the information regarding the absolute time at which a specific amplitude or frequency occurs is lost, thereby helping the PCA search in identifying relevant deviations.

2.1.1.3 Defining individuality through simple scalar variables

The simple scalar variables, identified by Tooze *et al.* (1990) and Palacios *et al.* (2007) that are necessary to identify individuals from their howls, were calculated for features extracted by both Praat and the bespoke Matlab code (listed with definitions and abbreviations in Table 2.2). For the bespoke Matlab code, the simple scalar variables necessary to describe the amplitudes of the first four harmonics were also calculated by normalising them to the maximum amplitude of each harmonic (Table 2.2).

Table 2.2 Variables of frequency used for individual identification in simple scalar variable analyses. '*' denotes variables used in Praat analysis.

Variable Name	Definition of Variable
FreqPaf	F_0 at the position of maximum amplitude of first harmonic
F0Mean*	Mean frequency of the fundamental at 0.0468s intervals over duration (Hz)
F0Max*	Maximum fundamental frequency (Hz)
F0Min*	Minimum fundamental frequency (Hz)
F0Range*	Range of the fundamental: Range = F0Max – F0Min (Hz)
CofM*	Coefficient of frequency modulation $= \frac{\sum \lvert f(t) - f(t+1) \rvert / (n-1)}{\text{F0Mean}} \times 100$
CofV*	Coefficient of frequency variation = (SD / Mean) x 100
Abrupt*	Number of discontinuities in the fundamental (change of more than 25Hz in one time step)
Posmax*	Position in the howl at which the maximum frequency occurs: Posmax = time of F0Max/Dur
Posmin*	Position in the howl at which the minimum frequency occurs: Posmin = time of F0Min/Dur
F0End*	Frequency at the end of the fundamental (Hz)
Dur*	Duration of the howl measured at the fundamental (s) = t(end) - t(start)
NorAmp1Range	Normalised range of the amplitude of the first harmonic (H1) = Range of Amplitude of H1/ Maximum of Amplitude of H1
NorAmp2Range	Normalised range of the amplitude of the second harmonic (H2) = Range of Amplitude of H2/ Maximum of Amplitude of H2
NorAmp3Range	Normalised range of the amplitude of the third harmonic (H3) = Range of Amplitude of H3/ Maximum of Amplitude of H3
NorAmp4Range	Normalised range of the amplitude of the fourth harmonic (H4) = Range of Amplitude of H4/ Maximum of Amplitude of H4
NorAmp1Min	Normalised minimum amplitude of the first harmonic (H1) = Minimum of Amplitude of H1/ Maximum of Amplitude of H1
NorAmp2Min	Normalised minimum amplitude of the second harmonic (H2) = Minimum of Amplitude of H2/ Maximum of Amplitude of H2

NorAmp3Min	Normalised minimum amplitude of the third harmonic (H3) = Minimum of Amplitude of H3/ Maximum of Amplitude of H3
NorAmp4Min	Normalised minimum amplitude of the fourth harmonic (H4) = Minimum of Amplitude of H4/ Maximum of Amplitude of H4
NorAmp2Max	Normalised maximum amplitude of the second harmonic (H2) = Maximum of Amplitude of H2/ Maximum of Amplitude of H2
NorAmp3Max	Normalised maximum amplitude of the third harmonic (H3) = Maximum of Amplitude of H3/ Maximum of Amplitude of H3
NorAmp4Max	Normalised maximum amplitude of the fourth harmonic (H4) = Maximum of Amplitude of H4/ Maximum of Amplitude of H4
NorAmp1Mean	Normalised mean amplitude of the first harmonic (H1) = Mean of Amplitude of H1/ Maximum of Amplitude of H1
NorAmp2Mean	Normalised mean amplitude of the second harmonic (H2) = Mean of Amplitude of H2/ Maximum of Amplitude of H2
NorAmp3Mean	Normalised mean amplitude of the third harmonic (H3) = Mean of Amplitude of H3/ Maximum of Amplitude of H3
NorAmp4Mean	Normalised mean amplitude of the fourth harmonic (H4) = Mean of Amplitude of H4/ Maximum of Amplitude of H4

2.3.3 Using PCA for automatic identification of deviations defining individuality

For automatic identification of deviations, the data were entered, in the form of a 'training database', to a PCA in Matlab, to enable automated identification of the largest, statistically-independent deviations found in the howl database. (Martin Bencsik and Alexandra Bourit developed the code in Matlab, which I then used in the analysis.) This supplied the information that may be missing from defined simple scalar variable analysis alone.

The main challenge in this newly developed method was supplying PCA with a training database that did not include the phase lag of the howl relative to the recording start and end (as this information was irrelevant), yet retained the rest of the information. The best results were obtained by computing the histogram distributions of the time courses of the parameters. These histograms (Figure 2.4) were then stacked and entered as a training database into a PCA search. Smoother histograms were obtained by interpolating the time course data by a factor of 10.

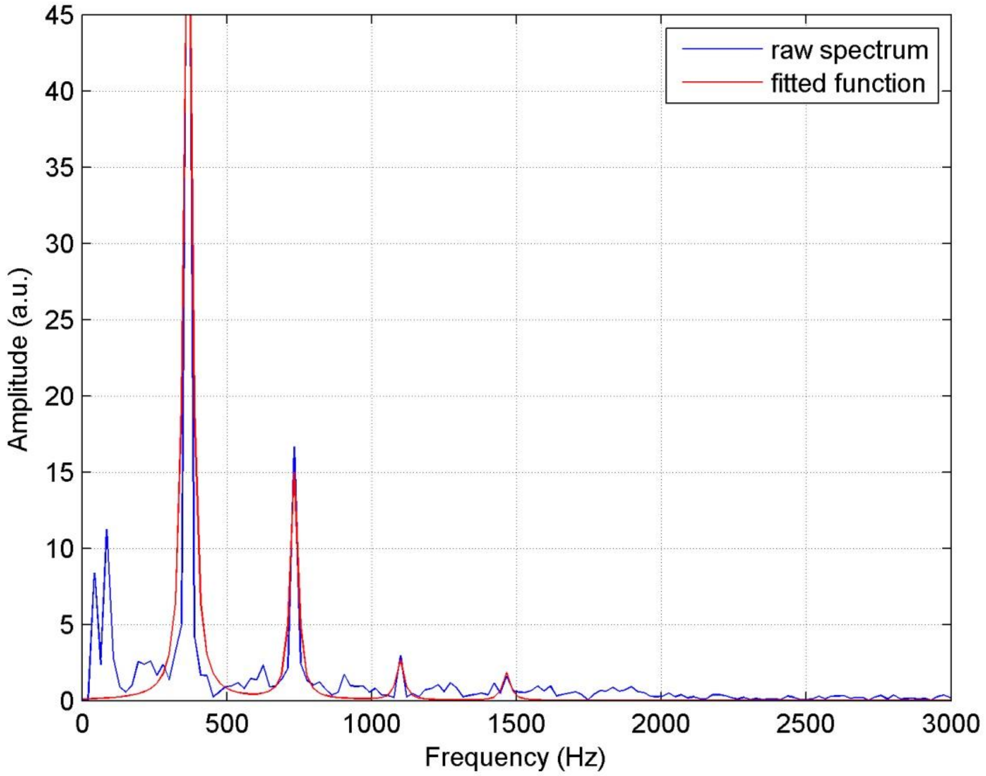


Figure 2.4 Raw spectrum (blue curve) superimposed with a five-parameter fitted function (red curve) as described in the text, using the same howl as in Figure 2.1 and Figure 2.2. Note the remarkable agreement between the fitted curve and the raw data, and the effective dismissal of non-howl-related information, such as the large background noise seen between 0 and 250 Hz.

PCA values based on the set of scalars were characterised, and the 40 greatest values were considered for further classification using DFA. Including more PCA values in the analysis added variation to the dataset that did not improve groupings of howls from individual wolves.

PCA values were generated for the six individuals for i) the 87 howls extracted by Praat, ii) these 87 howls extracted separately by the bespoke Matlab code and iii) the full set of 89 howls extracted by the bespoke Matlab code. The PCA values were obtained from the histograms of both the fundamental frequencies (F_0 probability) for all howls, and the amplitude of the first harmonic (amplitude probability), for the 89 howls extracted by the bespoke Matlab code. When both F_0 and amplitudes were used together, these were concatenated into arrays of 80 PCA values.

2.1.1.4 DFA classification of individuals using PCA values and simple scalar variables

For the dataset of 87 howls extracted by both Praat and the bespoke Matlab code, DFA was applied to two sets of descriptive variables: the simple scalar variables and the histogram-derived PCA values describing F_0. For the full set of 89 howls extracted by the bespoke Matlab code only, DFA was applied to three matched levels of analysis: it was applied to simple scalar variables and histogram-derived PCA values of F_0 alone, amplitudes alone, and F_0 and amplitudes together. The simple scalar variables and PCA values of each howl were labelled with their originator wolf name and the DFA was applied in SPSS 17 (SPSS Inc. 2010).

To optimise the DFA on the simple scalar variables, one-way analyses of variance (ANOVAs) were undertaken in SPSS 17 (SPSS Inc. 2010) on each of the 27 acoustic features to see if there was a significant difference in acoustic features between individuals which would be useful for DFA (Tooze *et al.* 1990). Variables which were non-significant were excluded from the DFA.

For the 89 howls extracted via the bespoke Matlab code only, stepwise DFA was then undertaken to establish which variables contributed the most to the clustering by changing which variables are included and removing them if they do not add to discrimination. Variables were entered in this analysis based on the change in Wilk's lambda (F to enter = 3.84; F to remove = 2.71) which is the probability that it is associated with the desired value.

Eight levels of analysis were applied to the data using 1) the 12 simple scalar variables describing F0 alone (Table 2.2) matched with 2) the 40 PCA values describing F0 alone obtained from the various training databases; 3) the three simple scalar variables describing amplitude change of harmonic one (Table 2.2) matched with 4) the 40 PCA values describing amplitudes of harmonic one alone; 5) all simple scalar variables of amplitude changes of harmonics one to four; 6) all 27 simple scalar variables describing F0 and its amplitude changes (Table 2.2) matched with 7) up to 80 PCA values describing F0 and amplitudes of harmonic one together; 8) all 27 simple scalar variables describing F0 and amplitude changes of harmonics one to four. Finally, the variables defined as the best indicators of individual identity were entered into a separate DFA to establish how accurately they alone could predict identity.

2.4 Results

2.4.1 Choice of significant variables using ANOVA and stepwise DFA

2.4.1.1 ANOVA results

One-way ANOVAs were used to test for differences in the acoustic variables between individuals. For the 87 howls extracted by Praat, 9 out of the 11 variables were significant indicators of individuality and 2 were excluded from DFA: the position in the howl at which the maximum frequency occurs (PosMax: $F_{5,86}=0.678$; $p=0.641$) and the number of discontinuities in the fundamental frequency (Abrupt: $F_{5,\ 86}=1.609$, $p=0.167$). For the matched 87 howls extracted by the Matlab code, position at which the maximum frequency occurs (PosMax: $F_{5,\ 86}=2.217$, $p=0.060$) and the position in the howl at which the minimum frequency occurs (PosMin: $F_{5,\ 86}=1.937$, $p=0.097$) were also found to be non-significant indicators of individuality so were excluded from DFA. However, Abrupt was not excluded ($F_{5,\ 86}=4.484$, $p=0.001$), possibly because the code was better at tracking the howls and created less steep jumps than Praat where the howl changed rapidly.

For the full dataset of 89 howls extracted by the bespoke Matlab code, PosMax (*d.f.=88;* $F_{5,\ 88}=2.157$, $p=0.067$) and PosMin (*d.f.=88;* $F_{5,\ 88}=1.902$, $p=0.103$) were again excluded from DFA. For the amplitude variables, the range of the normalised amplitude of harmonic 3 (Nor Amp3Range) (*d.f.=88;* $F_{5,\ 88}=2.090$, $p=0.075$) and the minimum of the normalised amplitude of harmonic 3 (NorAmp3Min) (*d.f.=88;* $F_{5,\ 88}=2.131$, $p=0.070$) were also excluded from DFA.

2.4.1.2 Stepwise DFA results

Stepwise DFA of the Matlab code's simple scalar variables showed that the four most important variables were the mean of the fundamental frequency (F0Mean: F to remove = 88.321, Wilks lambda = 0.156), coefficient of variation of fundamental frequency (CofV: F to remove = 19.919, Wilks lambda = 0.054), the normalised mean amplitude of the second harmonic (NorAmp2Mean: F to remove = 10.141, Wilks lambda = 0.039) and the normalised maximum amplitude of the third harmonic (NorAmp3Max: F to remove = 10.051, Wilks lambda = 0.039).

2.4.2 Benchmarking with Praat

Using Praat, 87 of the 89 howls were successfully analysed using nine simple scalar variables to describe the fundamental frequency (Table 2.2). Two of the 89 howls were excluded because Praat could not reliably extract them due to background noise interference. DFA of F_0 alone achieved 82.8% accuracy of individual identification (Table 2.3). However, when the histogram-derived PCA values were used in the analysis instead of the simple scalar variables the accuracy was improved by 11.5% to 94.3% (Table 2.3).

Table 2.3 Summary of the Discriminant Function Analysis accuracies using Principal Component Analysis values and simple scalar variables of fundamental frequency (F_0) changes, Amplitude changes and both fundamental frequency and amplitude changes together, and the difference between the PCA value and simple scalar variable analyses, for 89 howls extracted by the Matlab code.

Data Used	Variables used	Simple scalar variable accuracy %	PCA values accuracy %	Difference from simple scalar variable %
Praat 87 howls	F_0 changes	82.8	94.3	+11.5
Bespoke code 87 howls	F_0 changes	85.1	96.6	+11.5
Bespoke code 89 howls	F_0 changes (analyses 1 & 2)	83.1	92.1	+9.0
	Amplitude changes of harmonic 1 (analyses 3 & 4)	74.2	85.4	+11.2
	Amplitude changes of harmonics 1-4 (Analysis 5)	89.9	-	-
	F_0 & Amplitude changes of harmonic 1 (analyses 6 & 7)	88.8	100	+11.2
	F_0 & Amplitude changes of harmonics 1-4 (Analysis 8)	95.5	-	-
	Four best variables for identity: F0Mean, CofV, NorAmp2Mean, NorAmp2Max	89.9	-	-

Using the Matlab code in place of the Praat software improved howl extraction possibilities, allowing extraction of all 89 (100%) howls compared to Praat's 87 (97.8%) (Table 2.3). When the 87 Praat-extracted howls were matched with the howls extracted with the Matlab code, individual identification using the significant simple scalar variables of F0 alone were improved by 2.3% to 85.1% (Table 2.3).

When the analysis used the histogram-derived PCA values instead the accuracy was again improved by 11.5% to 96.6% (Table 2.3). This presented a further improvement on the histogram-derived PCA values of Praat-extracted howls by 2.3% (Table 2.3).

2.4.3 The application of bespoke code to extract howl features

The bespoke code was used to undertake eight analyses on all 89 Matlab-extracted howls (Table 2.3). The findings show that individual identity was present in the changes of F_0 and amplitudes. Using the four variables found to be most useful by stepwise DFA (F0Mean, CofV, NorAmp2Mean, NorAmp3Max), DFA achieved 89.9% accuracy of individual identification using just these simple scalar variables (Table 2.3). The findings also demonstrate that DFA of histogram-derived PCA values improved on results using the simple scalar variables alone (Figure 2.5). This suggests that more simple scalar variables are needed to fully describe the howls and to maximise the accuracy achieved.

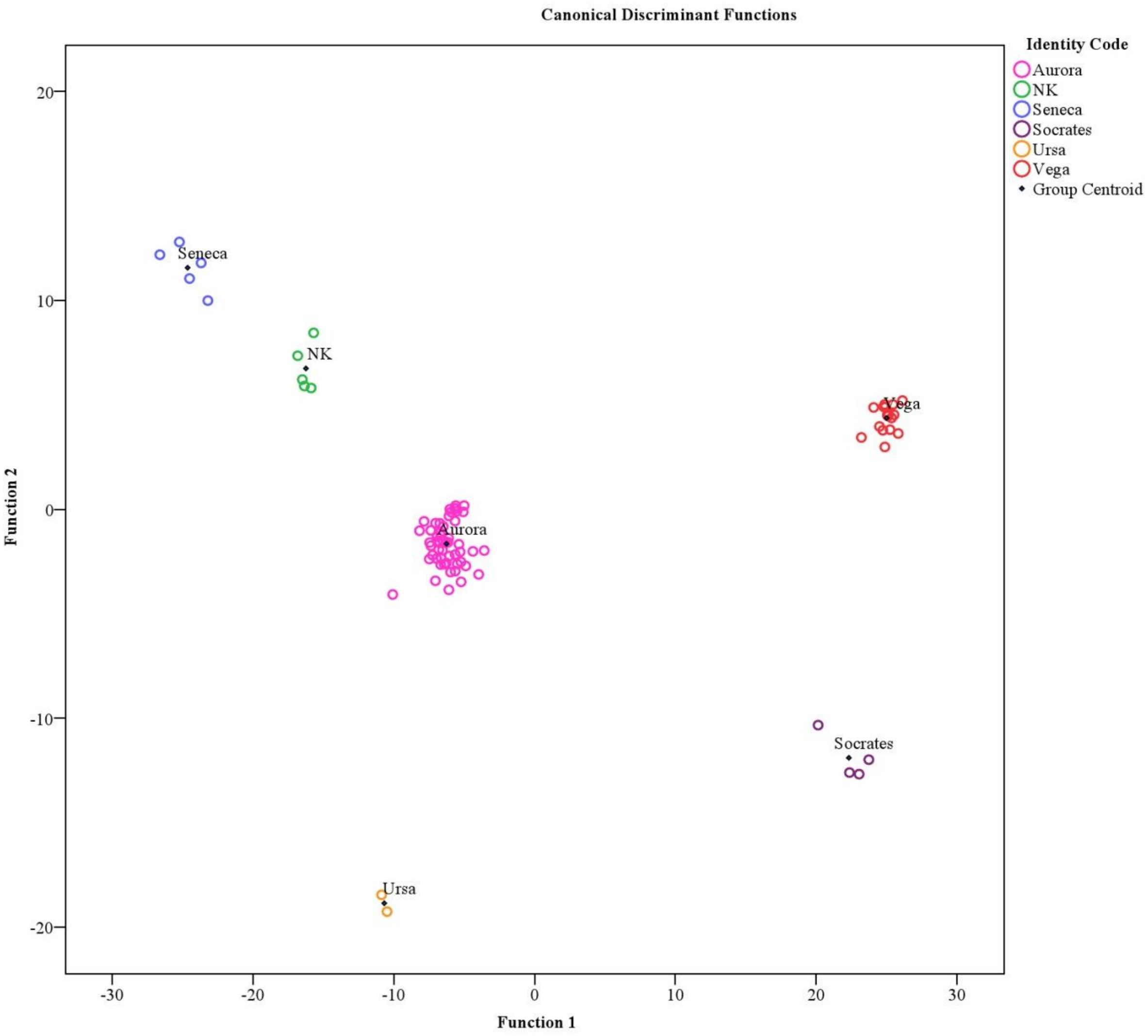


Figure 2.5 Discriminant Function Analysis (DFA) results for correct individual identification from analysis 7 which used histogram-derived PCA values of F_0 and amplitude of harmonic one.

2.5 Discussion

The inclusion of amplitude to improve identification of individual mammals has rarely been attempted due to the difficulty of reliably extracting amplitudes, with distance and background noise confounding fidelity (Frommolt *et al.* 2003). However, this is beginning to change, with more studies including amplitude data to improve identification accuracies (Charlton *et al.* 2009; Depraetere *et al.* 2012; Pitcher *et al.* 2012). My findings show that including normalised amplitudes of harmonics improved the individual identification accuracy of wolves in DFA of both simple scalar variables and histogram-derived PCA values. The previous best accuracy for captive Eastern wolves using all F_0 variables alone was 86.5% (Tooze *et al.* 1990). However, my accuracy of 100% (achieved with histogram-derived PCA values and the Matlab code) cannot be improved further and is the highest accuracy recorded compared to other canid species where F_0 alone was used (Darden *et al.* 2003), and to other species where amplitude changes have been used in addition to F_0 (Charrier and Harcourt 2006; Charlton *et al.* 2009; Rek and Osiejuk 2011).

I have shown that Eastern wolves express individuality in their howls through both temporal changes in F_0 variables and the amplitude they generate at different points in the howl. However, not all of the amplitude variables are of equal value in identifying individuals, and amplitude of harmonic two appeared to contribute most to identification, shown by stepwise DFA. Consequently, further work could investigate what defines the most important amplitude changes and how these arise, and the effect of distance on the transmission of the amplitudes of the different harmonics. Nevertheless, it is likely that by including amplitudes in analyses of other subspecies of wolves and canids, individual identification accuracy in these species will be improved further. In addition, although my new extraction code is directly applicable to the harmonic vocalisations of canids, the use of amplitudes alongside F_0 to increase the accuracy of individual identification should be extended to other species if needed.

One of the limitations of the approach utilised in this study is that my bespoke code was generated using the licensable software Matlab, whereas the less accurate but more accessible Praat software is free. However, my bespoke code achieved better extraction (100% of howls compared to 97.8%) and produced an automatic fit that also extracted amplitudes. In addition, my bespoke code achieved higher individual identification accuracy for F_0 alone (+2.3%) and achieved 100% accuracy in identifying individuals when it extracted amplitude alongside F_0 data. Again, this suggests that other species would also benefit from code specifically designed to extract their vocalisations. For long range vocalisations of canids my code could be used to improve identification accuracy, especially where background noise has previously prevented good quality extraction of data e.g. in barking foxes (*Alopex lagopus*) (Frommolt *et al.* 2003) where the amplitudes of recordings were affected by the sounds of waves on the beach.

Comparing the DFA findings for simple scalar variables and histogram-derived PCA values, it can be seen that when PCA values of F_0 or amplitudes were used, PCA achieved a higher individual identification accuracy than simple scalar variables. As PCA describes the differences between the individuals and simple scalar variables describe what these differences are, this suggests that further simple scalar variables should be added to describe howls if using this method alone. However, these two systems can be seen as complementary rather than antagonistic as although histogram-derived PCA values show a more complete image of the differences that exist between individuals, they do not provide information on how these differences are defined. Therefore, using histogram-derived PCA values in conjunction with simple scalar variables allows a fuller picture to emerge. I suggest that using histogram-derived PCA values could improve the accuracy of individual identification in mammals by identifying a larger number of significant deviations between individuals that may not be represented by simple scalar variables alone.

Amplitude is seldom used in bioacoustics studies because of the difficulty of reliably extracting it and controlling the conditions that it is recorded under (Lameira and Wich 2008). Also, it can attenuate over distance, although this does not mean that the information it carries is always lost (Lameira and Wich 2008). This study adds to the increasing evidence that amplitude does encode information about individual identity (Charrier and Harcourt 2006; Charlton *et al.* 2009; Pitcher *et al.* 2012), although it has rarely been tested at distance. It would be advantageous to have definite knowledge of the identity of the individual wolf howling as they often use howls to communicate over long distances with pack-mates and potential breeding partners (Joslin 1967). However, the application of amplitudes *in situ* requires more work to establish the rate of attenuation over distance and through different habitats, and how far this is affected by individuals, either consciously or through vocal tract differences (Bradbury and Vehrencamp 1998). For example, amplitude measurements may function better in certain environments with few obstacles between subject and observer but should be used with caution for species with high frequency calls or those in highly heterogeneous environments. The next step is to demonstrate whether including amplitudes could be effective in identifying wild wolves and this will be the focus of the next chapter. For these, the distance between observer and wolf would, by necessity, vary substantially and it would be important to show whether the amplitudes would remain reliable indicators of wolf identity. It is expected that they should be as robust to distance as orangutan (*Pongo pygmaeus wurmbii*) calls are, with fidelity up to at least 300m (Lameira and Wich 2008).

I limited my study to solo howls from individuals so as not to introduce any problems of crossover, seen in chorus howls, affecting amplitudes. Reliably extracting amplitudes from these more complex recordings poses a future challenge. However, Palacios *et al.* (2012) used chirplet transformation of recordings to separate and extract howls within choruses, where multiple wolves were howling at the same time, and their howls could not easily be separated. I suggest that using histogram-derived PCA values with this, or a similar technique, could allow the reliable separation and classification of howls to individuals using F_0 alone. This method could then be optimised by adding amplitude changes to the analysis.

Charrier and Harcourt (2006) were the first to use normalised amplitude data when using *in situ* wild recordings. Further work could focus on extending my result to wild wolves, and identifying differences between vocalisations of different wolf subspecies, packs and possibly genders. I propose that the use of amplitude data in captive mammal populations, where attenuation and degradation will be minimised, will be beneficial to studies trying to identify individuals from vocalisations. However, there have been few studies which have focused on captive and wild recordings of mammal species. Extending these results to other species, in particular canids known to carry individual identity information in their long-distance vocalisations such as coyotes (Mitchell *et al.* 2006) and African wild dogs (*Lycaon pictus*) (Hartwig 2005), could be possible.

2.6 Conclusion

I have demonstrated that my new bespoke Matlab code has substantially improved both the extraction of acoustic features of Eastern wolf howls and the accuracy of individual identity. Furthermore, I believe that using my combination of bespoke code to extract the features and the addition of histogram-derived PCA values could improve individual identification accuracies in other mammal species.

Identifying individual wild Eastern grey wolves (*Canis lupus lycaon*) using fundamental frequency and amplitude of howls

This chapter was published in Bioacoustics as Root-Gutteridge *et al.* (2014a).

3.1 Introduction

The science of bioacoustics has developed to enable the vocalisations of different species to be utilised in monitoring populations and in exploring the relationship between the animal and its individual call (Bradbury and Vehrencamp 1998). For example, acoustic sampling has successfully been used to monitor wild populations of bats (O'Farrell and Gannon 1999; Parsons and Jones 2000; Bohn *et al.* 2007) and marine mammals (Berrow *et al.* 2009; Frasier *et al.* 2011). However, application *in situ* is often limited by the accuracy of identification, whether to species, group or individual, so improving this accuracy is vital before surveys that can reliably identify individuals in the wild using vocalisations alone can be undertaken.

As acoustic monitoring systems become more advanced (Blumstein *et al.* 2011), recording vocalisations *in situ* has become easier and cheaper, and surveys relying on their analysis are now possible and affordable. The identification of individuals through non-invasive methods such as acoustic monitoring has the potential to produce accurate counts which are vital in conservation studies (for example, McGregor and Peake (1998)) where double-counting and miscounting need to be avoided. For example, a bioacoustic approach has recently been applied to monitor site fidelity in endangered European eagle owls (*Bubo bubo*) (Grava *et al.* 2008).

Increasingly, researchers have tried to determine whether vocalisations carry information about the individual and if these can be used as the basis of individual and life history surveys. This has been so successful in bats that entire software programmes have been developed around their calls, and a bat can now be identified to species (Parsons and Jones 2000), roost site (Fenton *et al.* 2004; Jameson and Hare 2009) and kinship group (Boughman 1997) from its echolocation characteristics alone. It is possible that vocalisations of many other species will carry similar information, and therefore bioacoustics has the potential to improve on current animal identification methodologies.

Acoustic monitoring has already been used to explore the distribution of populations of wild canids, often using elicited response techniques to monitor species with large territories (Joslin 1967). Howls from wild wolves have been used to track wolves in presence / absence surveys because they can be heard from distances of 10 km or more (Joslin 1967). These howls have been recorded using both observers with microphones and more recently with automated howl stimulation boxes (Ausband *et al.* 2011), which are reusable, movable and reliable for elicited wolf howl recordings. However, capture-mark-recapture surveying cannot be undertaken as there is currently no accurate method in place to individually identify wolves based on their howls alone. The accuracy of acoustic sampling of wild wolves is 75.7% when using fundamental frequency (Passilongo *et al.* 2012), less than the 80% threshold suggested by Terry and McGregor (2002). However, the inclusion of amplitude variables in sound analyses have been shown to be useful in improving identification accuracy in a number of species including California sea otters (Mcshane *et al.* 1995), giant pandas (Charlton *et al.* 2009) and Australian sea lions (Pitcher *et al.* 2012). In addition, when tested over a short distance of less than 20 m, amplitudes have been shown to improve the identification accuracy of captive Eastern wolves to 95.5% using simple scalar variables and to 100% when using histogram-derived PCA values (Chapter 2).

In Chapter 2, I reported the development of a bespoke Matlab (Mathworks Inc. 2005) code for extraction of howls from recordings of captive wolves (Chapter 2), which increased both the number of howls extracted and the accuracy achieved by the free speech analysis programme Praat (Boersma and Weenink 2005). The recordings were made at a short distance from the howling wolves to minimise interference, and little work has focused on amplitude differences over distance. Whether a similar result could be achieved for wild wolves is unknown as there are problems of amplitude attenuation with increasing distance (Bradbury and Vehrencamp 1998) and interference in amplitude fidelity under both different atmospheric conditions (Bradbury and Vehrencamp 1998) and in different habitats (Charrier *et al.* 2003).

3.2 Aims

Therefore, the aims of this chapter are to:

1. show whether the bespoke Matlab code developed in Chapter 2 can reliably improve the extraction of sound variables from poor quality and chorus howls which pose challenges to extraction (Palacios *et al.* 2012);

2. demonstrate whether amplitudes can be useful in distinguishing howls of individuals recorded in the wild, and increase the accuracy of identification shown through fundamental frequency alone, with the hope of establishing a baseline for potential *in situ* population surveys;

3. determine whether differences in microphone quality affects individual identification accuracy;

4. determine whether any differences between wolf pack vocalisations are a result of microphone recording fidelity or pack-association signature.

3.3 Materials and methods

A total of 179 howls from 119 individual wild wolves (mean per wolf = 1.53, *SD* = ±2.67) were obtained from 24 recordings from the British Library Sound Archive, Fred H. Harrington via PBS website, and Macaulay Library, New York, with the permission of the copyright owners. The howls were all cited as being from Eastern wolves, and individuals were visually identified at the time of recording. One hundred and fifty-six of the howls were recorded around Algonquin Park, Canada, between May 1959 and 2003. The howls were recorded on six different microphone set-ups in.wav form at 512 bit rate (see Table 3.1 for details).

Table 3.1 Sources of wolf recordings and number of individuals explored. Wolf identity was established either visually or by only sampling a limited number of howls from a recording.

Recording Area	**Recording Date**	**Recording Source**	**Microphone Type**	**Number of Howls**	**Number of Individual Wolves**
Unknown	Exact date unknown (1990s)	Fred H. Harrington	Unknown model	3	1
Ellesmere Island, Canada	Exact date unknown (1990s)	British Library Sound Archive	Unknown model	5	1
Algonquin Park, Canada	1980-1995	British Library Sound Archive	Dan Gibson P-650 and Sony P-206, third model unknown (BBC)	80	50 (maximum)
Algonquin Park, Canada	1959-1960	Macaulay Sound Archive	Nagra III recorded by William Gunn	91	67 (maximum)

Of the 179 howls, I sampled 67 solo howls from 10 individuals (mean per wolf = 6.7, *SD* = ±7.65), with a minimum of three howls per wolf. These were high quality individual howls without any background noise and were used to show whether measuring change in amplitude was suitable for identifying individuals in the wild. The remaining 112 howls were taken from a maximum of 109 wolves, with either one or two howls per wolf. These included poor quality howls (N=10), where the recordings were affected by wind or water noise, and chorus howls, where several wolves were howling at the same time i.e. where normalised amplitudes of harmonics were unsuitable for analysis, except for the normalised amplitude of the fundamental frequency. For the chorus howls, only howls that overlapped in time (the second howl starting before the first ended) were used. So, from a potential 40 howls per recording, often only two or three were actually included.

3.1.1.1 Feature extraction of howls

Howls were extracted from audio files using the bespoke code (Chapter 2) designed in Matlab (Mathworks Inc. 2005) and simple scalar variables were used to describe the features of the fundamental frequency and the amplitudes of the first four harmonics (see Table 3.1). Amplitudes of harmonics two to four could not be reliably extracted from the chorus because, although wolves howl on different fundamental frequencies, they may overlap at points on the same frequencies for the higher harmonics of their howls (Theberge and Falls 1967). Furthermore, poor quality howls were also expected to have less fidelity in amplitude (Bradbury and Vehrencamp 1998). Therefore the amplitudes of harmonics two to four were only used in the analyses for the solo howls.

3.1.1.2 Automatic identification of deviations by PCA

The howl feature extraction data were fed, in the form of a training data base, to a Principle Component Analysis (PCA) where the 40 greatest PCA values were considered for further identification using Discriminant Function Analysis (DFA). PCA values were obtained using two separate strategies (i) the histograms of the fundamental frequency (F_0 probability) only, undertaken on all 179 howls, and (ii) the histograms of the amplitude of the first harmonic (amplitude probability) only, limited to the 67 high quality solo howls. Therefore, the 67 solo howls had a total of 80 PCA values (F_0 and amplitude of harmonic one probability) for further identification via DFA.

3.3.1 Classification using DFA

DFA was optimised by using one-way analyses of variance (ANOVA) in SPSS 17 (SPSS Inc. 2010) on all data sets to determine whether there was a difference within individuals, microphones and packs for each of the 27 extracted simple scalar variables (Table 2.2) so that only variables which were significantly different between individuals were used in the DFA, following Palacios *et al.* (2007).

3.1.1.3 Analysis 1: Individual identification of wolves from chorus and poor quality howls

Using bespoke Matlab code (Chapter 2), I extracted acoustic features from 179 howls from a maximum of 119 wolves. DFA was applied to (i) the histogram-derived PCA values and (ii) simple scalar variables describing changes in F_0 only. In addition, DFA was applied to (i) the histogram-derived PCA values and (ii) simple scalar variables describing changes in both F_0 and normalised amplitude of harmonic one (NorAmp1) in an attempt to improve individual identification further (Chapter 2).

3.1.1.4 Analysis 2: Individual identification of wolves from solo howls

A further analysis was made of the 67 solo howls, from 10 wolves, where all amplitudes could be used. Therefore, in addition to simple scalar variables describing F_0 and normalised amplitude of harmonic one, the normalised amplitude of harmonics two to four (NorAmp2, NorAmp3 and NorAmp4) were included in the DFA. Analyses were undertaken for (i) F_0 alone, (ii) amplitudes of harmonics one to four alone, and (iii) both F_0 and amplitudes of harmonics one to four together. A stepwise DFA was then undertaken to establish which acoustic variables contributed most to the analysis, with variables considered based on the change in Wilk’s lambda (F to enter = 3.84; F to remove = 2.71).

3.1.1.5 Analysis 3: Identification of wolves using different microphone types

A separate DFA was performed for each of the three microphones that had recorded howls from more than one individual wolf (see Table 3.3 for details). Analyses were undertaken using the simple scalar variables describing (i) F_0 alone and (ii) F_0 plus the normalised amplitudes of harmonic one.

3.1.1.6 Analysis 4: Potential microphone and pack differences

DFA was applied to all 179 howls which were recorded using 6 different microphone types and were from 14 different packs. Microphone type, where unknown, was assumed to be different because of the different decades the recordings were made and the improbability of a 1990s' era recorder using the same as the 1960s' era recorder (Table 3.1). Analyses were undertaken using the simple scalar variables describing (i) F_0 alone and (ii) F_0 plus the normalised amplitudes of harmonic one.

3.4 Results

Following the optimisation of extracted sound variables for DFA, only the position in the howl at which the minimum frequency occurred (PosMin) was non-significant for all 119 wolves together ($F_{62, 116}$=1.259, p=0.162), the 10 wolves from the solo howls ($F_{9, 66}$=1.806, p=0.087), the 14 packs ($F_{13, 165}$=1.715, p=0.062) and the 6 microphone types ($F_{5, 173}$=1.724, p=0.131). This was in agreement with optimisation of extracted sound variable from captive Eastern wolves (Chapter 2).

3.1.1.7 Analysis 1: Individual identification of wolves from chorus and poor quality howls

When all 179 howls from the 119 wolves were analysed together, DFA using F_0 simple scalar variables alone, extracted by the bespoke Matlab code, achieved 82.7% identification accuracy (Table 3.2). This accuracy was improved to 97.4% when using histogram-derived PCA values, suggesting that individuality is strongly present in howls, despite the quality of howl recording or the extraction of acoustic variables from chorus howls. However, more simple scalar variables are required to define individuality to match the PCA values result.

Table 3.2 Summary of the Discriminant Function Analyses for individual identification of wild wolves.

Howls used	**Variables used**	**Identification accuracy from DFA using simple scalar variables**	**Identification accuracy from DFA using histogram-derived PCA values**	**Difference between DFA using simple scalar variables and histogram-derived PCA values**
179 howls, including solo and chorus	F_0	82.7%	97.4%	+14.7%
67 solo howls	F_0	88.1%	100%	+11.9%
	Amplitude of Harmonics 1-4	88.1%	100%	+11.9%
	F_0 and Amplitude of Harmonics 1-4	98.5%	100%	+1.5%

3.1.1.8 Analysis 2: Individual identification of wolves from solo howls

When the 67 best quality solo howls were analysed with DFA using F_0 simple scalar variables alone, 88.1% identification accuracy was achieved which was further improved to an accuracy of 100% when using histogram-derived PCA values (Table 3.2). These same percentages were also seen for amplitudes of harmonics one to four alone (Table 3.2). When DFA was applied to both F_0 and amplitudes of harmonics one to four, identification accuracy was increased to 98.5% (+10.4% over either F_0 or amplitude alone) and further improved to an accuracy of 100% when using histogram-derived PCA values of F_0 and amplitude one (Table 3.2; Figure 3.1). Therefore, wild wolves like captive wolvess can be accurately identified from solo howls using changes in both F_0 and amplitude of their howls, indicating that amplitudes carry information on wolf identity.

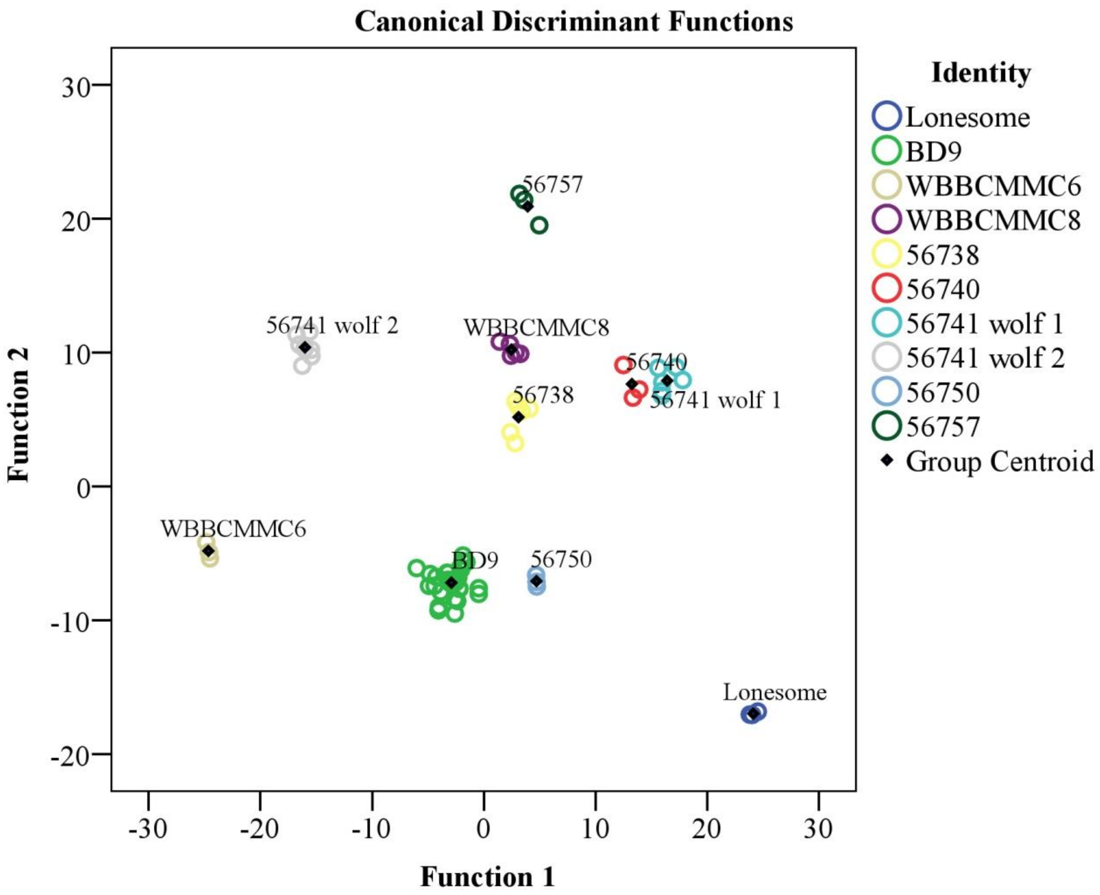


Figure 3.1 Plot of DFA output using histogram-derived PCA values for 67 solo howls from 10 wolves with 100% accuracy achieved.

Stepwise DFA of the bespoke Matlab code's simple scalar variables showed that the four most important variables were the normalised maximum amplitude of the third harmonic (NorAmp3Max: F to remove = 17.151, Wilks lambda = 0.018), duration (Dur: F to remove = 21.847, Wilks lambda = 0.021), F_0 at the position of maximum amplitude of first harmonic (FreqPAF: F to remove = 19.311, Wilks lambda = 0.019) and range of the F_0 (F0Range: F to remove = 13.764, Wilks lambda = 0.015). These four variables alone could achieve identification accuracies of 85.1%, compared to 98.5% using all 26 variables.

3.1.1.9 Analysis 3: Identification of wolves using different microphone types

Using simple scalar variables of F_0 alone, the lowest identification accuracy was 82.4%, achieved from the oldest microphone (Nagra III recordings made in 1959-1960), with the newer recordings achieving 90-100% accuracy (Table 3.3). However, this could not be separated from the effect of the larger sample size for the Nagra III recordings. When simple scalar variables of normalised amplitudes of harmonic 1 were also included in the analyses, I improved the accuracies achieved (apart from the Dan Gibson P650 microphone which remained at 100%; Table 3.3). The F_0 alone findings were similar to those for all 179 howls analysed together (82.7%) and for wild wolves (75.7%) (Passilongo *et al.* 2012). Therefore, it is likely that I detected differences between wolves rather than simply detecting differences in equipment.

Table 3.3 Discriminant Function Analysis for each microphone type.

	Nagra III	**Dan Gibson P650**	**Unknown BBC model**
Number of howls	91	49	10
Number of individuals	67	22	3
Identification Accuracy for F_0 only	82.4%	100%	90%
Identification Accuracy for F_0 & HAmp1	87.9%	100%	100%

3.1.1.10 Analysis 4: Potential microphone and pack differences

Using simple scalar variables of F_0 alone, the different microphones were identified with 74.9% accuracy (Figure 3.2) and the different packs with 66.5% accuracy (Figure 3.3). Viewing these figures together, it is clear that the groupings to microphones and packs are too similar to separate the effects of each and to know which is creating the groupings. However, when using simple scalar variables of both F_0 and the normalised amplitude of harmonic one, the howls recorded on different microphones were identified with 79.9% accuracy (+5.0%) and from different packs with 70.4% accuracy (+3.9%). Pack-association signature and microphone effect could not be separated further.

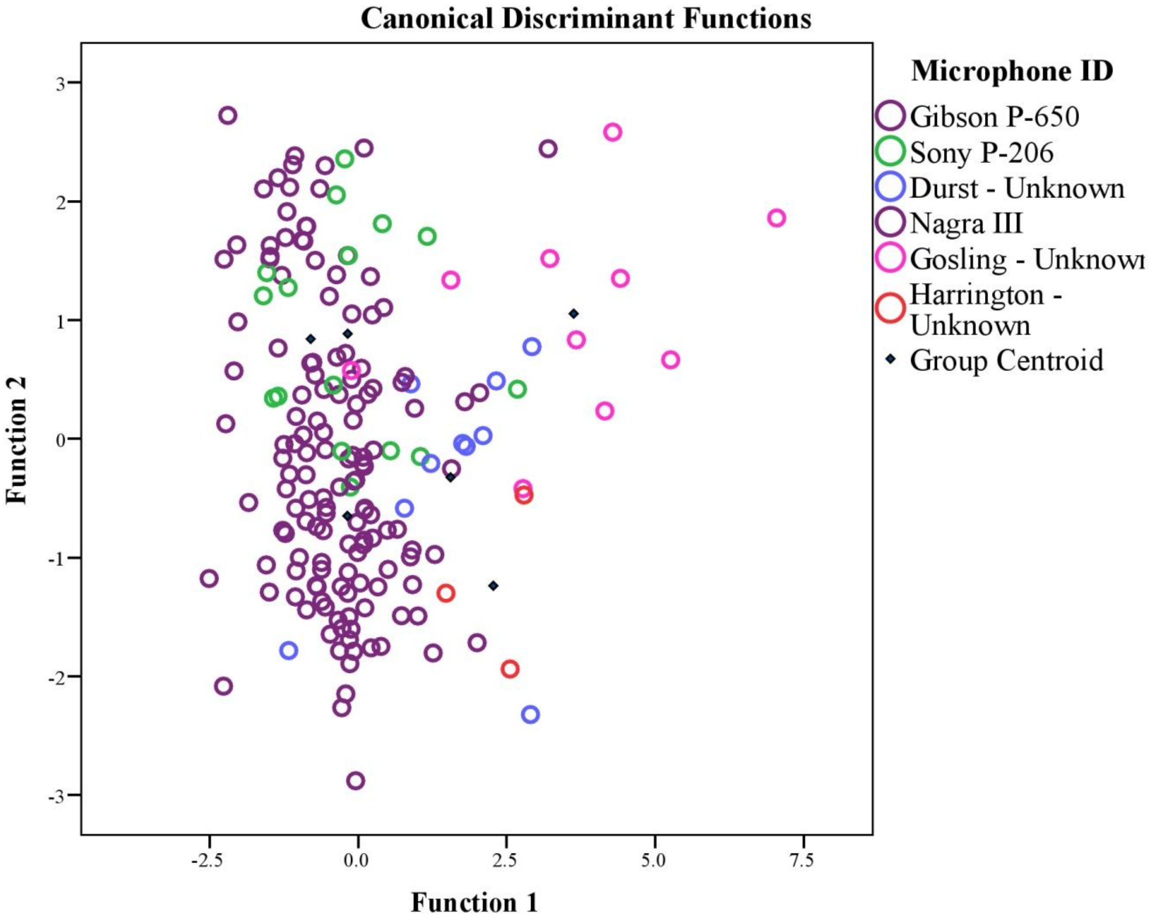


Figure 3.2 Plot of DFA output for 179 howls recorded across 6 microphones with 74.9% accuracy in microphone identification, using simple scalar variables. Clustering to microphone is stronger for some microphones (e.g. Nagra III) than others (e.g. Dan Gibson P-650 and Sony P-206 parabola microphones).

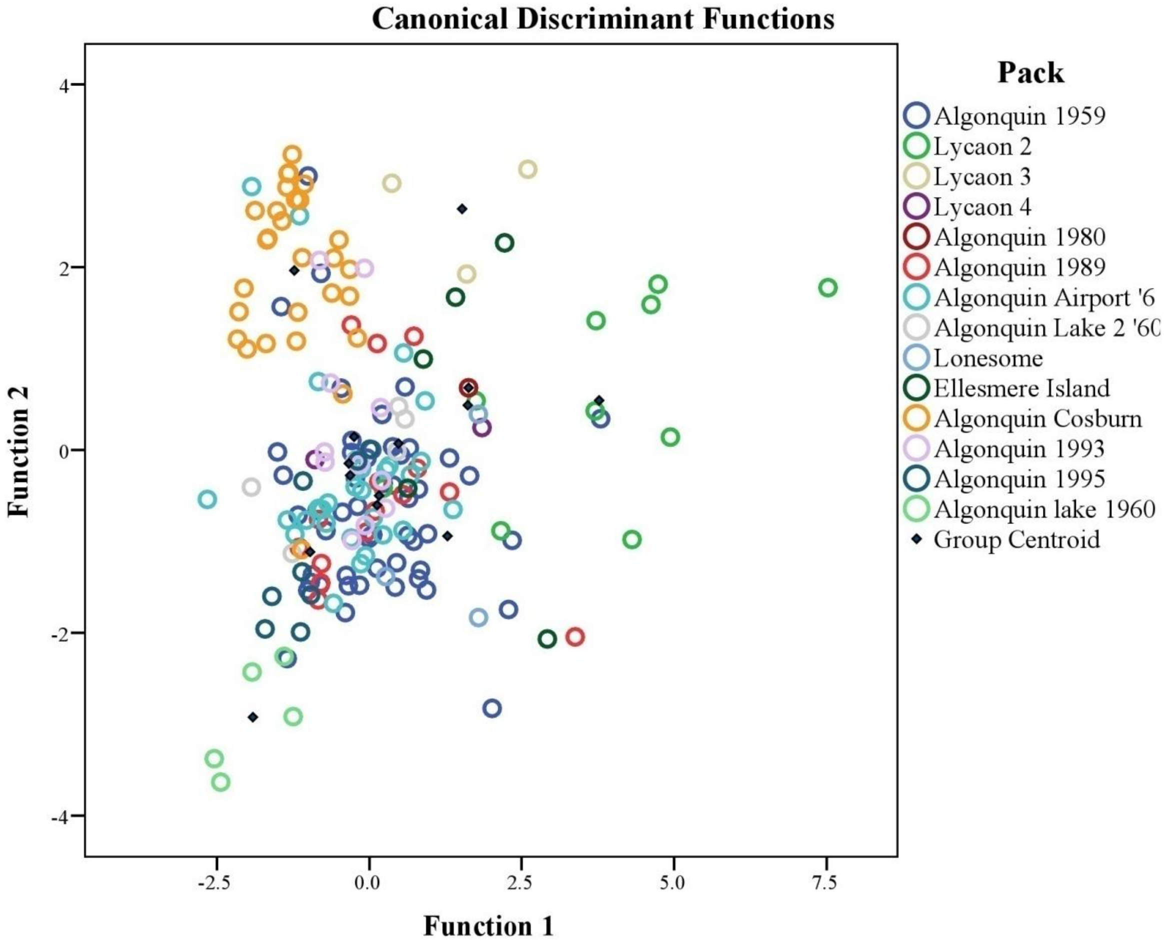


Figure 3.3 Plot of DFA output for 179 howls recorded with 66.5% accuracy in pack identification, using simple scalar variables. Note the similarity to Figure 3.2 in the distribution of wolves and that pack-association signature is weak compared to individual identification.

3.5 Discussion

We show that wild Eastern wolves can be individually identified with high accuracy using methods of howl extraction and analysis developed for captive Eastern wolves (Chapter 2). My findings improve upon other methods (Tooze *et al.* 1990; Passilongo *et al.* 2012), with DFA from histogram-derived PCA values for F_0 alone achieving 100% accuracy for wolf identity from solo howls (Table 3.2).

Normalised harmonic amplitudes were shown to improve individual identification accuracy of howls from wild wolves in their natural habitat, as it was for captive Eastern wolves (Chapter 2). It is likely that by including amplitudes in analyses of other canids, individual identification accuracy in these species may also be improved. I further suggest that the simple scalar variables used in previous bioacoustics studies to accurately assign wolf identity (Tooze *et al.* 1990; Palacios *et al.* 2007; Passilongo *et al.* 2012), can be improved by using DFA with histogram-derived PCA values.

Furthermore, utilisation of the new bespoke Matlab extraction code overcame the problem of reliably extracting amplitudes. This has formerly beset *in situ* recording studies (e.g. (Frommolt *et al.* 2003; Mitchell *et al.* 2006) due to the difficulty in reliably excluding background noise. The new bespoke Matlab extraction code substantially alleviates this difficulty and may allow recordings to be re-analysed with amplitude data included, thereby improving accuracy of identification of individuals from their vocalisations.

The application of encoding individual identity from amplitudes *in situ* requires more work to establish the rate of attenuation over distance, through different habitats and under different atmospheric conditions, and how far this is affected by individuals, either actively or through vocal tract differences. For my data, non-standard recording conditions including distance between recording equipment and wolf did not prevent us from accurately identifying individuals and correctly classifying howls, suggesting that differences in weather conditions and distance to howl will not prevent my method from working. Nevertheless, more detailed analysis of wolf howls in natural habitats is suggested to assess the rate of loss of amplitude across the lowest four harmonics and the effects of distance and weather, with changes in temperature and wind speed expected to have the largest impact on amplitude attenuation (Frommolt 2002; Frommolt *et al.* 2003). For this study, only recordings made under calm dry conditions were used when amplitudes were included.

My findings showed that not all of the amplitude variables were of equal value in identifying individuals, and changes in amplitude of harmonic three showed the greatest individuality, contributing the most to correct classification. Mitchell *et al.* (2006) suggested that coyotes (*Canis latrans*) may control amplitudes of vocalisations in order to achieve the highest fidelity at distances of over 1km. Whether wolves do the same is unknown but my findings suggest a field of further study, with a focus on whether there is a specific quality of the amplitude of harmonic three, which clearly carries more information on the individual animal than the other harmonics.

When all 179 howls were included in the analysis, the small number of howls per individual (often one chorus howl per individual by necessity) produced findings that (82.7-97.4% accuracy, Table 3.2) were more tentative than when only the ten wolves with at least three howls per individual were tested (88.1-100% accuracy, Table 3.2). Therefore it was easier to separate a few wolves represented by multiple howls than many wolves each represented by one or two howls. However, the finding for the full 119 is still the highest accuracy of individual identification for wild wolves using F_0 alone. Furthermore, the complicated chorus howls and low quality of the recordings did not prevent high accuracy of identification of all wolves. Being able to include chorus howls in acoustic analyses improves the usefulness of my method of individual identification as wolves are displaying individuality as part of a group as well as when howling solo (Theberge and Falls 1967; Palacios *et al.* 2007). Again, my new method of extraction and analysis could allow howl recordings to be re-analysed to include individual information from chorus howls.

As microphone technology has advanced, it is possible that differences in equipment used to collect howls and the associated differences in recording fidelity (particularly in amplitude) would affect the accuracy of individual identification. Overall, there were differences between the newer microphones and the oldest (Nagra III), with only the two newest microphones achieving 100% accuracy when using F_0 and amplitudes together. Microphone age, where uncertain, was estimated from the age of the recordings and because these spanned decades, it was unlikely that the microphone used by Gunn in the 1960s would be contemporaneous with those used by the BBC in the 1990s (Table 3.1). However, when only the best quality howls were used, 100% accuracy was still achieved (Table 3.2). This either indicates that there is no difference in recording quality with different microphones or, more likely, that the new bespoke Matlab code is capable of extracting howl data with minimum influence of microphone type as presumably poorer quality microphones would just produce poorer quality recordings akin to those with lots of background noise.

Classification of individuals (Figure 3.2 and Figure 3.3) did not show a clear effect of pack or microphone conclusively as there was some overlay between microphone type and pack identity which could not be separated further. There was also a possibility of regional association signature (Figure 3.3) where wolves from Algonquin Park grouped more closely to each other than wolves from more distant geographic regions. In addition, many of the recordings were from wolves in the same geographic region and therefore probably related to each other (e.g. packs from the 1990 s could have been descended from the 1960 s), making it impossible to compare pack-association signature with those found by Passilongo *et al.* (2010) in Italian wolves. I suggest that both pack and regional association signatures should be explored in Eastern wolves as they have been in Italian wolves (Passilongo *et al.* 2010; Zaccaroni *et al.* 2012), focusing either on differences between packs from the same geographic region in the same time frame or alongside genetic studies to compare potential pack-association signature with relatedness. Information on how pack-association signature is maintained or changes over time would be interesting for both learning-culture studies and genetic studies and could be used to show whether wolves retain their natal pack-association signature when removed from their natal pack.

3.6 Conclusion

The high accuracy of individual identification of captive Eastern wolves from howl recordings (Chapter 2) is repeated here for wild Eastern wolves, suggesting that the new bespoke Matlab extraction code and analysis based on histogram-derived PCA values could improve extraction of vocalisations from recordings of other canid species. This new method of analysis of vocalisations could form the basis of future survey techniques for the individual identification of wild canids.

Using Artificial Neural Networks to Identify Individual Wolves: A Novel Survey Method

4.1 Introduction

Knowing the population size of species is fundamental to providing effective wildlife management. However, accurate censusing of populations is notoriously difficult, especially for species that are nocturnal, elusive or at low density (Long 2008). To recognise individuals for surveys, they must have demonstrable inter-individual variation that can be assessed and used to uniquely identify them e.g. the distinctive arrangement of whiskers on a polar bear (Anderson *et al.* 2007). Chapters 2 and 3 showed that Eastern grey wolves have high individual variation in their howls that can be used to identify individuals with up to 100% accuracy using discriminant function analysis (DFA). This suggests that howls do indeed carry enough between-individual variation to function as a tool for *in situ* monitoring of wolves and may also be applicable to other canids which have shown individual variation in their vocalisations (Darden *et al.* 2003; Frommolt *et al.* 2003; Robbins and McCreery 2003; Hartwig 2005).

Wolf surveys are very difficult to perform accurately *in situ* because of their large home range sizes, long dispersal distances and fear of humans (Joslin 1967; Carlos Blanco and Cortes 2012). Current techniques such as radio telemetry and GPS collars are expensive, time-consuming to use and require the capture of individuals (Gogan *et al.* 2004), while remote wolf howl surveys by ear do not produce reliable counts of numbers or identify individuals (Harrington and Mech 1982). However, howls do provide a useful remote monitoring tool for both finding and tracking wolves *in situ* as they carry over distances of more than 6 miles (10 km) (Joslin 1967). Nevertheless, although current analytical methods (such as DFA) can identify individual wolves from recordings of howls, they can only do so where the identity of the wolf howling is already known and cannot identify unknown howls from new individuals (Tooze *et al.* 1990). Therefore, a further method of recognition capable of assigning novel data to novel individuals is required before vocalisations can be used for *in situ* surveys (Terry and McGregor 2002). Artificial neural networks (ANNs) present a potential non-invasive analytical tool to quantify and identify new wolves and to recognise their howls in new recordings.

Although artificial neural networks have been used for such diverse applications as disease classification (Lemetre *et al.* 2010), the identification of biomarkers (Lancashire *et al.* 2009), and the environmental effects of ozone on clover (*Trifolium repens*) (Ball *et al.* 1998), they have also been used for almost twenty years in the fields of mammal vocalisation research (Potter *et al.* 1994) and ecological modelling (Lek and Guegan 1999). In order for the ANN models to be applicable to wildlife surveys an accuracy of 80% or higher is desired from the validation data (Terry and McGregor 2002). Neural networks have already successfully identified known individuals from their vocalisations in fallow deer (90% recognition) (Reby *et al.* 1998), corncrakes (*Crex crex*) (96.5% recognition) (Terry and McGregor 2002), stellar sea lions (*Eumetopias jubatus*) (76.7% recognition) (Campbell *et al.* 2002), south-western willow flycatchers (*Empidonax traillii extimus*) (80.7% recognition) (Fernandez-Juricic *et al.* 2009) and blue monkeys (*Cercopithecus mitis stuhlmanni*) (73% recognition) (Mielke and Zuberbuehler 2013). Furthermore, while comparatively few studies have used ANNs to identify individuals, the results have been striking: ANNs achieved an accuracy of 92.5-95.6% for the classification of types of vocalisations in black lemurs

(*Eulemur macaco*), compared to DFA and PCA accuracies of only 76.1-88.4% (Pozzi *et al.* 2010). Similarly, when Jennings, Parsons and Pocock (2009) compared accuracies of human and ANN classifications of bat echolocation calls to species, ANNs were more accurate than 75% of humans and achieved higher accuracies overall. ANNs have even achieved accuracies of 100% for recognition and 90% for prediction success on fallow deer vocalisations (Reby *et al.* 1997). ANNs are therefore considered to be a good direction for the further improvement of classifying vocalisations.

4.1.1 Artificial Neural Networks methodology

Artificial neural networks were inspired by the human body's natural neurons, synapses, axons and membranes (Bishop 1994). ANNs can either reduce complex systems to simpler elements to make them easier to understand, or gather simple elements to form more complex systems (Rumelhart *et al.* 1986). Networks are characterised by components of a set of nodes and the connections between those nodes (Bishop 1994). Nodes receive inputs and process them to obtain an output, which can be as simple as a sum of the inputs or as complex as a node containing another network (Rumelhart *et al.* 1986; Bishop 1994). The connections can be either uni- or bi-directional between nodes and the connections control the flow of information between the nodes (Lemetre *et al.* 2010). The inputs are multiplied by the weight of the signal (the signal strength) and these weights can be positive or negative, with positive weights promoting and negative weights inhibiting the input's importance in the overall network (Lemetre *et al.* 2010). The desired output of the network can be obtained by weighting all the artificial neurons, a difficult process where hundreds or thousands are used, according to which specific inputs are used (Lemetre *et al.* 2010). The process of using algorithms to adjust these weights is referred to as learning or training (Rumelhart *et al.*

1986). This training can be corrected in back-propagation networks where weights are changed according to the desired outcomes, and inputs can be weighted differently to obtain different desired results (Newhall *et al.* 2012).

For wolf howls, this adjustment could be a case of weighting the mean of the fundamental frequency more heavily when looking to identify sex than when trying to ascertain individual identity, and negatively weighting those variables which are found to be less important for assessing sex or individual identity, such as the position of the minimum frequency. However, one criticism of ANNs is that they are a ‘black box’ where the user does not know which variables are being weighted during the analysis. However, this can be overcome by analysing the nature of the input layer as well as the information contained in the output layer (Schmid *et al.* 2005). In the case of howls, this could be tested by altering the variables used in the input and comparing the output accuracies e.g. ANOVA-selected variables versus all variables. This is a similar method to automated stepwise DFA where the importance of each variable to the classification is tested.

ANNs use back-propagation algorithms to construct models from examples of data with known outputs, known as a supervised approach, allowing the prediction of an output vector (e.g. wolf identity) for a given input vector (e.g. fundamental frequency) (Lek and Guegan 1999; Ball *et al.* 2002). The models use the data presented in the training database, which is assumed to be representative of any set of potential data. Therefore, for ANNs to work with wolf howls, there must be consistency in how wolf howls differ between individuals, which can be generalised to define differences between all possible individuals. In the case of ANNs, this means including enough howls to generate generalised rules for individual identity rather than specific rules for separating two particular wolves. This method can also be applied to sexes, packs, geographic regions etc with the expectation that including more examples in the training dataset will provide more useful generalised rules. A too small database will generalise rules according to differences between particular individuals which may have anomalous features in their howls.

4.1.2 Application of ANNs to population surveys

One of the advantages of ANNs is that they can recognise novel data not used in a training data set and can assign the novel data to an “unknown” category based on the rules built with the known training data. For example, DFA in Chapter 2 demonstrated that F0Mean, CofV, NorAmp2Mean and NorAmp2Max were the most important variables for individual identification, and therefore these would be more weighted in ANNs than PosMin which did not appear to carry information about the vocalising individual.

ANNs are capable of identifying unknown individuals (which DFA cannot), thus they can be used as a non-invasive identification method to assist population surveys (Terry and McGregor 2002). However, the effectiveness of ANNs for population surveys using the calls of individuals has only been assessed in corncrakes (Terry and McGregor 2002) and flycatchers (Fernandez-Juricic *et al.* 2009). Nevertheless, the accuracies of identification for these were good with ANNs achieving best identification and recognition accuracies of 91.3% and 94.7%, respectively, for corncrakes (Terry and McGregor 2002) and 81% identification and 81% recognition for flycatchers (Fernandez-Juricic *et al.* 2009). As the effectiveness of ANNs in vocal recognition is still being assessed, ANN accuracies are compared to the established DFA accuracies before applying ANNs to novel data (Fernandez-Juricic *et al.* 2009). However, both of these studies achieved the minimum of

80% recognition accuracy for *in situ* surveys, suggesting that ANNs can be used for future survey efforts.

It is expected that an ANN model that has achieved a high accuracy using only the training data will classify vocalisations to individuals with a high degree of confidence (Fernandez-Juricic *et al.* 2009). However, to maximise the accuracy of the predictions for novel data, a set of best models must be developed with the known training data. While ANNs have successfully classified novel data to individuals in corncrakes (Terry and McGregor 2002) and south-western willow flycatchers (Fernandez-Juricic *et al.* 2009), the potential of neural networks as survey tools has yet to be explored in wolves and other canids.

Achieving the best models for individual identification of wolves from howls will be a challenge because, while ANNs can cope with complex data, increasing the amount of data may impact on the accuracy of the model. For example, pack-association signature (Palacios *et al.* 2007), pack signature (Passilongo *et al.* 2010; Zaccaroni *et al.* 2012), the inclusion of howls from only a single pack of a single subspecies or multiple packs from multiple subspecies, and the number of howls per wolf might all affect model accuracy.

4.2 Aims

This chapter aims to show whether ANNs can be used to successfully attribute howls to unknown wolves and to establish the accuracy of the classification for:

1. Individual identity within Eastern wolves

   (Using the howl data from Chapters 2 and 3)
2. Individual identity within nine subspecies / species combined
3. Individual identity within European wolves
4. Individual identity within Mackenzie Valley (present in North America) wolves
5. The number of howls per wolf required to achieve over 80% correct classification
6. Pack identity for eleven subspecies / species
7. Subspecies identity for eleven subspecies / species

4.3 Method

4.3.1 Howl collection

A total of 1262 wolf howl recordings were collected from a variety of sources (see Table 10.1 in Appendix 4). These included both solo and chorus howls and were sampled from both wild and captive populations of as many subspecies as could be sourced (Table 4.1). A description of each subspecies can be found in Appendix 3.

Table 4.1 Total number of *Canis* howls sampled per subspecies

Subspecies	Scientific name	Number of chorus & solo howls*	Number of solo howls
Arctic	*C.l. arctos*	40	20
Eastern Timber	*C.l. lycaon*	724	286
European	*C.l. lupus*	195	139
Great Lakes	*C.l. lycaon x. nubilus*	9	9
Great Plains	*C.l. nubilus*	33	17
Iberian	*C.l. signatus*	25	25
Mackenzie Valley	*C.l. occidentalis*	134	72
Mexican	*C.l. baileyi*	42	29
North African Wolf / Golden Jackal	*Canis aureus lupaster / Canis aureus*	44	35
Red	*C.l. rufus*	7	0
Tibetan	*C.l. chanco*	9	0
Total	**11 subspecies**	**1262**	**632**

*Including those solo howls used in solo howl analysis

4.1.1.1 Howls suitable for individual identification

Although 1262 howls were sourced, identity was known for only some of these wolves, and samples for the individual identity analysis were limited to those where identity could be established (sources of howls and numbers of howls used per subspecies are listed in Appendix 4). Wherever possible, samples from both wild and captive populations were used for each subspecies. However, this was not possible for red wolves, where only captive individuals were recorded, or for Great Plains and Great Lakes wolves, where only wild wolves were recorded. Wild wolf identity was established as in Chapter 3 by using either only solo howls or only a limited selection of howls per chorus that overlapped in time.

Captive wolf howls were collected by the author using the elicited howling method developed by Harrington & Mech (1979). Where possible for captive wolves, video footage was also recorded using a Sanyo Xacti CG20 digital video recorder to aid later individual identification when wolves were howling in chorus.

4.3.2 General method for ANNs

The data were analysed using Statistica (StatSoft 2012), which automatically randomly separates the data into three: a training subsample (70% of the original dataset), a testing subsample (15%) and a validation subsample (15%), by a process known as Monte Carlo or Random Sample Cross-validation. These percentages can be manually altered but were left unchanged and the sub-sampling method was set to be random. The training subsample is used to form the model, the testing subsample is used while the model is being formed and the validation subsample is used to independently train the model on data completely new to the model (Bishop 1994; Lancashire *et al.* 2009).

The ANN was a form of multi-layer perceptron analysis, where multiple layers of activation nodes exist in a directed graph with each layer fully connected to the next layer, and is a modified form of standard linear perceptron. The minimum and maximum numbers of hidden units were set automatically by Statistica (typically 4-14 minimum units, 21-25 maximum units, depending on input number). The ANNs were programmed to run 1, 500 best-fit classification models and to select 10 models with the lowest errors. Following (Fernandez-Juricic *et al.* 2009), one model was selected as presenting the highest overall training and recognition performances: if ANN model 1 achieved 100% training classification and 85% correct recognition but ANN model 2 achieved 98% training classification and 95% correct recognition, ANN model 2 was selected.

To establish a baseline for identity using an accepted standard method, SPSS 17 (SPSS Inc. 2010) was used to match each ANN model with one using DFA, as for Chapters 2 and 3, in order to make direct comparisons (Fernandez-Juricic *et al.* 2009).

4.3.3 Variables used in the ANN

Although histogram-derived PCA values achieved the highest rates of accuracy for individual identification in Chapters 2 and 3, they were not used in the ANNs as PCA values alter when additional data are included. Therefore, applying a PCA to a dataset including an additional wolf howl would alter all the previous values and make models formed on the first dataset inapplicable to the new dataset.

As in Chapters 2 and 3, to optimise the variables used in the ANNs one-way analyses of variance (ANOVAs) were undertaken in SPSS 17 on each of the simple scalar variables to see if there was a significant difference between individuals which would be useful for the ANNs (Tooze *et al.* 1990). Variables which were non-significant were excluded from the models. Findings from analysis of the PCA values in Chapters 2 and 3 suggested that not enough simple scalar variables were being used to describe the changes in amplitude, so the coefficient of variation of the amplitude of each harmonic was added to the analysis for simple scalar variables, which matched the coefficient of variation of the fundamental frequency in Chapters 2 and 3. This was acquired using the same formula as the coefficient of variation of F_0 described in Table 2.2: 'Coefficient of amplitude variation = (SD / Mean) x 100', Chapter 2.

Finally, any survey method needs to be effective at classifying chorus howls as well as solo howls, so both were used in the analyses, with amplitude data included only where applicable (e.g. to solo howls). Therefore, two sets of model ANNs were developed, one where only fundamental frequency (F_0) was applicable for chorus and low quality howls, and a second where amplitude data could also be used for solo howls.

4.3.4 Individual Identity

Eight subsamples from the total dataset were compiled to explore the accuracy of ANNs to classify howls to individuals (Table 4.2). The datasets included different numbers of packs and subspecies (see Table 4.1 and Table 4.3). Analyses 1-4 were limited to Eastern wolves and the same 156 solo wolf howls that were used in Chapters 2 and 3 were again used as test models to find the most useful set of variables for the ANNs. Analyses 5 and 6 looked at a combination of howls from 9 subspecies with either chorus howls included (Analysis 5) or excluded (Analysis 6). Two subspecies of the 11 were excluded due to the very small sample sizes. Finally, analyses 7 and 8 looked at European and Mackenzie Valley wolves, two subspecies with the next largest datasets to Eastern wolves. The solo howls from European and Mackenzie Valley wolves used in Analysis 6 are the same as those used in Analyses 7 and 8, respectively. 1500 ANN models were built and the best 10 were retained for comparison.

Table 4.2 Analyses undertaken for individual identification for all subspecies using PCA values and simple scalar (SS) variables for either F_0 data alone or F_0 data plus various amplitude variables [* indicates howl used in chapters 2 and 3]

Subspecies	Analysis	Number of individuals	Number of howls	Variables used			
				F_0	F_0 & Nor Amp of Harmonic 1	F_0 & Nor Amp of Harmonics 1-4	F_0, Nor Amp of Harmonics 1-4 & Nor Amp CofV
Eastern	1	6	89 solo*	X	X	X	X
	2	10	67 solo*	X	X	X	X
	3	16	156 solo*	X	X	X	X
	4	134	430 chorus & solo	X	N/A		

All 9 subspecies	5	162	774 chorus & solo	X	N/A		
	6	118	632 solo	X	X	X	
European	7	19	139 solo	X	X	X	X
Mackenzie Valley	8	8	72 solo	X	X	X	X

Table 4.3 List of howls used per subspecies for Analyses 5 and 6

Subspecies	Number of howls used for Analysis 5	Number of howls used for Analysis 6
Arctic	35	20
Eastern	369	286
European	159	139
Great Lakes	9	9
Great Plains	17	17
Iberian	25	25
Mackenzie Valley	90	72
Mexican	29	29
North African / Golden jackal	41	35

4.1.1.2 Exploring the Effect of Howl Sample Size on ANN Classification Accuracy

Following Fernandez-Juricic, del Novo and Poston (2009), analyses 9 and 10 established how many howls were required to achieve the 80% recognition accuracy required for *in situ* monitoring (Terry and McGregor 2002) with simple scalar variables of either F_0 alone or F_0 with the normalised amplitudes of harmonics 1-4, as in Table 2.2. Therefore, ANNs were run on subsamples of howls of 3, 4, 5, 6, 7, 8, 9, 10 and 20 solo howls per wolf, in order to establish whether a minimum number of howls are required to successfully build models for surveying and at what point adding additional howls stopped increasing the accuracy of the models.

For Analysis 9, howls were selected randomly (using a random number table) from the total number of solo howls (N=632) used in Analysis 6, with 9 subspecies represented. Following Fernandez-Juricic, del Nevo and Poston (2009). For analysis 10, only Eastern wolf howls were used (N=430) to reflect the results of subspecies association signature in Analyses 11 and 12, using the same method as for Analysis 9. Analyses removing pack-association signature were not possible because there were not enough individuals per pack.

4.3.5 Exploring Pack & Subspecies Association Signatures in Howls

Analyses 11 and 12 were undertaken on data including and excluding chorus howls to determine whether pack or subspecies association signature existed and if this could affect the overall accuracy of the individual identification when the largest datasets were used. This used the same protocol as for individual identification, simply replacing the target variable 'individual ID' with 'pack ID' or 'subspecies ID'.

Analysis 11 used chorus howls where amplitudes were not applicable so F_0 alone was used, analysis 12 used only solo howls and therefore both amplitude and F_0 variables were applicable (Table 4.4). Two further subspecies (red and Tibetan wolves) were included in analysis 11, having been excluded before because they were only represented by chorus howls so were not suitable for individual identification analysis.

Table 4.4 Analyses using simple scalar variables for pack and subspecies identity for chorus and solos howl collections

Analysis	**No. of subspecies**	**No. of howls**	**F_0**		**F_0 & NorAmp1-4**	
			Pack	**Subspecies**	**Pack**	**Subspecies**
11	11	1262 (chorus & solos)	X	X		
12	9	632 (solos only)	X	X	X	X

4.4 Results

4.4.1 Individual Identity

The most accurate ANN models were always achieved using simple scalar variables of F_0 and normalised amplitudes of harmonics 1 – 4, and were always more accurate than the DFA method of identification for the training ANN (where possible when DFA did not achieve 100% accuracy) but not the validation ANN (Table 4.5). However, the validation performances for the single subspecies analyses (1-3, 7 & 8) of 82.6 - 100% were all above the accepted 80% correct classification accuracy for *in situ* surveys (Terry and McGregor 2002). For wolves compared across packs, as well as across individuals, the validation results were increased to 100% in analysis 2 (Table 4.5). Although not all models achieved the 80% accuracy threshold, exceptions occurred where there were very large differences in the number of cases representing each wolf so that a wolf might not be included in the randomly selected training dataset (as in Analysis 4) or when many subspecies were analysed together (as in Analyses 5 and 6). Therefore, ANNs worked when single howls were included to separate individuals but random selection is not advised when training databases.

Table 4.5 Findings from the most accurate models for individual identification of wolves for analyses 1-8, achieved using simple scalar variables (SS) of fundamental frequency (F_0) and normalised amplitudes of harmonics 1-4 (NorAmp1-4) where applicable for solo howls

Subspecies	Analysis	DFA accuracy (%)	ANN accuracy (%)		Difference in accuracy between DFA and training ANN (%)	Variables used
			Training	Validation		
Eastern	1	95.5	100	92.3	+4.5	SS F_0 & NorAmp1-4 inc. CofV NorAmp1-4
	2	100	100	100	0.0	SS F_0 & NorAmp1-4 inc. CofV NorAmp1-4
	3	95.5	100	82.6	+4.5	SS F_0 & NorAmp1-4 inc. CofV NorAmp1-4
	4	74.0	87.1	42.2	+13.1	SS F_0
All 9 subspecies	5	64.7	73.4	40.5	+8.7	SS F_0
	6	78.0	85.6	52.1	+7.6	SS F_0 & NorAmp1-4
European	7	95.0	100	85.0	+5.0	SS F_0 & NorAmp1-4 inc. CofV NorAmp1-4
Mackenzie Valley	8	98.6	100	90.0	+1.4	SS F_0 & NorAmp1-4 inc. CofV NorAmp1-4

For all 9 subspecies combined (analysis 9) it was shown that using 3 howls in the ANN model was sufficient to achieve 80% or higher individual classification accuracy, using simple scalar variables of F_0 alone (Figure 4.1a) or F_0 with amplitude data (Figure 4.1b). However, findings from the ANN validation data for all 9 subspecies combined showed that to achieve 80% or higher individual classification accuracy, 9 howls were required using simple scalar variables of F_0 alone (Figure 4.1a) and 7 howl were required when amplitude data (Figure 4.1b) was included in the models.

A further analysis using only Eastern wolf howls (analysis 10) was undertaken to determine how many howls were required from each wolf to achieve an accuracy of 80%. The analysis revealed that when the sample was limited to a single subspecies, Eastern wolves, 9 howls were required per wolf to build models with at least 80% recognition accuracy using simple scalar variables of F_0 alone (Figure 4.2a) but only 6 howls were required per wolf when amplitude data (Figure 4.2b) was included in the model. In addition, higher training classification accuracies were achieved for 3, 4 and 5 howls per wolf (Figure 4.2). Therefore, it is likely that including different subspecies of wolves in the same database will decrease the accuracy of the ANN classification.

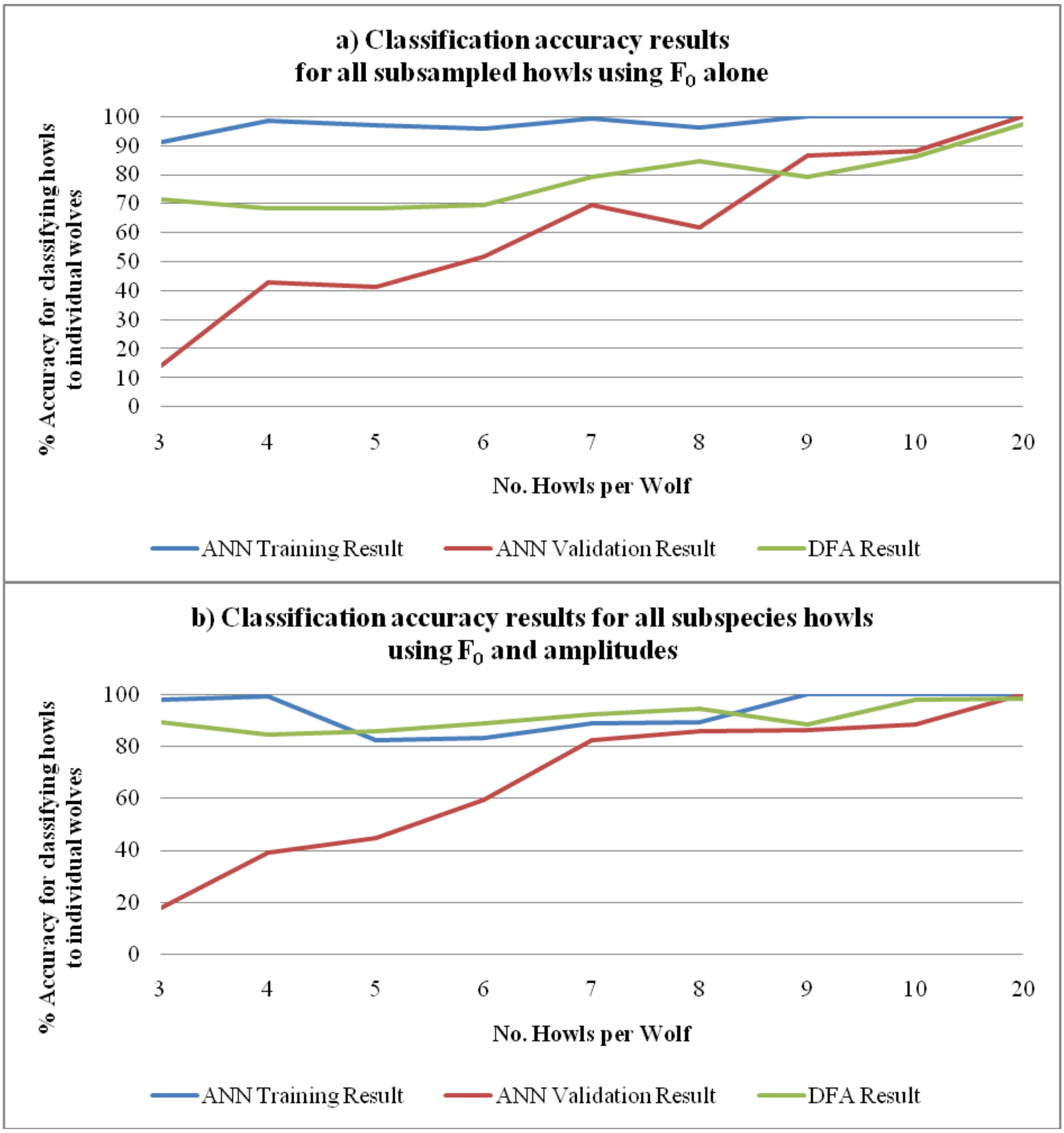


Figure 4.1 All subspecies: classification accuracies for ANN training and validation data and DFA data for howls using simple scalar variables of a) F_0 alone and b) both F_0 and amplitudes.

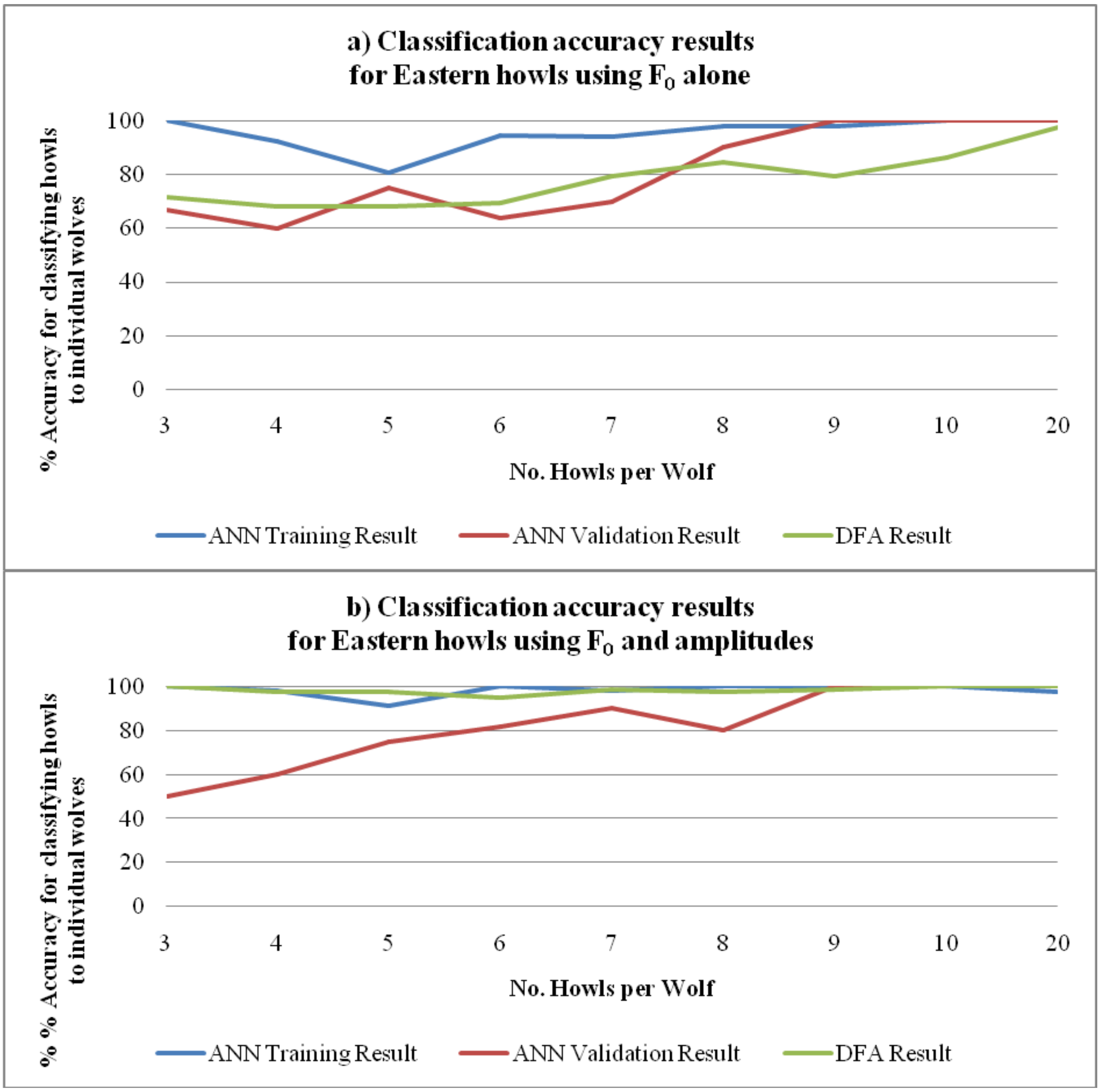


Figure 4.2 Eastern wolves only: classification accuracies for ANN training and validation data and DFA data for howls using simple scalar variables of a) F_0 alone and b) F_0 and amplitudes.

4.4.2 Exploring Pack & Subspecies Association Signature in Howls

For all 1262 chorus and solo howls (analysis 11), the variable coefficient of variation (CofV) was excluded by ANOVA for pack identity as there was no significant difference between individuals ($F_{79,\ 1261}$=0.856, p=0.806). No variables were excluded for subspecies identity. The mean number of howls for packs = 16.18 with SD = ±26.48. The mean number of howls for subspecies = 114.73, SD = ±210.38.

ANN models achieved recognition accuracies of 66.5% and 80.2% when using training data for packs and subspecies, respectively (Table 4.6). This suggests that both pack and subspecies show unique vocal signatures so should be taken into account when collating databases as they may interfere with the correct classification of individuals when they are collated.

Table 4.6 Results for pack and subspecies identity for all 1262 howls, chorus and solo, using simple scalar variables of F_0

1262 howls	DFA accuracy (%)	ANN accuracy (%)		Difference in accuracy between DFA and training ANN
		Training	Validation	
78 packs	42.7	66.5	44.6	+23.8
11 subspecies	64.7	80.2	67.6	+15.5

For the 632 solo howls (analysis 12), no variables were excluded by ANOVA. ANN models achieved accuracies of 86.2% and 85.8% when using training data for packs and subspecies, respectively (Table 4.6). This provides further evidence to suggest that both pack and subspecies show unique vocal signatures.

Table 4.7 Pack and subspecies results for 632 solo howls from 64 packs across all 9 subspecies and 9 subspecies, using simple scalar variables of F_0 and amplitudes 1-4

632 howls	**DFA accuracy (%)**	**ANN accuracy (%)**		**Difference in accuracy between DFA and training ANN**
		Training	**Validation**	
64 Packs	66.0	86.2	53.5	+20.2
9 Subspecies	67.7	85.8	75.5	+18.1

4.5 Discussion

4.5.1 Individual Identity

The 89 howls used in chapter 2 and Analysis 1 were from a single pack and ANNs were effective at using rules built from the known (training) subsample to classify howls in the unknown (validation) subsample. As was shown in chapters 2 and 3 for DFA, the most accurate models for simple scalar variables used both the normalised amplitude variables and the F_0 variables with 92.3% accuracy of classification for unknown solo howls (Table 4.5). This was further borne out in analysis 2 which looked at wolf howls from multiple packs where identification classification accuracy increased to 100% for unknown howls, which cannot be improved (Table 4.5). Overall, these findings concur with those from chapters 2 and 3 that individual identity is present in both F_0 and amplitude changes of howls. Analyses 3 and 4 compared results across packs and showed again that including amplitude data achieved more accurate classifications (Table 4.5). Furthermore, analyses 5, 6, 7 and 8 extended this result to other subspecies, showing that including amplitude data improved the identification of individuals for all nine subspecies tested (Table 4.5). The results for individual identification compare well with the results achieved for other mammals using ANNs including fallow deer (90% recognition) (Reby *et al.* 1998), stellar sea lions (76.7% recognition) (Campbell *et al.* 2002), and blue monkeys (73% recognition) (Mielke and Zuberbuehler 2013).

4.5.2 Exploring the Effect of Howl Sample Size on ANN Classification Accuracy

As validation accuracies of over 80% were achieved for most analyses, satisfying the requirement for *in situ* surveys (Terry and McGregor 2002), this suggests that ANNs are suitable as a survey method for identifying wolves using their howls as a capture-mark-recapture model. However, the survey method needs to be optimised for highest accuracies for *in situ* monitoring. While the results showed that the ANNs always outperformed DFAs in the identification of individuals, the identification accuracy for both methods fell as sample size increased, whether using chorus or solo howls (Table 4.6). Superficially, this contradicts the idea that increasing sample sizes should improve ANNs (Rumelhart *et al.* 1986). Nevertheless, analyses 8 and 9 showed that increasing the number of howls per wolf directly increased the identification accuracy, whether F_0 alone or F_0 with normalised amplitudes were used (Figure 4.1 and Figure 4.2). This agreed with the results of Fernandez-Juricic *et al.* (2009) where increasing the number of vocalisations per bird to over 15 fitz-bew calls decreased the error margins of recognition by ~11%. Furthermore, when only a single subspecies of wolf was analysed the number of howls required to achieve over 80% accuracy of identification dropped from 9 to just 6 howls per wolf (Figure 4.2). To achieve 100% accuracy of recognition, 9 howls per wolf were required (Figure 4.2).

4.5.3 Exploring Pack & Subspecies Association Signature in Howls

Both subspecies and pack identity were present in howls so were likely to interfere with the correct identification of individuals (Table 4.6 and Table 4.7). Therefore, increasing the sample set of training databases of a subspecies with a small sample size by adding howls from a second subspecies could lower the accuracy of the models and should be avoided. For instance, Tibetan wolves were represented by a single wolf. Therefore, more data from Tibetan wolves with known identity should be collected before attempting to use this method *in situ*, instead of using a model based on the lone Tibetan wolf plus other howls sourced from European or Eastern wolves. However, Eastern and European wolves already have sample sizes large enough in this database to form reliable models for recognising new wolves (over 100 solo howls per subspecies, Table 4.1).

Subspecies and species association signature was defined in both chorus and solo howl models (analyses 10 and 11). This was despite the large sample size differences between the subspecies and species, with Eastern wolves represented by over 700 howls from captive and wild wolves, and Tibetan wolves represented by only 9 howls from a single captive wolf. Subspecies association signature has been suggested before by Palacios *et al.* (2007) but has not yet been properly quantified. This chapter has not attempted to quantify the differences between the subspecies and species. Instead, it has aimed to show that there are pack and subspecies association signatures which should be taken into account when compiling ANN training databases. Furthermore, this could be of interest to wolf taxonomists looking for further characteristics which may define differences between the subspecies and species, and to match against genetic changes. Vocalisation data has been used to investigate relatedness and species identity in bats (Ramasindrazana *et al.* 2011), frogs (Smith *et al.* 2012) and primates (Thinh *et al.* 2011; Meyer *et al.* 2012). Therefore, more work could investigate patterns of vocal inheritance in wolves and determine whether it is purely genetic or has a learnt-aspect.

For *in situ* surveys, effective dataset size should also be limited to samples from only local wolves to maximise accuracy, whether the aim is to assign solo howls to the wolf's pack or to assign chorus howls to individual wolves, predicting identity for novel wolves. This limit to samples was previously suggested for south-western willow flycatchers, where ANNs also showed the effect of population level signature on their vocalisations (Fernandez-Juricic *et al.* 2009). The most accurate ANN models were based on 6 or more solo howls per wolf where amplitudes were applicable and included. However, as wolves often howl in chorus, a chorus model is also desirable. For the chorus model, where only F_0 variables are applicable, at least 7 to 8 howls per wolf were required to achieve the 80% recognition for *in situ* monitoring (Terry and McGregor 2002). Therefore a solo model, where amplitudes are applicable, and a second model for low quality and chorus howls, where amplitudes are not applicable, could be used in tandem to produce the most accurate survey method. Furthermore, I suggest an existing database of wolf howls should be used to train the models for classifying chorus howls, with a preference for the highest quality howls. There is an expected decrease in the recognition accuracy of individuals when chorus and not solo howls are used because of the decrease in the available number of descriptive variables when amplitudes are not applicable. It is possible that older microphone models may also have recorded less faithfully so the age of recording should be considered when judging whether to include the howls in the database.

Pack identity has previously been shown from howls of Italian wolves (Passilongo *et al.* 2010) with an identification accuracy of 95.5% using DFA of F_0 variables alone. This degree of accuracy could not be matched in the current study where only 42.7% accuracy was achieved for all wolves in analysis 11 (Table 4.6). Although ANNs increased the accuracy by 23.9% (Table 4.6), these results were comparatively weak, suggesting that other factors were affecting classification accuracies. However, Passilongo *et al.*'s (2010) 95.5% result was achieved for howls from a single subspecies and geographic area, whereas the results in analyses 11 and 12 were across all subspecies.

Mirroring the advantages for individuality, there are advantages for advertising species or kinship group for both avoiding inter-species conflict and preventing breeding or attacks between closely-related groups (Bradbury and Vehrencamp 1998). However, what is still unclear is how far pack-association signature is the result of genetics and how far it may be the result of social learning (Passilongo *et al.* 2010). Further investigations into pack-association signature would be enlightening as to whether social learning or genetic influences control this association signature. Again, as for individual identity, it may be that differences across subspecies are affecting the rate of classification, even if it is as simple as many geographic areas being compared. Also, in the current study, howls were included from the same geographic area of Algonquin Park, Canada, over a period of more than 30 years, so if pack-association signature is heritable (Passilongo *et al.* 2010), packs classified as different may have been related and therefore not truly separate. In greater sac-winged bats (*Saccopteryx bilineata*), group signature is acquired through social modification, a form of social learning, rather than genetically inheriting it (Knoernschild *et al.* 2012). (For a review of vocal learning see Janik and Slater (1997; 2000)). Further work could establish how far pack-association signature and subspecies signature are maintained across geographic distances and time, and if the regional differences seen in other species such as American pikas exist in wolves (Trefry and Hik 2010).

4.6 Conclusion

ANN models achieved identification accuracies of 100% whenever a single subspecies was considered and amplitude data were included. In addition, validation accuracies of over 80% were achieved for these analyses, satisfying the requirement for *in situ* surveys (Terry and McGregor 2002). This suggests that ANNs can be used to identify individuals and use known patterns within small groups to attribute identity to unknown individual wolves, with up to 100% accuracy (73.4% to 100%, mean=93.3%), and could therefore be used *in situ* to monitor wolves. To optimise *in situ* surveys using wolf howls to capture-mark-recapture individuals, findings show that it is best to use a minimum of 6 to 7 howls per wolf, solo howls wherever possible, and only howl samples from the same subspecies (and where possible from the same pack). Using historic recordings, as in chapter 3, did not alter the findings so if there are existing recordings of other individuals of the same subspecies, these could be used as a starting point.

ANNs have previously been used to identify species of bats (Parsons and Jones 2000; Walters *et al.* 2012), birds (Connor *et al.* 2012) and insects (Ganchev and Potamitis 2007); and individuals in both deer (Reby *et al.* 1997) and birds (Terry and McGregor 2002; Peake *et al.* 1998; Fernandez-Juricic *et al.* 2009). The trend of results show that ANNs improve on DFA classification performances and that they can be used for both species identification (Walters *et al.* 2012) and population monitoring at an individual level (Terry and McGregor 2002; Fernandez-Juricic *et al.* 2009). The results in this chapter mirror these findings, suggesting that ANNs are also appropriate for wolf howls and could potentially be used to classify unknown individuals as a future capture-mark-recapture survey method.

Discussion of Results

5.1 Howls in Context

Wolf howls have been used to survey wolf populations for more than forty years (Joslin 1967). However, this thesis has aimed to improve the method used for surveying from simple presence/absence surveys to a systematic approach of attributing howls to individuals. To this end, two forms of data description (simple scalar variables and histogram-derived PCA values) and two forms of statistical analysis (Discriminant Function Analysis and Artificial Neural Networks) were used. While Discriminant Function Analysis achieved up to 100% accuracy when attributing howls to known individuals using histogram-derived PCA values for both captive and wild recorded individuals (64.7% to 100%), Artificial Neural Networks were required to extend this to unknown individuals where they correctly discriminated between and classified individuals in both the training and recognition samples with up to 100% accuracy (73.4% to 100%, mean=93.3%). The most accurate results for both methods were achieved when the howls sampled were from the same subspecies and from either a small number of packs or the same pack, which agreed with the results from analyses 10 and 11 in Chapter 4 where both pack and subspecies association signature was established.

Wolves can hear and respond to howls over distances of 10 km or more (Joslin 1967), thus howls are useful for territorial defence, mate-seeking and social bonding. Individual recognition is a complex subject but wolves are known to recognise other wolves using both physical features and scent (Mech 1970), although these are short-range cues. With no visual or olfactory clues available over long ranges, wolves have evolved so that their howls carry information that allows individual recognition, although whether this information affects response rate needs investigating. The results for all subspecies in Chapter 4 support the universality of this, which is hardly surprising as long calls are known to encode identity in coyotes (Mitchell *et al.* 2006), African wild dogs (Hartwig 2005) and many non-canid species (Caudron *et al.* 1998; Spillmann *et al.* 2010; Cheng *et al.* 2012; Gamba *et al.* 2012).

The results achieved for individual identification using fundamental frequency alone for wolves (61.7% - 100%) compare well with the published results for other species using fundamental frequency alone koalas (87.7%) (Charlton *et al.* 2011b) and African wild dogs (67%) (Hartwig 2005). The inclusion of amplitude data always improved the identification accuracy achieved when using F0 alone (78% - 100%). Artificial neural networks further improved the results achieved with DFA and can be used to classify novel howls to individuals with up to 100% confidence in recognition. Comparing these findings with the information explored in the introduction (Table 1.1) it can be seen that vocalisations carrying information on individual identity are near universal.

This universality of identity advertisement is not surprising as where multiple individuals act and react as part of repeated interactions, individual recognition can grant many advantages. These include the ability to make knowledge-based judgements in the future. Previous knowledge alters what any individual will do to optimise outcomes (Hamilton 1964). Equally, identifying a number of known individuals within an unknown pack may alter the interaction. Dogs advertise their identity in their barks (Yin and McCowan 2004) and discriminate between callers (Molnar *et al.* 2009). It is also possible that, as for goats (Briefer and McElligott 2011b), wolf pups will respond more readily to their mother’s howls than to others, and this vocal recognition may help to protect them by altering their responses so that they do not advertise their position to unrelated hostile wolves.

Wolves are already known to respond differently to howls depending on whether they are solo or part of a chorus (Harrington and Mech 1982; Harrington and Mech 1983); this is a numerical assessment ability that has been more thoroughly tested in lions (Mccomb *et al.* 1994) and spotted hyena (Benson-Amram *et al.* 2011). However, wolves have already demonstrated the ability to assess the quantity of objects using discrimination between food items (Utrata *et al.* 2012), and may be similarly capable of assessing the number of individuals in a chorus. If wolves can similarly assess which individuals are making up the chorus howl, this grants more information to the listener and will correspondingly alter their behaviour. The stronger the advertising signal, the more useful it is to the listener, so the 100% correct classification by statistical analysis may be matched with 100% correct recognition by wolves where their hearing is good enough.

5.2 Howling as a remote monitoring tool

Wolf howls have been used for presence / absence surveys since the 1960 s (Joslin 1967). However, these have been criticised for failing to assess numbers accurately (Fuller and Sampson 1988). For capture-mark-recapture surveys, inter-individual differences have to exist and be recognisable by researchers. Chapters 2 and 3 demonstrated that wolf howls show individual differences that can be successfully used to classify wolves to individual with up to 100% accuracy (95.5% to 100% for solo howls, 82.7% to 97.4% for chorus howls). Chapter 4 explored how these differences could also be used to classify novel howls to individuals and showed that given at least 6 howls per wolf, a recognition accuracy of 80%

to 100% could be achieved. This is the first time that unknown wolves have been able to be individually identified via their howls and thus provides a non-invasive method of remotely assessing populations via acoustic monitoring. This also has implications for the ability to accurately monitor individuals from a distance because it changes surveys from presence / absence to capture-mark-recapture. Using this system, multiple howls per wolf are recorded for comparison and can indicate many factors such as population size and individual movements by comparing different recordings of howls and demonstrating if they are the same or different individuals.

Although individual-specific acoustic monitoring is not yet widely used, it has been used for diverse purposes including monitoring occupancy of nest sites (Holschuh and Otter 2005), population monitoring (Terry and McGregor 2002; Fernandez-Juricic *et al.* 2009), evolution in real time (Irwin *et al.* 2008), attracting individuals close enough to count visually (Mills *et al.* 2001) and the effects of migration on shared social behaviour (Walcott *et al.* 2006). In addition, several studies have used bioacoustics to monitor population sizes (O'Farrell and Gannon 1999; Tripp and Otter 2006; Thompson *et al.* 2010b; Walters *et al.* 2012; Xia *et al.* 2012). Of these studies, elephants represent the most similar model species as they also transmit calls over long distances, live in family groups and have large home ranges (Thompson *et al.* 2010b). Acoustic monitoring of elephants revealed that the area used by the species was considerably larger than that indicated from dung surveys alone, thus the overall methodology was proposed as a tool for acoustically active but visually elusive species (Thompson *et al.* 2010b). Like that study, the results here suggest that precise acoustic surveys are a neglected field in long-range monitoring and that by optimising studies for the species, whether by using simple counts or by the complex monitoring achieved for European eagle owls (Grava *et al.* 2008), higher accuracies and better detail can be obtained.

5.3 Future directions

In considering the future use of bioacoustics for terrestrial mammal tracking, researchers should consider the work already undertaken on marine mammal species. Wolves and whales show similarities in their life patterns: both live in family groups, communicating with other individuals over huge distances, with complex social lives, and both species are extremely difficult to track. Whale song has been found to be information-rich, with complex interactions between individuals taking place at distances of tens of miles, and showing specific geographic accents (McDonald *et al.* 2001), kin-specific qualities to their calls (Miller and Bain 2000; Schulz *et al.* 2011) and individual identification (Schulz *et al.* 2011). The findings from this thesis show that individuality is present in wolf howls in many subspecies but the defining characteristics differ between subspecies, suggesting that wolves may show patterns of vocal complexity similar to whales.

It is likely that individual identity will also be shown in other subspecies of wolf when it is possible to collect howl recordings of them for analysis. Increasing sample size for the subspecies studied here, and adding more samples from other subspecies where available, is a clear next step: using these new howls, it should be conceivable to establish possible differences between populations, regions or subspecies. A huge amount of further work is possible in this field including comparing differences between subspecies and vocalisations, as has been found in crested gibbons (Thinh *et al.* 2011) and leaf monkeys (Meyer *et al.* 2012), and exploring the way that different wolf subspecies encode identity – whether all the subspecies have the same differences between individuals or if different subspecies encode identity differently as suggested by (Palacios *et al.* 2007).

Other further work could focus on whether the same methodology applied here could improve the individual identification rates for other canid species with harmonic vocalisations. Initial tests show that the bespoke Matlab code is applicable to other canid howls and barks (unpublished data) and the histogram-derived PCA values are applicable to any extracted datasets, regardless of source. The analysis of howls from other canid species could be used to investigate many concepts. For example, the implications of honest signalling in knowing who is calling can extend beyond simple one-on-one interactions into complex social associations. Amongst others these can include the effects of social-group size and pack spacing on kin-specific association signatures. Furthermore, research could address how far social ecology and vocalisation qualities are controlled by either genetic influences, social learning or a combination of both (Janik and Slater 2000). More research should also assess how well wild-type call characteristics are retained over long-term captivity (generations) and also the stability of calls over time in both wild and captivity (Matrasova et al. 2010). Many of these issues have not been addressed directly in the literature but pose important points for investigation in the future.

Other work could focus on establishing how the differences in vocalisations between individuals arise. Body size, condition and sex have been shown to be present in vocal patterns of koala (Charlton *et al.* 2011b), common loons (Mager *et al.* 2007b) and goats (Briefer and McElligott 2011a). As our knowledge of vocal communication increases, further fields of examination open up with the possibility of howls encoding more information about individuals including position in dominance-hierarchy, age and health.

Wolves howl for a variety of purposes and express their identity through their howls. Also, they may express simple signals of communication and information. For example, it has been suggested that different howls are used for long range communication, gathering the pack for hunts and social bonding (Joslin 1967; Theberge and Falls 1967; Mech 1970). However, much more work is required to show whether wolves use different howl patterns for different purposes of the howl.

Finally, the sound analysis and collection programme iBats uses volunteers to record bat vocalisations and upload them to a database where they can be analysed to determine the presence of species and track differences within and between populations (Walters *et al.* 2012). A similar system could be used for wolves, with conservation groups, tourism groups and researchers all recording howls and uploading them to an international database for analysis. As there are already several acoustic libraries, this should not be difficult to implement. Dr Karl-Heinz Frommolt, who was kind enough to allow permission to his collection, has begun such a project with a vast collection of 120 000 sound files from many species as part of the Museum für Naturkunde (http://www.animalsoundarchive.org/). Analyses of the differences between wolf howls, coyote howls and dog howls would be useful to both phylogenetic studies and *in situ* assessments of the species of animal recorded howling. In summary, wolf howls contain information which has only just begun to be decoded and there is still much work to be done to add to the method of identification of individuals described here.

5.4 Summary

This thesis presents the first attempt to use amplitude changes to identify individuals in canid species. The results showed that by using amplitude changes, identification of individuals could be achieved with up to 100% accuracy and that this was robust to distance. The results add to the growing number of studies where including amplitude data improves the classification of vocalisations to above the desired 80% accuracy for surveys and suggests that, like fundamental frequency changes, changes in amplitudes carry information about individuals in many different species. Therefore, it is suggested that amplitudes should be included in other mammal vocalisation studies. Furthermore, artificial neural networks could be developed as a reliable survey tool for all canid species. This is already taking place *in situ* as since the publication of Chapters 2 and 3 in the journal 'Bioacoustics' (published online in July 2013), three separate wolf research groups have approached Nottingham Trent University interested in exploiting the new method of extraction and DFA for monitoring their wolves. Additionally, the bespoke Matlab code and DFA method is already being used for the identification of golden jackals in Greece by volunteers with the Archipelagos Institute of Marine Conservation and to analyse new howls from Eastern wolves by a student of Oregon State University.

Compared to bats and birds, little work has been undertaken on canid vocalisations and the results detailed here show that there is still much information to be decoded from their howls, including pack and phylogenetic identity, cultural transmission and learning. However, we can already hear the wolf's individual identity loud and clear in its famous 'call of the wild'.

References

Abgrall, P., Terhune, J.M. and Burton, H.R., 2003. Variation of Weddell seal (*Leptonychotes weddellii*) underwater vocalizations over mesogeographic ranges. *Aquatic Mammals*, 29 (2), 268-277.

Adams, L.G., Farley, S.D., Stricker, C.A., Demma, D.J., Roffler, G.H., Miller, D.C. and Rye, R.O., 2010a. Are inland wolf-ungulate systems influenced by marine subsidies of Pacific salmon? *Ecological Applications*, 20 (1), 251-262.

Adams, M.D., Law, B.S. and Gibson, M.S., 2010b. Reliable automation of bat call identification for eastern New South Wales, Australia, using classification trees and AnaScheme software. *Acta Chiropterologica,* 12 (1), 231-245.

Agnarsson, I., Kuntner, M. and May-Collado, L.J., 2010. Dogs, cats, and kin: A molecular species-level phylogeny of Carnivora. *Molecular Phylogenetics and Evolution,* 54 (3), 726-745.

Anderson, C.J.R., Roth, J.D. and Waterman, J.M., 2007. Can whisker spot patterns be used to identify individual polar bears? *Journal of Zoology*, 273 (4), 333-339.

Anonymous, 2012. *US Fish and Wildlife Service* [online]. US Fish and Wildlife Service. Available at: http://ecos.fws.gov/ [Accessed 06/25 2012].

Anonymous, 1973. *The little flowers of St. Francis: The mirror of perfection St. Bonaventure's life of St. Francis.* London: Dent: Everyman's Library.

Ansmann, I.C., Goold, J.C., Evans, P.G.H., Simmonds, M. and Keith, S.G., 2007. Variation in the whistle characteristics of short-beaked common dolphins, *Delphinus delphis*, at two locations around the British Isles. *Journal of the Marine Biological Association of the United Kingdom*, 87 (1), 19-26.

Arjo, W.M., and Pletscher, D.H., 1999. Behavioral responses of coyotes to wolf recolonization in northwestern Montana. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 77 (12), 1919-1927.

Aspi, J., Roininen, E., Kiiskila, J., Ruokonen, M., Kojola, I., Bljudnik, L., Danilov, P., Heikkinen, S. and Pulliainen, E., 2009. Genetic structure of the northwestern Russian wolf populations and gene flow between Russia and Finland. *Conservation Genetics,* 10 (4), 815-826.

Aubin, T., Mathevon, N., Staszewski, V. and Boulinier, T., 2007. Acoustic communication in the Kittiwake *Rissa tridactyla*: potential cues for sexual and individual signatures in long calls. *Polar Biology,* 30 (8), 1027-1033.

Ausband, D.E., Skrivseth, J. and Mitchell, M.S., 2011. An automated device for provoking and capturing wildlife calls. *Wildlife Society Bulletin,* 35 (4), 498-503.

Balint, A., Farago, T., Doka, A., Miklosi, A. and Pongracz, P., 2013. 'Beware, I am big and non-dangerous!' - Playfully growling dogs are perceived larger than their actual size by their canine audience. *Applied Animal Behaviour Science,* 148 (1-2), 128-137.

Ball, G., Mian, S., Holding, F., Allibone, R.O., Lowe, J., Ali, S., Li, G., McCardle, S., Ellis, I.O., Creaser, C. and Rees, R.C., 2002. An integrated approach utilizing artificial neural networks and SELDI mass spectrometry for the classification of human tumours and rapid identification of potential biomarkers. *Bioinformatics,* 18 (3), 395-404.

Ball, G.R., Benton, J., Palmer-Brown, D., Fuhrer, J., Skarby, L., Gimeno, B.S. and Mills, G., 1998. Identifying factors which modify the effects of ambient ozone on white clover (*Trifolium repens*) in Europe. *Environmental Pollution,* 103 (1), 7-16.

Ballard, W.B., Carbyn, L.N. and Smith, D.W., 2003. *Wolf Interactions with Non-Prey.* 1st ed. Chicago: University of Chicago Press.

Ballintijn, M.R., and tenCate, C., 1997. Sex differences in the vocalizations and syrinx of the Collared Dove (*Streptopelia decaocto*). *Auk,* 114 (1), 22-39.

Bard, S.C., Hau, M., Wikelski, M. and Wingfield, J.C., 2002. Vocal distinctiveness and response to conspecific playback in the Spotted Antbird, a Neotropical suboscine. *Condor,* 104 (2), 387-394.

Baril, L.M., Hansen, A.J., Renkin, R. and Lawrence, R., 2011. Songbird response to increased willow (*Salix spp*.) growth in Yellowstone's northern range. *Ecological Applications*, 21 (6), 2283-2296.

Barja, I., 2009. Prey and prey-age preference by the Iberian wolf *Canis lupus signatus* in a multiple-prey ecosystem. *Wildlife Biology,* 15 (2), 147-154.

Baumgartner, M.F., Van Parijs, S.M., Wenzel, F.W., Tremblay, C.J., Esch, H.C. and Warde, A.M., 2008. Low frequency vocalizations attributed to sei whales (*Balaenoptera borealis*). *Journal of the Acoustical Society of America*, 124 (2), 1339-1349.

Bee, M.A., 2004. Within-individual variation in bullfrog vocalizations: Implications for a vocally mediated social recognition system. *Journal of the Acoustical Society of America,* 116 (6), 3770-3781.

Bee, M.A., and Gerhardt, H.C., 2002. Individual voice recognition in a territorial frog (*Rana catesbeiana*). *Proceedings of the Royal Society of London Series B-Biological Sciences,* 269 (1499), 1443-1448.

Bee, M.A., and Gerhardt, H.C., 2001. Neighbour-stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): I. Acoustic basis. *Animal Behaviour*, 62, 1129-1140.

Bee, M.A., Perrill, S.A. and Owen, P.C., 2000. Male green frogs lower the pitch of acoustic signals in defense of territories: a possible dishonest signal of size? *Behavioral Ecology,* 11 (2), 169-177.

Bee, M.A., Cook, J.M., Love, E.K., O'Bryan, L.R., Pettitt, B.A., Schrode, K. and Velez, A., 2010. Assessing Acoustic Signal Variability and the Potential for Sexual Selection and Social Recognition in Boreal Chorus Frogs (*Pseudacris maculata*). *Ethology,* 116 (6), 564-576.

Bencsik, M., Bencsik, J., Baxter, M., Lucian, A., Romieu, J. and Millet, M., 2011. Identification of the honey bee swarming process by analysing the time course of hive vibrations. *Computers and Electronics in Agriculture,* 76 (1), 44-50.

Benedict, L., and Bowie, R.C.K., 2009. Macrogeographical variation in the song of a widely distributed African warbler. *Biology Letters,* 5 (4), 484-487.

Benson-Amram, S., Heinen, V.K., Dryer, S.L. and Holekamp, K.E., 2011. Numerical assessment and individual call discrimination by wild spotted hyaenas, *Crocuta crocuta*. *Animal Behaviour,* 82 (4), 743-752.

Berg, K.S., Delgado, S., Okawa, R., Beissinger, S.R. and Bradbury, J.W., 2011. Contact calls are used for individual mate recognition in free-ranging green-rumped parrotlets, *Forpus passerinus*. *Animal Behaviour,* 81 (1), 241-248.

Berger, J., 1999. Anthropogenic Extinction of Top Carnivores and Interspecific Animal Behaviour: Implications of the Rapid Decoupling of a Web Involving Wolves, Bears, Moose and Ravens. *Proceedings: Biological Sciences,* 266 (1435), 2261-2267.

Berrow, S., O'Brien, J., O'Connor, I. and McGrath, D., 2009. Abundance Estimate and Acoustic Monitoring of Harbour Porpoises (*Phocoena Phocoena (L.)*) in the Blasket Islands' Candidate Special Area of Conservation. *Biology and Environment-Proceedings of the Royal Irish Academy,* 109B (1), 35-46.

Beschta, R.L., and Ripple, W.J., 2008. Wolves, trophic cascades, and rivers in the Olympic National Park, USA. *Ecohydrology,* 1 (2), 118-130.

Bishop, C.M., 1994. Neural Networks and their Applications. *Review of Scientific Instruments,* 65 (6), 1803-1832.

Blumstein, D.T., Mennill, D.J., Clemins, P., Girod, L., Yao, K., Patricelli, G., Deppe, J.L., Krakauer, A.H., Clark, C., Cortopassi, K.A., Hanser, S.F., McCowan, B., Ali, A.M. and Kirschel, A.N.G., 2011. Acoustic monitoring in terrestrial environments using microphone arrays: applications, technological considerations and prospectus. *Journal of Applied Ecology*, 48 (3), 758-767.

Boersma, P., and Weenink, D., 2005. *Praat: doing phonetics by computer [Computer program]*. http://www.praat.org/:.

Bohling, J.H., and Waits, L.P., 2011. Assessing the prevalence of hybridization between sympatric Canis species surrounding the red wolf (*Canis rufus*) recovery area in North Carolina. *Molecular Ecology*, 20 (10), 2142-2156.

Bohn, K.M., Wilkinson, G.S. and Moss, C.F., 2007. Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*. *Animal Behaviour*, 73, 423-432.

Boitani, L., 2003. *Wolf Conservation and Recovery.* 1st ed. Chicago: University of Chicago Press.

Boitani, L., 2000. *Action plan for the conservation of wolves in Europe (Canis lupus).* Strasbourg: Council of Europe Pub.

Bouchet, H., Blois-Heulin, C., Pellier, A., Zuberbuehler, K. and Lemasson, A., 2012. Acoustic Variability and Individual Distinctiveness in the Vocal Repertoire of Red-Capped Mangabeys (*Cercocebus torquatus*). *Journal of Comparative Psychology*, 126 (1), 45-56.

Boughman, J.W., 1997. Greater spear-nosed bats give group-distinctive calls. *Behavioral Ecology and Sociobiology*, 40 (1), 61-70.

Bozarth, C.A., Hailer, F., Rockwood, L.L., Edwards, C.W. and Maldonado, J.E., 2011. Coyote colonization of northern Virginia and admixture with Great Lakes wolves. *Journal of Mammalogy*, 92 (5), 1070-1080.

Bradbury, J.W., and Vehrencamp, S.L., 1998. *Principles of animal communication.* Sunderland, MA: Sinauer Associates.

Bragina, E.V., and Beme, I.R., 2010. Siberian crane duet as an individual signature of a pair: comparison of visual and statistical classification techniques. *Acta Ethologica,* 13 (1), 39-48.

Breitenmoser, U., 1998. Large predators in the Alps: The fall and rise of man's competitors. *Biological Conservation,* 83 (3), 279-289.

Briefer, E.F., de la Torre, M.P. and McElligott, A.G., 2012. Mother goats do not forget their kids' calls. *Proceedings of the Royal Society B-Biological Sciences,* 279 (1743), 3749-3755.

Briefer, E., and McElligott, A.G., 2011a. Indicators of age, body size and sex in goat kid calls revealed using the source-filter theory. *Applied Animal Behaviour Science,* 133 (3-4), 175-185.

Briefer, E., and McElligott, A.G., 2011b. Mutual mother-offspring vocal recognition in an ungulate hider species (*Capra hircus*). *Animal Cognition,* 14 (4), 585-598.

Brown, D., and Smith, R., 1976. Predicting Hunting Success from Call Counts of Mourning and White-Winged Doves. *Journal of Wildlife Management,* 40 (4), 743-749.

Brown, W.M., and Parsons, D.R., 2001. *Restoring the Mexican gray wolf to the mountains of the southwest.*

Bump, J.K., Peterson, R.O. and Vucetich, J.A., 2009. Wolves modulate soil nutrient heterogeneity and foliar nitrogen by configuring the distribution of ungulate carcasses. *Ecology,* 90 (11), 3159-3167.

Burton, J.A., and Nietsch, A., 2010. Geographical Variation in Duet Songs of Sulawesi Tarsiers: Evidence for New Cryptic Species in South and Southeast Sulawesi. *International Journal of Primatology,* 31 (6), 1123-1146.

Busch, R.H., 2007. *The wolf almanac : a celebration of wolves and their world.* New & rev. / revised by Robert Busch; foreword by Rick Bass. ed. Guilford, CT: Lyons Press.

Camaclang, A.E., Hollis, L. and Barclay, R.M.R., 2006. Variation in body temperature and isolation calls of juvenile big brown bats, *Eptesicus fuscus*. *Animal Behaviour,* 71, 657-662.

Campbell, G.S., Gisiner, R.C., Helweg, D.A. and Milette, L.L., 2002. Acoustic identification of female Steller sea lions (*Eumetopias jubatus*). *Journal of the Acoustical Society of America*, 111 (6), 2920-2928.

Caniglia, R., Fabbri, E., Cubaynes, S., Gimenez, O., Lebreton, J. and Randi, E., 2012. An improved procedure to estimate wolf abundance using non-invasive genetic sampling and capture-recapture mixture models. *Conservation Genetics*, 13 (1), 53-64.

Caniglia, R., Fabbri, E., Greco, C., Galaverni, M. and Randi, E., 2010. Forensic DNA against wildlife poaching: Identification of a serial wolf killing in Italy. *Forensic Science International-Genetics,* 4 (5), 334-338.

Carlos Blanco, J., and Cortes, Y., 2012. Surveying wolves without snow: a critical review of the methods used in Spain. *Hystrix-Italian Journal of Mammalogy,* 23 (1), 35-48.

Carroll, C., McRae, B.H. and Brookes, A., 2012. Use of Linkage Mapping and Centrality Analysis Across Habitat Gradients to Conserve Connectivity of Gray Wolf Populations in Western North America. *Conservation Biology,* 26 (1), 78-87.

Catchpole, C., Slater, P.J.B., Mann, N. and ebrary, I., 2008. *Bird song.* 2nd ed. Cambridge England; New York: Cambridge University Press.

Caudron, A.K., Kondakov, A.A. and Siryanov, S.V., 1998. Acoustic structure and individual variation of grey seal (*Halichoerus grypus*) pup calls. *Journal of the Marine Biological Association of the United Kingdom,* 78 (2), 651-658.

Chapman, C.A., and Weary, D.M., 1990. Variability in Spider Monkeys Vocalizations may Provide Basis for Individual Recognition. *American Journal of Primatology*, 22 (4), 279-284.

Charlton, B.D., Ellis, W.A.H., McKinnon, A.J., Brumm, J., Nilsson, K. and Fitch, W.T., 2011a. Perception of Male Caller Identity in Koalas (*Phascolarctos cinereus*): Acoustic Analysis and Playback Experiments. *Plos One,* 6 (5), e20329.

Charlton, B.D., Ellis, W.A.H., McKinnon, A.J., Cowin, G.J., Brumm, J., Nilsson, K. and Fitch, W.T., 2011b. Cues to body size in the formant spacing of male koala (*Phascolarctos cinereus*) bellows: honesty in an exaggerated trait. *The Journal of Experimental Biology,* 214 (Pt 20), 3414-3422.

Charlton, B.D., and Reby, D., 2011. Context-Related Acoustic Variation in Male Fallow Deer (*Dama dama*) Groans. *Plos One,* 6 (6), e21066.

Charlton, B.D., Reby, D., Ellis, W.A.H., Brumm, J. and Fitch, W.T., 2012. Estimating the Active Space of Male Koala Bellows: Propagation of Cues to Size and Identity in a Eucalyptus Forest. *Plos One,* 7 (9), e45420.

Charlton, B.D., Zhang Zhihe and Snyder, R.J., 2009. Vocal cues to identity and relatedness in giant pandas (*Ailuropoda melanoleuca*). *Journal of the Acoustical Society of America,* 126 (5), 2721-2732.

Charrier, I., Mathevon, N. and Jouventin, P., 2003. Individuality in the voice of fur seal females: An analysis study of the pup attraction call in *Arctocephalus tropicalis*. *Marine Mammal Science,* 19 (1), 161-172.

Charrier, I., and Harcourt, R.G., 2006. Individual vocal identity in mother and pup Australian sea lions (*Neophoca cinerea*). *Journal of Mammalogy,* 87 (5), 929-938.

Chavez, A., and Gese, E., 2005. Food habits of wolves in relation to livestock depredations in northwestern Minnesota. *American Midland Naturalist,* 154 (1), 253-263.

Chen Jiu-Yi, Zhang Li-Jia, Wang An-Meng, Nasendelger, B., Yuan Li and Bao Wei -Dong, 2011. Population, distribution and food composition of wolves (*Canis lupus*) at Saihanwula Nature Reserve, Inner Mongolia. *Zoological Research,* 32 (2, Sp. Iss. SI), 232-235.

Cheng, J., Xie, B., Lin, C. and Ji, L., 2012. A comparative study in birds: call-type-independent species and individual recognition using four machine-learning methods and two acoustic features. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 21 (2), 157-171.

Chulkina, M.M., Volodin, I.A. and Volodina, E.V., 2006. Individual, intersexual, and interbreed variability of barks in the dog *Canis familiaris* (*Carnivora, Canidae*). *Zoologichesky Zhurnal,* 85 (4), 544-555.

Clark, J.A., and Leung, J., 2011. Vocal Distinctiveness and Information Coding in a Suboscine with Multiple Song Types: Eastern Wood-Pewee. *Wilson Journal of Ornithology,* 123 (4), 835-840.

Collins, K.T., Terhune, J.M., Rogers, T.L., Wheatley, K.E. and Harcourt, R.G., 2006. Vocal individuality of in-air weddell seal (*Leptonychotes weddellii*) pup "primary" calls. *Marine Mammal Science,* 22 (4), 933-951.

Conner, D.A., 1982. Geographic-Variation in Short Calls of Pikas (*Ochotona princeps*). *Journal of Mammalogy,* 63 (1), 48-52.

Connor, E.F., Li, S. and Li, S., 2012. Automating identification of avian vocalizations using time-frequency information extracted from the Gabor transform. *Journal of the Acoustical Society of America,* 132 (1), 507-517.

Creel, S., and Christianson, D., 2009. Wolf presence and increased willow consumption by Yellowstone elk: implications for trophic cascades. *Ecology,* 90 (9), 2454-2466.

Cure, C., Antunes, R., Samarra, F., Alves, A.C., Visser, F., Kvadsheim, P.H. and Miller, P.J.O., 2012. Pilot Whales Attracted to Killer Whale Sounds: Acoustically-Mediated Interspecific Interactions in Cetaceans. *Plos One,* 7 (12), e52201.

Darden, S.K., Dabelsteen, T. and Pedersen, S.B., 2003. A potential tool for swift fox (*Vulpes velox*) conservation: Individuality of long-range barking sequences. *Journal of Mammalogy,* 84 (4), 1417-1427.

Davidson, S.M., and Wilkinson, G.S., 2002. Geographic and individual variation in vocalizations by male *Saccopteryx bilineata* (*Chiroptera : Emballonuridae*). *Journal of Mammalogy,* 83 (2), 526-535.

Davies, N.B., and Krebs, J.R., 1997. *Behavioural ecology.* 4th ed. Oxford; Malden, MA: Blackwell Science.

Dawkins, M.S., and Guilford, T., 1991. The corruption of honest signalling. *Animal Behaviour,* 41 (5), 865-873.

Deecke, V.B., Barrett-Lennard, L.G., Spong, P. and Ford, J.K.B., 2010. The structure of stereotyped calls reflects kinship and social affiliation in resident killer whales (*Orcinus orca*). *Naturwissenschaften,* 97 (5), 513-518.

Delgado, R.A., Jr., 2007. Geographic variation in the long calls of male orangutans (*Pongo* spp.). *Ethology,* 113 (5), 487-498.

Delport, W., Kemp, A.C. and 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 (1), 30-39.

Depraetere, M., Pavoine, S., Jiguet, F., Gasc, A., Duvail, S. and Sueur, J., 2012. Monitoring animal diversity using acoustic indices: Implementation in a temperate woodland. *Ecological Indicators,* 13 (1), 46-54.

Digweed, S.M., Rendall, D. and Imbeau, T., 2012. Who's your neighbor? Acoustic cues to individual identity in red squirrel *Tamiasciurus hudsonicus* rattle calls. *Current Zoology,* 58 (5), 758-764.

Dijk, J.v., Gustavsen, L., Mysterud, A., May, R., Flagstad, Ø, Brøseth, H., Andersen, R., Andersen, R., Steen, H. and Landa, A., 2008. Diet Shift of a Facultative Scavenger, the Wolverine, following Recolonization of Wolves. *Journal of Animal Ecology,* 77 (6), 1183-1190.

Duchamp, C., Boyer, J., Briaudet, P., Leonard, Y., Moris, P., Bataille, A., Dahier, T., Delacour, G., Millisher, G., Miquel, C., Poillot, C. and Marboutin, E., 2012. A dual frame survey to assess time- and space-related changes of the colonizing wolf population in France. *Hystrix-Italian Journal of Mammalogy,* 23 (1), 14-28.

Dugnol, B., Fernández, C. and Galiano, G., 2007a. Wolf population counting by spectrogram image processing. *Applied Mathematics and Computation,* 186 (1), 820-830.

Dugnol, B., Fernández, C., Galiano, G. and Velasco, J., 2008. On a chirplet transform-based method applied to separating and counting wolf howls. *Signal Processing,* 88 (7), 1817-1826.

Dugnol, B., Fernández, C., Galiano, G. and Velasco, J., 2007b. Implementation of a diffusive differential reassignment method for signal enhancement: An application to wolf population counting. *Applied Mathematics and Computation,* 193 (2), 374-384.

Eakle, W.L., Mannan, R.W. and Grubb, T.G., 1989. Identification of Individual Breeding Bald Eagles by Voice Analysis. *Journal of Wildlife Management,* 53 (2), 450-455.

Eda-Fujiwara, H., Yamamoto, A., Sugita, H., Takahashi, Y., Kojima, Y., Sakashita, R., Ogawa, H., Miyamoto, T. and Kimura, T., 2004. Sexual dimorphism of acoustic signals in the oriental white stork: Non-invasive identification of sex in birds. *Zoological Science,* 21 (8), 817-821.

Eichholz, D.E., 1962. *Natural History : with an English Translation vol 10 Libri 36-37.* Heinemann.

Ericsson, G., and Heberlein, T.A., 2003. Attitudes of hunters, locals, and the general public in Sweden now that the wolves are back. *Biological Conservation,* 111 (2), 149-159.

Ey, E., and Fischer, J., 2009. The "Acoustic Adaptation Hypothesis" - a Review of the Evidence from Birds, Anurans and Mammals. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 19 (1-2), 21-48.

Fain, S.R., Straughan, D.J. and Taylor, B.F., 2010. Genetic outcomes of wolf recovery in the western Great Lakes states. *Conservation Genetics,* 11 (5), 1747-1765.

Farago, T., Pongracz, P., Miklosi, A., Huber, L., Viranyi, Z. and Range, F., 2010. Dogs' Expectation about Signalers' Body Size by Virtue of Their Growls. *Plos One,* 5 (12), e15175.

Fenton, M.B., Jacobs, D.S., Richardson, E.J., Taylor, P.J. and White, E., 2004. Individual signatures in the frequency-modulated sweep calls of African large-eared, free-tailed bats *Otomops martiensseni* (*Chiroptera : Molossidae*). *Journal of Zoology,* 262, 11-19.

Fernandez-Juricic, E., del Nevo, A.J. and Poston, R., 2009. Identification of Individual and Population-Level Variation in Vocalizations of the Endangered Southwestern Willow Flycatcher (*Empidonax traillii extimus*). *Auk,* 126 (1), 89-99.

Filatova, O.A., Deecke, V.B., Ford, J.K.B., Matkin, C.O., Barrett-Lennard, L.G., Guzeev, M.A., Burdin, A.M. and Hoyt, E., 2012. Call diversity in the North Pacific killer whale populations: implications for dialect evolution and population history. *Animal Behaviour,* 83 (3), 595-603.

Fox, E.J.S., Roberts, J.D. and Bennamoun, M., 2008. Call-independent individual identification in birds. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 18 (1), 51-67.

Frasier, K.E., Soldevilla, M.S., McDonald, M.A., Merkens, K.P., Wiggins, S.M., Hildebrand, J.A. and Roch, M.A., 2011. Acoustic monitoring of dolphin populations in the Gulf of Mexico. *The Journal of the Acoustical Society of America,* 130 (4), 2537.

Frommolt, K.-., 2002. Acoustic and temporal features of long distance wolf vocalisations and their possible consequences for signal transmission. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 13 (1), 88.

Frommolt, K.H., Goltsman, M.E. and 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., and Sampson, B.A., 1988. Evaluation of a Simulated Howling Survey for Wolves. *The Journal of Wildlife Management*, 52 (1), 60-63.

Galaverni, M., Palumbo, D., Fabbri, E., Caniglia, R., Greco, C. and Randi, E., 2012. Monitoring wolves (*Canis lupus*) by non-invasive genetics and camera trapping: a small-scale pilot study. *European Journal of Wildlife Research*, 58 (1), 47-58.

Gamba, M., Colombo, C. and Giacoma, C., 2012. Acoustic cues to caller identity in lemurs: a case study. *Journal of Ethology,* 30 (1), 191-196.

Ganchev, T., and Potamitis, I., 2007. Automatic acoustic identification of singing insects. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 16 (3), 281-328.

Garrott, R.A., Jason E. Bruggeman, Becker, M.S., Kalinowski, S.T. and White, P.J., 2007. Evaluating Prey Switching in Wolf-Ungulate Systems. *Ecological Applications,* 17 (6), 1588-1597.

Gasser, H., Amezquita, A. and Hoedl, W., 2009. Who is Calling? Intraspecific Call Variation in the Aromobatid Frog *Allobates femoralis*. *Ethology,* 115 (6), 596-607.

Gaubert, P., Bloch, C., Benyacoub, S., Abdelhamid, A., Pagani, P., Djagoun, C.A.M.S., Couloux, A. and Dufour, S., 2012. Reviving the African Wolf *Canis lupus lupaster* in North and West Africa: A Mitochondrial Lineage Ranging More than 6,000 km Wide. *PloS One,* 7 (8), e42740.

Gese, E.M., Ruff, R.L. and Crabtree, R.L., 1996. Social and nutritional factors influencing the dispersal of resident coyotes. *Animal Behaviour*, 52, 1025-1043.

Gipson, P.S., Ballard, W.B., Nowak, R.M. and Mech, L.D., 2000. Accuracy and precision of estimating age of gray wolves by tooth wear. *Journal of Wildlife Management*, 64 (3), 752-758.

Gogan, P.J.P., Route, W.T., Olexa, E.M., Thomas, N., Kuehn, D. and Podruzny, K.M., 2004. *Grey Wolves in and Adjacent to Voyageurs National Park, Minnesota. Research and Synthesis 1987-1991*. Technical Report NPS/MWR/NRTR/2004-01 ed. Omaha, Nebraska: National Park Service.

Grava, T., Mathevon, N., Place, E. and Balluet, P., 2008. Individual acoustic monitoring of the European Eagle Owl *Bubo bubo*. *Ibis*, 150 (2), 279-287.

Growcott, A., Miller, B., Sirguey, P., Slooten, E. and Dawson, S., 2011. Measuring body length of male sperm whales from their clicks: The relationship between inter-pulse intervals and photogrammetrically measured lengths. *The Journal of the Acoustical Society of America*, 130 (1), 568.

Gude, J.A., Mitchell, M.S., Russell, R.E., Sime, C.A., Bangs, E.E., Mech, L.D. and Ream, R.R., 2012. Wolf population dynamics in the US Northern Rocky Mountains are affected by recruitment and human-caused mortality. *Journal of Wildlife Management*, 76 (1), 108-118.

Gula, R., 2008. Legal protection of wolves in Poland: implications for the status of the wolf population. *European Journal of Wildlife Research*, 54 (2), 163-170.

Hall, M.L., Kingma, S.A. and Peters, A., 2013. Male Songbird Indicates Body Size with Low-Pitched Advertising Songs. *Plos One*, 8 (2), e56717.

Hamilton, W.D., 1964. The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, 7 (1), 17-52.

Hammerschmidt, K., and Todt, D., 1995. Individual-Differences in Vocalizations of Young Barbary Macaques (*Macaca sylvanus*) - a Multi-Parametric Analysis to Identify Critical Cues in Acoustic Signaling. *Behaviour,* 132, 381-399.

Han, N.C., Muniandy, S.V. and Dayou, J., 2011. Acoustic classification of Australian anurans based on hybrid spectral-entropy approach. *Applied Acoustics,* 72 (9), 639-645.

Harrington, F.H., and Asa, C.S., 2003. *Wolf Communication.* 1st ed. Chicago: University of Chicago Press.

Harrington, F.H., 1978. Ravens Attracted to Wolf Howling. *The Condor,* 80 (2), 236-237.

Harrington, F.H., and Mech, L.D., 1983. Wolf Pack Spacing: Howling as a Territory-Independent Spacing Mechanism in a Territorial Population. *Behavioral Ecology and Sociobiology,* 12 (2), 161-168.

Harrington, F.H., and Mech, L.D., 1982. An Analysis of Howling Response Parameters Useful for Wolf Pack Censusing. *The Journal of Wildlife Management,* 46 (3), 686-693.

Harrington, F.H., and Mech, L.D., 1979. Wolf Howling and Its Role in Territory Maintenance. *Behaviour,* 68 (3/4), 207-249.

Hartwig, S., 2005. Individual acoustic identification as a non-invasive conservation tool: An approach to the conservation of the African wild dog *Lycaon pictus* (Temminck, 1820). *Bioacoustics-the International Journal of Animal Sound and its Recording,* 15 (1), 35-50.

Hedrick, P.W., and Fredrickson, R.J., 2008. Captive breeding and the reintroduction of Mexican and red wolves. *Molecular Ecology,* 17 (1), 344-350.

Herting, B.L., and Belthoff, J.R., 2001. Bounce and double trill songs of male and female Western Screech-Owls: Characterization and usefulness for classification of sex. *Auk,* 118 (4), 1095-1101.

Hoffmann, F., Musolf, K. and Penn, D.J., 2012. Spectrographic analyses reveal signals of individuality and kinship in the ultrasonic courtship vocalizations of wild house mice. *Physiology & Behavior,* 105 (3), 766-771.

Hollen, L.I., and Manser, M.B., 2006. Ontogeny of alarm call responses in meerkats, *Suricata suricatta*: the roles of age, sex and nearby conspecifics. *Animal Behaviour,* 72, 1345-1353.

Holschuh, C.I., and Otter, K.A., 2005. Using vocal individuality to monitor Queen Charlotte Saw-whet owls (*Aegolius acadicus brooksi*). *Journal of Raptor Research,* 39 (2), 134-141.

Insley, S.J., 1992. Mother-Offspring Separation and Acoustic Stereotypy - a Comparison of Call Morphology in 2 Species of Pinnipeds. *Behaviour,* 120, 103-122.

Irwin, D.E., Thimgan, M.P. and Irwin, J.H., 2008. Call divergence is correlated with geographic and genetic distance in greenish warblers (*Phylloscopus trochiloides*): a strong role for stochasticity in signal evolution? *Journal of Evolutionary Biology,* 21 (2), 435-448.

Jameson, J.W., and Hare, J.F., 2009. Group-specific signatures in the echolocation calls of female little brown bats (*Myotis lucifugus*) are not an artefact of clutter at the roost entrance. *Acta Chiropterologica,* 11 (1), 163-172.

Janicke, T., Ritz, M.S., Hahn, S. and Peter, H., 2007. Sex recognition in brown skuas: do acoustic signals matter? *Journal of Ornithology,* 148 (4), 565-569.

Janik, V.M., and Slater, P.J.B., 2000. The different roles of social learning in vocal communication. *Animal Behaviour,* 60, 1-11.

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

Jedrzejewksi, W., Branicki, W., Veit, C., Medugorac, I., Pilot, M., Bunevich, A.N., Jedrzejewska, B., Schmidt, K., Theuerkauf, J., Oyarma, H., Gula, R., Szymura, L. and Forster, M., 2005. Genetic diversity and relatedness within packs in an intensely hunted population of wolves *Canis lupus*. *Acta Theriologica,* 50 (1), 3-22.

Jedrzejewski, W., Jedrzejewska, B., Zawadzka, B., Borowik, T., Nowak, S. and Myszajek, R.W., 2008. Habitat suitability model for Polish wolves based on long-term national census. *Animal Conservation,* 11 (5), 377-390.

Jones, D.N., and Smith, G.C., 1997. Vocalisations of the marbled frogmouth - II: An assessment of vocal individuality as a potential census technique. *Emu*, 97, 296-304.

Jones, G., Gordon, T. and Nightingale, J., 1992. Sex and Age-Differences in the Echolocation Calls of the Lesser Horseshoe Bat, *Rhinolophus hipposideros*. *Mammalia*, 56 (2), 189-193.

Jones, G., Morton, M., Hughes, P.M. and Budden, R.M., 1993. Echolocation, Flight Morphology and Foraging Strategies of some West-African *Hipposiderid* Bats. *Journal of Zoology*, 230, 385-400.

Jones, G., and Ransome, R.D., 1993. Echolocation Calls of Bats are Influenced by Maternal Effects and Change over a Lifetime. *Proceedings: Biological Sciences*, 252 (1334), 125-128.

Joslin, P.W.B., 1967. Movements and Home Sites of Timber Wolves in Algonquin Park. *American Zoologist*, 7 (2), 279-288.

Kaartinen, S., Kojola, I. and Colpaert, A., 2005. Finnish wolves avoid roads and settlements. *Annales Zoologici Fennici*, 42 (5), 523-532.

Kaartinen, S., Luoto, M. and Kojola, I., 2009. Carnivore-livestock conflicts: determinants of wolf (*Canis lupus*) depredation on sheep farms in Finland. *Biodiversity and Conservation,* 18 (13), 3503-3517.

Karlin, M., and Chadwick, J., 2012. Red wolf natal dispersal characteristics: comparing periods of population increase and stability. *Journal of Zoology,* 286 (4), 266-276.

Karlsson, J., Broseth, H., Sand, H. and Andren, H., 2007. Predicting occurrence of wolf territories in Scandinavia. *Journal of Zoology,* 272 (3), 276-283.

Kauffman, M.J., Varley, N., Smith, D.W., Stahler, D.R., MacNulty, D.R. and Boyce, M.S., 2007. Landscape heterogeneity shapes predation in a newly restored predator-prey system. *Ecology Letters,* 10 (8), 690-700.

Kauffman, M.J., Brodie, J.F. and Jules, E.S., 2010. Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade. *Ecology,* 91 (9), 2742-2755.

Kazial, K.A., Burnett, S.C. and Masters, W.M., 2001. Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus* (*Chiroptera : Vespertilionidae*). *Journal of Mammalogy,* 82 (2), 339-351.

Kazial, K.A., and Masters, W.M., 2004. Female big brown bats, *Eptesicus fuscus,* recognize sex from a caller's echolocation signals. *Animal Behaviour,* 67, 855-863.

Kiefer, S., Sommer, C., Scharff, C. and Kipper, S., 2010. Singing the Popular Songs? Nightingales Share More Song Types with Their Breeding Population in Their Second Season than in Their First. *Ethology,* 116 (7), 619-626.

Kingston, T., and Rossiter, S.J., 2004. Harmonic-hopping in Wallacea's bats. 429 (6992), 657.

Kittle, A.M., Fryxell, J.M., Desy, G.E. and Hamr, J., 2008. The Scale-Dependent Impact of Wolf Predation Risk on Resource Selection by Three Sympatric Ungulates. *Oecologia,* 157 (1), 163-175.

Klinck, H., Mellinger, D.K., Klinck, K., Bogue, N.M., Luby, J.C., Jump, W.A., Shilling, G.B., Litchendorf, T., Wood, A.S., Schorr, G.S. and Baird, R.W., 2012. Near-Real-Time Acoustic Monitoring of Beaked Whales and Other Cetaceans Using a Seaglider (TM). *Plos One,* 7 (5), e36128.

Knoernschild, M., Nagy, M., Metz, M., Mayer, F. and von Helversen, O., 2012. Learned vocal group signatures in the polygynous bat Saccopteryx bilineata. *Animal Behaviour,* 84 (4), 761-769.

Kojola, I., Aspi, J., Hakala, A., Heikkinen, S., Ilmoni, C. and Ronkainen, S., 2006. Dispersal in an Expanding Wolf Population in Finland. *Journal of Mammalogy,* 87 (2), 281-286.

Kojola, I., and Kuittinen, J., 2002. Wolf Attacks on Dogs in Finland. *Wildlife Society Bulletin,* 30 (2), 498-501.

Kojola, I., Ronkainen, S., Hakala, A., Heikkinen, S. and Kokko, S., 2004. Interactions between wolves *Canis lupus* and dogs *C. famillaris* in Finland. *Wildlife Biology,* 10 (2), 101-105.

Koren, L., and Geffen, E., 2011. Individual identity is communicated through multiple pathways in male rock hyrax (*Procavia capensis*) songs. *Behavioral Ecology and Sociobiology,* 65 (4), 675-684.

Koren, L., and Geffen, E., 2009. Complex call in male rock hyrax (Procavia capensis): a multi-information distributing channel. *Behavioral Ecology and Sociobiology,* 63 (4), 581-590.

Lameira, A.R., and Wich, S.A., 2008. Orangutan long call degradation and individuality over distance: A playback approach. *International Journal of Primatology,* 29 (3), 615-625.

Lancashire, L.J., Lemetre, C. and Ball, G.R., 2009. An introduction to artificial neural networks in bioinformatics-application to complex microarray and mass spectrometry datasets in cancer studies. *Briefings in Bioinformatics*, 10 (3), 315-329.

Lanszki, J., Markus, M., Ujvary, D., Szabo, A. and Szemethy, L., 2012. Diet of wolves *Canis lupus* returning to Hungary. *Acta Theriologica,* 57 (2), 189-193.

Lapshina, E.N., Volodin, I.A., Volodina, E.V., Frey, R., Efremova, K.O. and Soldatova, N.V., 2012. The ontogeny of acoustic individuality in the nasal calls of captive goitred gazelles, *Gazella subgutturosa*. *Behavioural Processes,* 90 (3), 323-330.

Latham, A.D.M., and Boutin, S., 2011. Wolf, *Canis lupus*, Pup Mortality: Interspecific Predation or Non-Parental Infanticide? *Canadian Field-Naturalist*, 125 (2), 158-161.

Lehman, N., Clarkson, P., Mech, L.D., Meier, T.J. and Wayne, R.K., 1992. A Study of the Genetic Relationships within and among Wolf Packs Using DNA Fingerprinting and Mitochondrial DNA. *Behavioral Ecology and Sociobiology*, 30 (2), 83-94.

Lek, S., and Guegan, J., 1999. Artificial neural networks as a tool in ecological modelling, an introduction. *Ecological Modelling,* 120 (2-3), 65-73.

Leliveld, L.M.C., Scheumann, M. and Zimmermann, E., 2011. Acoustic correlates of individuality in the vocal repertoire of a nocturnal primate (*Microcebus murinus*). *Journal of the Acoustical Society of America,* 129 (4), 2278-2288.

Lemasson, A., Boutin, A., Boivin, S., Blois-Heulin, C. and Hausberger, M., 2009. Horse (*Equus caballus*) whinnies: a source of social information. *Animal Cognition,* 12 (5), 693-704.

Lemetre, C., Lancashire, L.J. and Ball, G.R., 2010. A Novel Artificial Neural Network Based Algorithm to Analyse the Interaction Patterns Existing in Gene Microarrays: an Application to Breast Cancer Genomic Data. *Ejc Supplements,* 8 (6), 8-9.

Leonard, J.A., Vila, C. and Wayne, R.K., 2005. Legacy lost: genetic variability and population size of extirpated US grey wolves (*Canis lupus*). *Molecular Ecology,* 14 (1), 9-17.

Leonard, J.A., and Wayne, R.K., 2008. Native Great Lakes wolves were not restored. *Biology Letters,* 4 (1), 95-98.

Leopold, A., 1949. *A Sand County almanac, and Sketches here and there.* London: Oxford University Press.

Liberg, O., Chapron, G., Wabakken, P., Pedersen, H.C., Hobbs, N.T. and Sand, H., 2012. Shoot, shovel and shut up: cryptic poaching slows restoration of a large carnivore in Europe. *Proceedings.Biological Sciences / the Royal Society,* 279 (1730), 910-5.

Linnell, J.D.C., Andersen, R., Andersone, Z., Balciauskas, L., Blanco, J.C., Boitani, L., Brainerd, S., Breitenmoser, U., Kojola, I., Liberg, O., Løe, J., Okarma, H., Pedersen, H.C., Promberger, C., Sand, H., Solberg, E.J., Valdmann, H. and Wabakken, P., 2002. *The Fear of Wolves: A Review of Wolf Attacks on Humans.* 731:1 ed. Trondheim, Sweden: NINA Oppdragsmelding.

Long, R.A., 2008. *Noninvasive survey methods for carnivores.* Washington, DC: Island Press.

Lopez-Rivas, R.M., and Bazua-Duran, C., 2010. Who is whistling? Localizing and identifying phonating dolphins in captivity. *Applied Acoustics,* 71 (11), 1057-1062.

Lucchini, V., Fabbri, E., Marucco, F., Ricci, S., Boitani, L. and Randi, E., 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. *Molecular Ecology,* 11 (5), 857-868.

Macdonald, D.W., and Sillero-Zubiri, C., 2004. *The biology and conservation of wild canids.* Oxford: Oxford University Press.

Maeda, T., and Masataka, N., 1987. Locale-specific Vocal Behaviour of the Tamarin (*Saguinus I. labiatus*). 75 (1), 30.

Mager, J.N., Walcott, C. and Evers, D., 2007a. Macrogeographic variation in the body size and territorial vocalizations of male Common Loons (*Gavia immer*). *Waterbirds,* 30 (1), 64-72.

Mager, J.N., Walcott, C. and Piper, W.H., 2007b. Male common loons, *Gavia immer,* communicate body mass and condition through dominant frequencies of territorial yodels. *Animal Behaviour,* 73, 683-690.

Markov, G., and Lanszki, J., 2012. Diet composition of the golden jackel, *Canis aureus* in an agricultural environment. *Folia Zoologica,* 61 (1), 44-48.

Marucco, F., Pletscher, D.H., Boitani, L., Schwartz, M.K., Pilgrim, K.L. and Lebreton, J.D., 2009. Wolf survival and population trend using non-invasive capture-recapture techniques in the Western Alps. *Journal of Applied Ecology,* 46 (5), 1003-1010.

Mathevon, N., Charrier, I. and Jouventin, P., 2003. Potential for individual recognition in acoustic signals: a comparative study of two gulls with different nesting patterns. *Comptes Rendus Biologies,* 326 (3), 329-337.

Mathworks Inc., T., 2005. *Matlab & Simulink student version electronic resource.* Release 14 Service Pack 3. ed. Natick, Mass.: MathWorks.

Matrosova, V.A., Blumstein, D.T., Volodin, I.A. and Volodina, E.V., 2011. The potential to encode sex, age, and individual identity in the alarm calls of three species of *Marmotinae*. *Naturwissenschaften,* 98 (3), 181-192.

Matrosova, V.A., Volodin, I.A., Volodina, E.V. and Babitsky, A.F., 2007. Pups crying bass: vocal adaptation for avoidance of age-dependent predation risk in ground squirrels? *Behavioral Ecology and Sociobiology,* 62 (2), 181-191.

Maurer, G., Smith, C., Suesser, M. and Magrath, R.D., 2008. Solo and duet calling in the pheasant coucal: sex and individual call differences in a nesting cuckoo with reversed size dimorphism. *Australian Journal of Zoology,* 56 (3), 143-149.

Mccomb, K., Packer, C. and Pusey, A., 1994. Roaring and Numerical Assessment in Contests between Groups of Female Lions, *Panthera Leo*. *Animal Behaviour,* 47 (2), 379-387.

McCowan, B., and 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 (3), 1157-1160.

McDonald, M.A., Calambokidis, J., Teranishi, A.M. and Hildebrand, J.A., 2001. The acoustic calls of blue whales off California with gender data. *Journal of the Acoustical Society of America,* 109 (4), 1728-1735.

McDonald, M.A., Mesnick, S.L. and Hildebrand, J.A., 2006. Biogeographic characterisation of blue whale song worldwide: using song to identify populations. *Journal of Cetacean Research and Management,* 8 (1), 55-65.

McGregor, P., and Peake, T., 1998. The role of individual identification in conservation biology. *Behavioral Ecology and Conservation Biology,*, 31-55.

McPhee, H.M., Webb, N.F. and Merrill, E.H., 2012. Time-to-kill: measuring attack rates in a heterogenous landscape with multiple prey types. *Oikos*, 121 (5), 711-720.

Mcshane, L.J., Estes, J.A., Riedman, M.L. and Staedler, M.M., 1995. Repertoire, Structure, and Individual Variation of Vocalizations in the Sea Otter. *Journal of Mammalogy,* 76 (2), 414-427.

Mech, L.D., 1970. *The Wolf: The Ecology and Behaviour of an Endangered Species.* 2nd ed. Garden City, N.Y.: Natural History Proceedings for American Museum of Natural History.

Mech, L.D., and Boitani, L., 2003. *Wolf Social Ecology.* 1st ed. Chicago: University of Chicago Press.

Mech, L.D., and Peterson, R.O., 2003. *Wolf-Prey Relationships.* 1st ed. Chicago: University of Chicago Press.

Mech, L.D., 2009. Crying wolf: concluding that wolves were not restored. *Biology Letters,* 5 (1), 65-66.

Mech, L.D., 2007. Annual arctic wolf pack size related to arctic hare numbers. *Arctic,* 60, 309-311.

Mech, L.D., 1999. Alpha status, dominance, and division of labor in wolf packs. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 77 (8), 1196-1203.

Mech, L.D., 1995. A ten-year history of the demography and productivity of an arctic wolf pack. *Arctic*, 48 (4), 329-332.

Mech, L.D., and Paul, W.J., 2008. Wolf body mass cline across Minnesota related to taxonomy? *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 86 (8), 933-936.

Mech, L.D., 1997. *The Arctic wolf : ten years with the pack.* Updated 10th anniversary ed. Shrewsbury: Swan Hill.

Mech, L.D., and Boitani, L., 2006. *Wolves : behavior, ecology, and conservation.* Chicago, Ill.; Bristol: University of Chicago Press; University Presses Marketing distributor.

Mech, L.D., and Cluff, H.D., 2011. Movements of Wolves at the Northern Extreme of the Species' Range, Including during Four Months of Darkness. *PloS One,* 6 (10), e25328.

Mech, L.D., Nowak, R.M. and Weisberg, S., 2011. Use of cranial characters in taxonomy of the Minnesota wolf (*Canis sp*.). *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 89 (12), 1188-1194.

Melendez, K.V., and Feng, A.S., 2010. Communication calls of little brown bats display individual-specific characteristics. *Journal of the Acoustical Society of America*, 128 (2), 919-923.

Mendes, S., Colino-Rabanal, V.J. and Peris, S.J., 2011. Bird song variations along an urban gradient: The case of the European blackbird (*Turdus merula*). *Landscape and Urban Planning,* 99 (1), 51-57.

Meriggi, A., Brangi, A., Schenone, L., Signorelli, D. and Milanesi, P., 2011. Changes of wolf (*Canis lupus*) diet in Italy in relation to the increase of wild ungulate abundance. *Ethology Ecology & Evolution,* 23 (3), 195-210.

Meriggi, A., and Lovari, S., 1996. A review of wolf predation in southern Europe: Does the wolf prefer wild prey to livestock? *Journal of Applied Ecology*, 33 (6), 1561-1571.

Merkle, J.A., Krausman, P.R., Stark, D.W., Oakleaf, J.K. and Ballard, W.B., 2009. Summer Diet of the Mexican Gray Wolf (*Canis lupus baileyi*). *Southwestern Naturalist*, 54 (4), 480-485.

Meyer, D., Hodges, J.K., Rinaldi, D., Wijaya, A., Roos, C. and Hammerschmidt, K., 2012. Acoustic structure of male loud-calls support molecular phylogeny of Sumatran and Javanese leaf monkeys (genus *Presbytis*). *Bmc Evolutionary Biology,* 12, 16.

Mielke, A., and Zuberbuehler, K., 2013. A method for automated individual, species and call type recognition in free-ranging animals. *Animal Behaviour*, 86 (2), 475-482.

Milanesi, P., Meriggi, A. and Merli, E., 2012. Selection of wild ungulates by wolves *Canis lupus* (L. 1758) in an area of the Northern Apennines (North Italy). *Ethology Ecology & Evolution*, 24 (1), 81-96.

Milheiras, S., and Hodge, I., 2011. Attitudes towards compensation for wolf damage to livestock in Viana do Castelo, North of Portugal. *Innovation-the European Journal of Social Science Research,* 24 (3), 333-351.

Miller, P.J.O., and Bain, D.E., 2000. Within-pod variation in the sound production of a pod of killer whales, *Orcinus orca*. *Animal Behaviour*, 60, 617-628.

Miller, P.J.O., Samarra, F.I.P. and Perthuison, A.D., 2007. Caller sex and orientation influence spectral characteristics of "two-voice" stereotyped calls produced by free-ranging killer whales. *Journal of the Acoustical Society of America,* 121 (6), 3932-3937.

Mills, M.G.L., Juritz, J.M. and Zucchini, W., 2001. Estimating the size of spotted hyaena (*Crocuta crocuta*) populations through playback recordings allowing for non-response. *Animal Conservation,* 4, 335-343.

Mitchell, B.R., Makagon, M.M., Jaeger, M.M. and Barrett, R.H., 2006. Information content of coyote barks and howls. *Bioacoustics-the International Journal of Animal Sound and its Recording*, 15 (3), 289-314.

Miththapala, S., Seidensticker, J., Phillips, L.G., Fernando, S.B.U. and Smallwood, J.A., 1989. Identification of Individual Leopards (*Panthera pardus kotiya*) using Spot Pattern Variation. *Journal of Zoology*, 218, 527-536.

Moehlman, P.D., 1987. Social Organization in Jackals: The complex social system of jackals allows the successful rearing of very dependent young. *American Scientist,* 75 (4), pp. 366-375.

Molnar, C., Pongracz, P., Doka, A. and Miklosi, A., 2006. Can humans discriminate between dogs on the base of the acoustic parameters of barks? *Behavioural Processes*, 73 (1), 76-83.

Molnar, C., Pongracz, P., Farago, T., Doka, A. and Miklosi, A., 2009. Dogs discriminate between barks: The effect of context and identity of the caller. *Behavioural Processes,* 82 (2), 198-201.

Mora, E.C., Rodriguez, A., Macias, S., Quinonez, I. and Mellado, M.M., 2005. The echolocation behaviour of *Nycticeius cubanus* (*Chiroptera : Vespertilionidae*): Inter- and intra-individual plasticity in vocal signatures. *Bioacoustics-the International Journal of Animal Sound and its Recording*, 15 (2), 175-193.

Morner, T., Eriksson, H., Brojer, C., Nilsson, K., Uhlhorn, H., Agren, E., af Segerstad, C.H., Jansson, D.S. and Gavier-Widen, D., 2005. Diseases and mortality in free-ranging brown bear (*Ursus arctos*), gray wolf (*Canis lupus*), and wolverine (*Gulo gulo*) in Sweden. *Journal of Wildlife Diseases,* 41 (2), 298-303.

Mulard, H., Aubin, T., White, J.F., Wagner, R.H. and Danchin, E., 2009. Voice variance may signify ongoing divergence among black-legged kittiwake populations. *Biological Journal of the Linnean Society,* 97 (2), 289-297.

Murray, A., Cerchio, S., McCauley, R., Jenner, C.S., Razafindrakoto, Y., Coughran, D., McKay, S. and Rosenbaum, H., 2012. Minimal similarity in songs suggests limited exchange between humpback whales (*Megaptera novaeangliae*) in the southern Indian Ocean. *Marine Mammal Science,* 28 (1), E41-E57.

Murray, D.L., Smith, D.W., Bangs, E.E., Mack, C., Oakleaf, J.K., Fontaine, J., Boyd, D., Jiminez, M., Niemeyer, C., Meier, T.J., Stahler, D., Holyan, J. and Asher, V.J., 2010. Death from anthropogenic causes is partially compensatory in recovering wolf populations. *Biological Conservation,* 143 (11), 2514-2524.

Nelson, M.P., Vucetich, J.A., Peterson, R.O. and Vucetich, L.M., 2011. The Isle Royale Wolf-Moose Project (1958-present) and the Wonder of Long-Term Ecological Research. *Endeavour,* 35 (1), 30-38.

Newhall, A.E., Lin, Y., Lynch, J.F., Baumgartner, M.F. and Gawarkiewicz, G.G., 2012. Long distance passive localization of vocalizing sei whales using an acoustic normal mode approach. *Journal of the Acoustical Society of America,* 131 (2), 1814-1825.

Nikol'skii i D, 1984. Scientific and Applied Aspects of the Activity of Zoological Phonetic Libraries. *Biologicheskie Nauki (Moscow),* (8), 39-43.

Nowak, R.M., and Federoff, N.E., 1996. Systematics of Wolves of eastern North America. *In: Proceedings of Defenders of Wildlife Wolves of America Conference,* Washington, D.C.: pp. 187-203.

Nowak, R.M., 2003. *Wolf Evolution and Taxonomy.* 1st ed. Chicago: University of Chicago Press.

Oakleaf, J.K., Murray, D.L., Oakleaf, J.R., Bangs, E.E., Mack, C.M., Smith, D.W., Fontaine, J.A., Jimenez, M.D., Meier, T.J. and Niemeyer, C.C., 2006. Habitat Selection by Recolonizing Wolves in the Northern Rocky Mountains of the United States. *The Journal of Wildlife Management,* 70 (2), 554-563.

O'Farrell, M.J., and Gannon, W.L., 1999. A comparison of acoustic versus capture techniques for the inventory of bats. *Journal of Mammalogy,* 80 (1), 24-30.

Oleson, E.M., Calambokidis, J., Barlow, J. and Hildebrand, J.A., 2007. Blue whale visual and acoustic encounter rates in the southern California bight. *Marine Mammal Science,* 23 (3), 574-597.

Oswald, J.N., Rankin, S., Barlow, J. and Lammers, M.O., 2007. A tool for real-time acoustic species identification of delphinid whistles. *Journal of the Acoustical Society of America,* 122 (1), 587-595.

Oyakawa, C., Koda, H. and Sugiura, H., 2007. Acoustic features contributing to the individuality of wild agile gibbon (*Hylobates agilis agilis*) songs. *American Journal of Primatology,* 69 (7), 777-790.

Packard, J.M., 2003. *Wolf Behaviour: Reproductive, Social, and Intelligent.* 1st ed. Chicago: University of Chicago Press.

Pahl, B.C., Terhune, J.M. and Burton, H.R., 1997. Repertoire and geographic variation in underwater vocalisations of Weddell seals (*Leptonychotes weddellii, Pinnipedia: Phocidae*) at the Vestfold Hills, Antarctica. *Australian Journal of Zoology,* 45 (2), 171-187.

Palacios, V., Llaneza, L., Dugnol, B., Fernández, C., Galiano, G. and Velasco, J., 2012. Bloodhound, a tool based on the chirplet transform for the analysis of wolf chorus howls recordings. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 21 (1), 50.

Palacios, V., Font, E. and Márquez, R., 2007. Iberian Wolf Howls: Acoustic Structure, Individual Variation, and a Comparison with North American Populations. *Journal of Mammalogy,* 88 (3), 606-613.

Paquet, P.C., and Carbyn, L.N., 2003. *Gray Wolf, Canis lupus and Allies.* Baltimore, MD: John Hopkins University Press.

Paradiso, J.L., and Nowak, R.M., 1972. *Canis rufus*. *Mammalian Species,* (22, Canis rufus), pp. 1-4.

Parsons, S., and Jones, G., 2000. Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. *Journal of Experimental Biology,* 203 (17), 2641-2656.

Passilongo, D., Dessi-Fulgheri, F., Gazzola, A., Zaccaroni, M. and Apollonio, M., 2012. Wolf counting and individual acoustic discrimination by spectrographic analysis. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 21 (1), 78-79.

Passilongo, D., Buccianti, A., Dessi-Fulgheri, F., Gazzola, A., Zaccaroni, M. and Apollonio, M., 2010. The Acoustic Structure of Wolf Howls in some Eastern Tuscany (Central Italy) Free Ranging Packs. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 19 (3), 159-175.

Payne, K.B., Thompson, M. and 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 (1), 99-107.

Payne, R.S., and McVay, S., 1971. Songs of Humpback Whales. *Science,* 173 (3997), 585-597.

Peake, T.M., McGregor, P.K., Smith, K.W., Tyler, G., Gilbert, G. and Green, R.E., 1998. Individuality in corncrake *Crex crex* vocalizations. *Ibis,* 140 (1), 120-127.

Pearson, K., 1901. On lines and planes of closest fit to systems of points in space. *Philosophical Magazine,* 2 (6), 559-572.

Petitgas, P., Masse, J., Beillois, P., Lebarbier, E. and Le Cann, A., 2003. Sampling variance of species identification in fisheries-acoustic surveys based on automated procedures associating acoustic images and trawl hauls. *ICES Journal of Marine Science,* 60 (3), 437-445.

Pfefferle, D., West, P.M., Grinnell, J., Packer, C. and Fischer, J., 2007. Do acoustic features of lion, *Panthera leo*, roars reflect sex and male condition? *Journal of the Acoustical Society of America,* 121 (6), 3947-3953.

Philips, M.K., Henry, V.G. and Kelly, B.T., 2003. *Restoration of the Red Wolf.* 1st ed. Chicago: University of Chicago Press.

Pilot, M., Jedrzejewski, W., Branicki, W., Sidorovich, V.E., Jedrzejewska, B., Stachura, K. and Funk, S.M., 2006. Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology,* 15 (14), 4533-4553.

Pitcher, B.J., Harcourt, R.G. and Charrier, I., 2012. Individual identity encoding and environmental constraints in vocal recognition of pups by Australian sea lion mothers. *Animal Behaviour,* 83 (3), 681-690.

Policht, R., Petru, M., Lastimoza, L. and Suarez, L., 2009. Potential for the use of vocal individuality as a conservation research tool in two threatened Philippine hornbill species, the Visayan Hornbill and the Rufous-headed Hornbill. *Bird Conservation International,* 19 (1), 83-97.

Potter, J., Mellinger, D. and Clark, C., 1994. Marine Mammal Call Discrimination using Artificial Neural Networks. *Journal of the Acoustical Society of America,* 96 (3), 1255-1262.

Pozzi, L., Gamba, M. and Giacoma, C., 2010. The Use of Artificial Neural Networks to Classify Primate Vocalizations: A Pilot Study on Black Lemurs. *American Journal of Primatology,* 72 (4), 337-348.

Price, T., Arnold, K., Zuberbuehler, K. and Semple, S., 2009. Pyow but not hack calls of the male putty-nosed monkey (*Cercopithcus nictitans*) convey information about caller identity. *Behaviour,* 146, 871-888.

Proops, L., and McComb, K., 2012. Cross-modal individual recognition in domestic horses (*Equus caballus*) extends to familiar humans. *Proceedings of the Royal Society B-Biological Sciences,* 279 (1741).

Ramasindrazana, B., Goodman, S.M., Schoeman, M.C. and Appleton, B., 2011. Identification of cryptic species of *Miniopterus* bats (*Chiroptera: Miniopteridae*) from Madagascar and the Comoros using bioacoustics overlaid on molecular genetic and morphological characters. *Biological Journal of the Linnean Society,* 104 (2), 284-302.

Randall, J.A., McCowan, B., Collins, K.C., Hooper, S.L. and Rogovin, K., 2005. Alarm signals of the great gerbil: Acoustic variation by predator context, sex, age, individual, and family group. *Journal of the Acoustical Society of America,* 118 (4), 2706-2714.

Reby, D., Joachim, J., Lauga, J., Lek, S. and Aulagnier, S., 1998. Individuality in the groans of fallow deer (*Dama dama*) bucks. *Journal of Zoology,* 245, 79-84.

Reby, D., Lek, S., Dimopoulos, I., Joachim, J., Lauga, J. and Aulagnier, S., 1997. Artificial neural networks as a classification method in the behavioural sciences. *Behavioural Processes,* 40 (1).

Reby, D., Andre-Obrecht, R., Galinier, A., Farinas, J. and Cargnelutti, B., 2006. Cepstral coefficients and hidden Markov models reveal idiosyncratic voice characteristics in red deer (*Cervus elaphus*) stags. *Journal of the Acoustical Society of America,* 120 (6), 4080-4089.

Reby, D., and McComb, K., 2003. Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Animal Behaviour,* 65 (3), 519-530.

Reed, J.E., Ballard, W.B., Gipson, P.S., Kelly, B.T., Krausman, P.R., Wallace, M.C. and Wester, D.B., 2006. Diets of free-ranging Mexican gray wolves in Arizona and New Mexico. *Wildlife Society Bulletin,* 34 (4), 1127-1133.

Rek, P., and Osiejuk, T.S., 2011. No male identity information loss during call propagation through dense vegetation: The case of the corncrake. *Behavioural Processes,* 86 (3), 323-328.

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

Riede, T., and Fitch, T., 1999. Vocal tract length and acoustics of vocalization in the domestic dog (*Canis familiaris*). *Journal of Experimental Biology,* 202 (20), 2859-2867.

Riesch, R., Ford, J.K.B. and Thomsen, F., 2006. Stability and group specificity of stereotyped whistles in resident killer whales, *Orcinus orca*, off British Columbia. *Animal Behaviour,* 71, 79-91.

Riesch, R., and Deecke, V.B., 2011. Whistle communication in mammal-eating killer whales (*Orcinus orca*): further evidence for acoustic divergence between ecotypes. *Behavioral Ecology and Sociobiology,* 65 (7), 1377-1387.

Robbins, R.L., and McCreery, E.K., 2003. Acoustic stimulation as a tool in African wild dog conservation. *Biological Conservation,* 111 (2), 263-267.

Rodhouse, T.J., Vierling, K.T. and Irvine, K.M., 2011. A Practical Sampling Design for Acoustic Surveys of Bats. *Journal of Wildlife Management,* 75 (5), 1094-1102.

Rogala, J.K., Hebblewhite, M., Whittington, J., White, C.A., Coleshill, J. and Musiani, M., 2011. Human Activity Differentially Redistributes Large Mammals in the Canadian Rockies National Parks. *Ecology and Society,* 16 (3), 16.

Rogers, T.L., and Cato, D.H., 2002. Individual variation in the acoustic behaviour of the adult male leopard seal, *Hydrurga leptonyx*. *Behaviour,* 139, 1267-1286.

Rogers, T.L., Ciaglia, M.B., Klinck, H. and Southwell, C., 2013. Density can be misleading for low-density species: benefits of passive acoustic monitoring. *PloS One,* 8 (1), e52542.

Rognan, C.B., Szewczak, J.M. and Morrison, M.L., 2009. Vocal Individuality of Great Gray Owls in the Sierra Nevada. *Journal of Wildlife Management,* 73 (5), 755-760.

Root-Gutteridge, H., Bencsik, M., Chebli, M., Gentle, L.K., Terrell-Nield, C., Bourit, A. and Yarnell, R.W., 2014a. Identifying individual wild Eastern grey wolves (*Canis lupus lycaon*) using fundamental frequency and amplitude of howls. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 23 (1), 55-66.

Root-Gutteridge, H., Bencsik, M., Chebli, M., Gentle, L.K., Terrell-Nield, C., Bourit, A. and Yarnell, R.W., 2014b. Improving individual identification in captive Eastern grey wolves (*Canis lupus lycaon*) using the time course of howl amplitudes. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 23 (1), 39-53.

Rueness, E.K., Asmyhr, M.G., Sillero-Zubiri, C., Macdonald, D.W., Bekele, A., Atickem, A. and Stenseth, N.C., 2011. The Cryptic African Wolf: *Canis aureus lupaster* Is Not a Golden Jackal and Is Not Endemic to Egypt. *PLoS ONE,* 6 (1), e16385.

Rumelhart, D., Hinton, G. and Williams, R., 1986. Learning Representations by Back-Propagating Errors. *Nature,* 323 (6088), 533-536.

Salvatori, V., and Linnell, J.D.C., 2005. *Report on the Conservation Status and Threats for Wolf (Canis lupus) in Europe.* 25th ed. Strasbourg: Convention on the Conservation of European Wildlife and Natural Habitats, Standing Committee.

Sand, H., Wikenros, C., Wabakken, P. and Liberg, O., 2006. Cross-continental differences in patterns of predation: will naive moose in Scandinavia ever learn? *Proceedings of the Royal Society B-Biological Sciences,* 273 (1592), 1421-1427.

Sandom, C., Bull, J., Canney, S. and Macdonald, D.W., 2012. *Exploring the Value of Wolves (Canis lupus) in Landscape-Scale Fenced Reserves for Ecological Restoration in the Scottish Highlands.* Springer New York.

Schibler, F., and Manser, M.B., 2007. The irrelevance of individual discrimination in meerkat alarm calls. *Animal Behaviour,* 74, 1259-1268.

Schmid, O., Ball, G., Lancashire, L., Culak, R. and Shah, H., 2005. New approaches to identification of bacterial pathogens by surface enhanced laser desorption/ionization time of flight mass spectrometry in concert with artificial neural networks, with special reference to *Neisseria gonorrhoeae*. *Journal of Medical Microbiology,* 54 (12), 1205-1211.

Schneiderova, I., and Policht, R., 2010. Alarm Calls of the European Ground Squirrel *Spermophilus citellus* and the Taurus Ground Squirrel *S. taurensis* Encode Information about Caller Identity. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 20 (1), 29-43.

Schrader, L., and Hammerschmidt, K., 1997. Computer-Aided Analysis of Acoustic Parameters in Animal Vocalisations: A Multi-Parametric Approach. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 7 (4), 247-265.

Schulz, T.M., Whitehead, H., Gero, S. and Rendell, L., 2011. Individual vocal production in a sperm whale (*Physeter macrocephalus*) social unit. *Marine Mammal Science,* 27 (1), 149-166.

Sebe, F., Duboscq, J., Aubin, T., Ligout, S. and Poindron, P., 2010. Early vocal recognition of mother by lambs: contribution of low- and high-frequency vocalizations. *Animal Behaviour,* 79 (5).

Servin, J., 1997. The mating, birth and growth periods of the Mexican wolf (*Canis lupus baileyi*). *Acta Zoologica Mexicana Nueva Serie,* 0 (71), 45-56.

Sharma, D.K., Maldonado, J.E., Jhala, Y.V. and Fleischer, R.C., 2004. Ancient wolf lineages in India. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 271, S1-S4.

Siemers, B.M., and Kerth, G., 2006. Do echolocation calls of wild colony-living Bechstein's bats (*Myotis bechsteinii*) provide individual-specific signatures? *Behavioral Ecology and Sociobiology,* 59 (3), 443-454.

Sinezhuk, E.B., and Krechmar, E.A., 2010. Geographic Variation in Advertising Song of the Scarlet Rosefinch (*Carpodacus Erythrinus*). *Zoologichesky Zhurnal,* 89 (3), 346-359.

Skaric, G.V., 2008. Speaker Verification in Forensic Phonetics. *Govor,* 25 (1), 31-44.

Skogland, T., 1991. What are the Effects of Predators on Large Ungulate Populations. *Oikos,* 61 (3), 401-411.

Smith, D.W., and Ferguson, G., 2005. *Decade of the wolf : returning the wild to Yellowstone.* Guilford, Conn.: Lyons Press.

Smith, K.L., Oliver, P.M. and Littlejohn, M.J., 2012. Morphological and acoustic evidence for hybridisation between two broadly sympatric south-eastern Australian tree frogs *Litoria ewingii* and *L. verreauxii* (*Anura: Hylidae*). *Australian Journal of Zoology,* 60 (1).

Sousa-Lima, R.S., Paglia, A.P. and 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.

Sousa-Lima, R.S., Paglia, A.P. and da Fonseca, G.A.B., 2008. Gender, age, and identity in the isolation calls of Antillean manatees (*Trichechus manatus manatus*). *Aquatic Mammals,* 34 (1), 109-122.

Sparkman, A.M., Waits, L.P. and Murray, D.L., 2011. Social and Demographic Effects of Anthropogenic Mortality: A Test of the Compensatory Mortality Hypothesis in the Red Wolf. *Plos One,* 6 (6), e20868.

Spillmann, B., Dunkel, L.P., van Noordwijk, M.A., Amda, R.N.A., Lameira, A.R., Wich, S.A. and van Schaik, C.P., 2010. Acoustic Properties of Long Calls Given by Flanged Male Orang-Utans (*Pongo pygmaeus wurmbii*) Reflect Both Individual Identity and Context. *Ethology,* 116 (5), 385-395.

SPSS Inc., 2010. *SPSS.* 17th ed. Chicago, Illinois: SPSS Inc.

Srivastav, A., and Nigam, P., 2009. *National Studbook of Tibetan wolf (Canis lupus chanco).* Dehradun and Central Zoo Authority, New Delhi: Wildlife Institute of India.

Stahler, D., Heinrich, B. and Smith, D., 2002. Common ravens, *Corvus corax*, preferentially associate with grey wolves, *Canis lupus*, as a foraging strategy in winter. *Animal Behaviour*, 64, 283-290.

Stahler, D.R., Smith, D.W. and Guernsey, D.S., 2006. Foraging and feeding ecology of the gray wolf (*Canis lupus*): Lessons from Yellowstone National Park, Wyoming, USA. *Journal of Nutrition*, 136 (7), 1923S-1926S.

StatSoft, I., 2012. *STATISTICA (data analysis software system).* Version 11 ed. www.statsoft.com.

Takagi, M., 2013. A Typological Analysis of the Hoot of the Ryukyu Scops Owl Across Island Populations in the Ryukyu Archipelago and Two Oceanic Islands. *Wilson Journal of Ornithology*, 125 (2), 358-369.

Taylor, A.M., and Reby, D., 2010. The contribution of source-filter theory to mammal vocal communication research. *Journal of Zoology*, 280 (3), 221-236.

Taylor, A.M., Reby, D. and McComb, K., 2010. Size communication in domestic dog, Canis familiaris, growls. *Animal Behaviour*, 79 (1), 205-210.

Terry, A.M.R., and McGregor, P.K., 2002. Census and monitoring based on individually identifiable vocalizations: the role of neural networks. *Animal Conservation*, 5, 103-111.

Terry, A.M.R., Peake, T.M. and McGregor, P.K., 2005. The role of vocal individuality in conservation. *Frontiers in Zoology*, 2 (1), 10-10.

Theberge, J.A., and Falls, J.B., 1967. Howling as a Means of Communication in Timber Wolves. *American Zoologist*, 7 (2), 331-338.

Theuerkauf, J., 2009. What Drives Wolves: Fear or Hunger? Humans, Diet, Climate and Wolf Activity Patterns. *Ethology*, 115 (7), 649-657.

Theuerkauf, J., and Rouys, S., 2008. Habitat selection by ungulates in relation to predation risk by wolves and humans in the Bialowieza Forest, Poland. *Forest Ecology and Management,* 256 (6), 1325-1332.

Theuerkauf, J., Rouys, S. and Jedrzejewski, W., 2003. Selection of den, rendezvous, and resting sites by wolves in the Bialowieza Forest, Poland. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 81 (1), 163-167.

Thinh, V.N., Hallam, C., Roos, C. and Hammerschmidt, K., 2011. Concordance between vocal and genetic diversity in crested gibbons. *Bmc Evolutionary Biology,* 11.

Thompson, M.E., Schwager, S.J. and Payne, K.B., 2010a. Heard but not seen: an acoustic survey of the African forest elephant population at Kakum Conservation Area, Ghana. *African Journal of Ecology*, 48 (1), 224-231.

Thompson, M.E., Schwager, S.J., Payne, K.B. and Turkalo, A.K., 2010b. Acoustic estimation of wildlife abundance: methodology for vocal mammals in forested habitats. *African Journal of Ecology,* 48 (3), 654-661.

Tooze, Z.J., Harrington, F.H. and Fentress, J.C., 1990. Individually Distinct Vocalizations in Timber Wolves, *Canis lupus*. *Animal Behaviour,* 40, 723-730.

Trefry, S.A., and Hik, D.S., 2010. Variation in pika (*Ochotona collaris, O. princeps*) vocalizations within and between populations. *Ecography,* 33 (4), 784-795.

Tripp, T.M., and Otter, K.A., 2006. Vocal individuality as a potential long-term monitoring tool for Western Screech-owls, *Megascops kennicottii*. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 84 (5), 744-753.

Tveraa, T., Fauchald, P., Yoccoz, N.G., Ims, R.A., Aanes, R. and Hogda, K.A., 2007. What regulate and limit reindeer populations in Norway? *Oikos,* 116 (4), 706-715.

Utrata, E., Viranyi, Z. and Range, F., 2012. Quantity Discrimination in Wolves (*Canis lupus*). *Frontiers in Psychology,* 3, 505.

Van Opzeeland, I.C., Corkeron, P.J., Risch, D., Stenson, G. and Van Parijs, S.M., 2009. Geographic variation in vocalizations of pups and mother-pup behavior of harp seals *Pagophilus groenlandicus*. *Aquatic Biology,* 6 (1-3), 109-120.

Vannoni, E., and McElligott, A.G., 2009. Fallow bucks get hoarse: vocal fatigue as a possible signal to conspecifics. *Animal Behaviour,* 78 (1), 3-10.

Vannoni, E., and McElligott, A.G., 2007. Individual acoustic variation in fallow deer (*Dama dama*) common and harsh groans: A source-filter theory perspective. *Ethology,* 113 (3), 223-234.

Vila, C., Amorim, I.R., Leonard, J.A., Posada, D., Castroviejo, J., Petrucci-Fonseca, F., Crandall, K.A., Ellegren, H. and Wayne, R.K., 1999. Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Molecular Ecology,* 8 (12), 2089-2103.

Volodin, I.A., Volodina, E.V., Klenova, A.V. and Filatova, O.A., 2005. Individual and sexual differences in the calls of the monomorphic White-faced Whistling Duck *Dendrocygna viduata*. *Acta Ornithologica,* 40 (1), 43-52.

Volodin, I., Kaiser, M., Matrosova, V., Volodina, E., Klenova, A., Filatova, O. and Kholodova, M., 2009. The Technique of Noninvasive Distant Sexing for Four Monomorphic Dendrocygna Whistling Duck Species by their Loud Whistles. *Bioacoustics-the International Journal of Animal Sound and its Recording,* 18 (3), 277-290.

Volodina, E.V., Volodin, I.A., Isaeva, I.V. and Unck, C., 2006. Biphonation may function to enhance individual recognition in the dhole, *Cuon alpinus*. *Ethology,* 112 (8), 815-825.

Vos, J., 2000. Food habits and livestock depredation of two Iberian wolf packs (*Canis lupus signatus*) in the north of Portugal. *Journal of Zoology,* 251, 457-462.

Wabakken, P., Kvam, T. and Sorensen, O.J., 1984. Wolves *Canis lupus* in Southeastern Norway. *Fauna Norvegica Series A,* 5 (5), 50-52.

Wabakken, P., Sand, H., Kojola, I., Zimmermann, B., Arnemo, J.M., Pedersen, H.C. and Liberg, O., 2007. Multistage, long-range natal dispersal by a global positioning system-collared Scandinavian wolf. *Journal of Wildlife Management,* 71 (5), 1631-1634.

Wagner, C., Holzapfel, M., Kluth, G., Reinhardt, I. and Ansorge, H., 2012. Wolf (*Canis lupus*) feeding habits during the first eight years of its occurrence in Germany. *Mammalian Biology - Zeitschrift Für Säugetierkunde,* 77 (3), 196-203.

Walcott, C., Mager, J.N. and Piper, W., 2006. Changing territories, changing tunes: male loons, *Gavia immer*, change their vocalizations when they change territories. *Animal Behaviour,* 71 (3), 673-683.

Wallner, A., 1998. The Role of Fox, Lynx and Wolf in Mythology. *Workshop on Human Dimension in Large Carnivore Conservation: KORA Bericht,* 3 (-), 31-33.

Walters, C.L., Freeman, R., Collen, A., Dietz, C., Fenton, M.B., Jones, G., Obrist, M.K., Puechmaille, S.J., Sattler, T., Siemers, B.M., Parsons, S. and Jones, K.E., 2012. A continental-scale tool for acoustic identification of European bats. *Journal of Applied Ecology,* 49 (5), 1064-1074.

Watkins, W.A., and Moore, K.E., 1982. An Under Water Acoustic Survey for Sperm Whales *Physeter catodon* and Other Cetaceans in the Southeast Caribbean. *Cetology,* (46), 1-7.

Watts, D.E., Butler, L.G., Dale, B.W. and Cox, R.D., 2010. The Ilnik wolf *Canis lupus* pack: use of marine mammals and offshore sea ice. *Wildlife Biology,* 16 (2), 144-149.

Wayne, R., and Hedrick, P., 2011. Genetics and wolf conservation in the American West: lessons and challenges Genetics of Wolf Conservation. *Heredity,* 107 (1), 16-19.

White, C., 2005. Hunters Ring Dinner Bell for Ravens: Experimental Evidence of a Unique Foraging Strategy. *Ecology,* 86 (4), 1057-1060.

White, S.J., White, R.E.C. and Thorpe, W.H., 1970. Acoustic Basis for Individual Recognition by Voice in Gannet. *Nature,* 225 (5238), 1156-&.

Whitehead, H., 2009. Estimating Abundance From One-Dimensional Passive Acoustic Surveys. *Journal of Wildlife Management,* 73 (6), 1000-1009.

Wilmers, C.C., Crabtree, R.L., Smith, D.W., Murphy, K.M. and Getz, W.M., 2003. Trophic Facilitation by Introduced Top Predators: Grey Wolf Subsidies to Scavengers in Yellowstone National Park. *Journal of Animal Ecology,* 72 (6), 909-916.

Wright, T.F., and Wilkinson, G.S., 2001. Population genetic structure and vocal dialects in an Amazon parrot. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 268 (1467), 609-616.

Wright, T.F., 1996. Regional Dialects in the Contact Call of a Parrot. *Proceedings: Biological Sciences,* 263 (1372), pp. 867-872.

Wyman, M.T., Mooring, M.S., McCowan, B., Penedo, M.C.T. and Hart, L.A., 2008. Amplitude of bison bellows reflects male quality, physical condition and motivation. *Animal Behaviour,* 76, 1625-1639.

Xia, C., Lin, X., Liu, W., Lloyd, H. and Zhang, Y., 2012. Acoustic Identification of Individuals within Large Avian Populations: A Case Study of the Brownish-Flanked Bush Warbler, South-Central China. *Plos One,* 7 (8), e42528.

Xia, C., Xiao, H. and Zhang, Y., 2010. Individual Variation in Brownish-Flanked Bush Warbler Songs. *Condor,* 112 (3), 591-595.

Yamaguchi, A., 1998. A sexually dimorphic learned birdsong in the Northern Cardinal. *Condor,* 100 (3), 504-511.

Yin, S., and McCowan, B., 2004. Barking in domestic dogs: context specificity and individual identification. *Animal Behaviour,* 68 (2), 343-355.

Yoshino, H., Armstrong, K.N., Izawa, M., Yokoyama, J. and Kawata, M., 2008. Genetic and acoustic population structuring in the Okinawa least horseshoe bat: are intercolony acoustic differences maintained by vertical maternal transmission? *Molecular Ecology,* 17 (23), 4978-4991.

Yu, X., Peng, Y., Aowphol, A., Ding, L., Brauth, S.E. and Tang, Y.-., 2011. Geographic variation in the advertisement calls of *Gekko gecko* in relation to variations in morphological features: implications for regional population differentiation. *Ethology Ecology & Evolution,* 23 (3), 211-228.

Zaccaroni, M., Passilongo, D., Buccianti, A., Dessi-Fulgheri, F., Facchini, C., Gazzola, A., Maggini, I. and Apollonio, M., 2012. Group specific vocal signature in free-ranging wolf packs. *Ethology Ecology & Evolution,* 24 (4), 322-331.

Ziegler, L., Arim, M. and Narins, P.M., 2011. Linking amphibian call structure to the environment: the interplay between phenotypic flexibility and individual attributes. *Behavioral Ecology,* 22 (3), 520-526.

Zimen, E., 1981. *The wolf : his place in the natural world.* London: Souvenir.

Zimmermann, B., Wabakken, P. and Dotterer, M., 2001. Human-carnivore interactions in Norway: How does the re-appearance of large carnivores affect people's attitudes and levels of fear? *Forest Snow and Landscape Research,* 76 (1-2), 137-153.

Zsebok, S., Estok, P. and Goerfoel, T., 2012. Acoustic Discrimination of *Pipistrellus Kuhlii* and *Pipistrellus Nathusii (Chiroptera: Vespertilionidae*) and its Application to Assess Changes in Species Distribution. *Acta Zoologica Academiae Scientiarum Hungaricae,* 58 (2).

Appendix 1 Bioacoustics Reviews

Sexual dimorphism is seen in many different species. A review using Web of Knowledge included 26 studies which had investigated sexual dimorphism present in vocalisations and the results are presented in Table 7.1.

Table 7.1 Sexual dimorphism and sex encoding vocalisations

Order	Common Name	Latin Name	Species sexually dimorphic?	Sex encoded?	Paper
Amphibia	Boreal chorus frog	*Pseudacris maculate*	No	Yes	(Bee *et al.* 2010)
Anseriformes	Cuban whistling duck	*Dendrocygna arborea*	No	Yes	(Volodin *et al.* 2009)
Anseriformes	Fulvous whistling duck	*Dendrocygna bicolour*	No	Yes	(Volodin *et al.* 2009)
Anseriformes	Red-billed whistling duck	*Dendrocygna autumnalis*	No	Yes	(Volodin *et al.* 2009)
Anseriformes	White-faced whistling duck	*Dendrocygna viduata*	No	Yes	(Volodin *et al.* 2005; Volodin *et al.* 2009)
Caprimulgiformes	Marbled frogmouth	*Podargus ocellatus*	No	Yes	(Jones and Smith 1997)
Carnivora	African Lion	*Panthera leo*	Yes	Yes	(Pfefferle *et al.* 2007)
Carnivora	Domestic dog	*Canis lupus familiaris*	No	Yes	(Riede and Fitch 1999; Chulkina *et al.* 2006; Farago *et al.* 2010)
Carnivora	Meerkat	*Suricata suricatta*	No	Yes	(Hollen and Manser 2006)
Cetacea	Antillean manatee	*Trichechus manatus manatus*	Yes	Yes	(Sousa-Lima *et al.* 2008)
Cetacea	Orca	*Orcinus orca*	Yes	Yes	(Miller *et al.* 2007)
Charadriiformes	Black-legged kittiwake	*Rissa tridactyla*	Yes	No	(Mulard *et al.* 2009)
Charadriiformes	Black-legged kittiwake	*Rissa tridactyla*	Yes	Yes	(Aubin *et al.* 2007)
Charadriiformes	Brown skua	*Catharacta antarctica*	No	No	(Janicke *et al.* 2007)

		lonnbergii			
Chiroptera	Big brown bat	*Eptesicus fuscus*	No	No	(Kazial *et al.* 2001)
Chiroptera	Big brown bat	*Eptesicus fuscus*	No	Yes	(Kazial and Masters 2004)
Chiroptera	Lesser horseshoe bat	*Rhinolophus hipposideros*	No	Yes	(Jones *et al.* 1992)
Chiroptera	Trident-leaf nosed bat	*Asellia tridens*	No	Yes	(Jones *et al.* 1993)
Ciconiiformes	Oriental white stork	*Ciconia boyciana*	Yes	Yes	(Eda-Fujiwara *et al.* 2004)
Columbiformes	Collared dove	*Streptopelia decaocto*	No	Yes	(Ballintijn and tenCate 1997)
Cuculiformes	Pheasant coucal	*Centropus phasianinus*	Yes	Yes	(Maurer *et al.* 2008)
Passeriformes	Northern cardinal	*Cardinalis cardinalis*	No	Yes	(Yamaguchi 1998)
Perissodactyla	Horse	*Equus caballus*	No	Yes	(Lemasson *et al.* 2009)
Rodentia	Great gerbil	*Rhombomys opinus*	No	Yes	(Randall *et al.* 2005)
Rodentia	Ground squirrels, marmots	*Marmotinae spp.*	No	Yes	(Matrosova *et al.* 2011)
Strigiformes	Western screech owl	*Otus kennicottii*	Yes	Yes	(Herting and Belthoff 2001)

A review of current papers (Table 7.2) suggests that regional and geographical differences in vocalisations are widespread enough across genera that they can be used to identify the home territory of individuals in other untried species. A further area of research should focus on the fidelity of individuals to this particular accent and under what circumstances it possibly can be lost. Changes of accent with region are seen in common loons (Walcott *et al.* 2006).

Table 7.2 List of species where geographic or regional accent has been shown

Common name	**Latin Name**	**Purpose of Study**	**Paper**
African warbler	*Cisticola erythrops*	Geographic / Species	(Benedict and Bowie 2009)
Bats genus *Microchiroptera*	*Microchiroptera*	Geographic	(Adams *et al.* 2010b)
Blue whale	*Balaenoptera musculus*	Geographic	(McDonald *et al.* 2006)
Common Loon	*Gavia immer*	Geographic	(Mager *et al.* 2007a)
Crested gibbons	*Nomascus nasutus, N. concolor, N. leucogenys, N. siki, N. annamensis, N. gabriellae*	Geographic	(Thinh *et al.* 2011)
European blackbird	*Turdus merula*	Geographic	(Mendes *et al.* 2011)
Gibbon	*Hylobates agilis*	Geographic	(Sharma *et al.* 2004)
Greater sac-winged bat	*Saccopteryx bilineata*	Geographic	(Davidson and Wilkinson 2002)
Harp Seals	*Pagophilus groenlandicus*	Geographic	(Van Opzeeland *et al.* 2009)
Okinawa least horseshoe bat	*Rhinolophus cornutus pumilus*	Regional	(Yoshino *et al.* 2008)
Pika	*Ochotona-Princeps*	Geographic	(Conner 1982)
Scarlet rosefinch	*Carpodacus erythrinus*	Geographic	(Sinezhuk and Krechmar 2010)

Short-beaked common dolphin	*Delphinus delphis*	Geographic	(Ansmann *et al.* 2007)
Sulawesi Tarsiers	*Tarsier tarsius, T. dianae, T. Pelengensis*	Geographic	(Burton and Nietsch 2010)
Tamarin	*Saguinus I. Labiatus*	Geographic	(Maeda and Masataka 1987)
Wallacea’s bat	*Rhinolophus philippinensis*	Geographic	(Kingston and Rossiter 2004)
Yellow-naped Amazon parrot	*Amazona auropalliata*	Geographic / group	(Wright 1996)
Yellow-naped Amazon parrot	*Amazona auropalliata*	Geographic	(Wright 1996)
Yellow-naped Amazon parrot	*Amazona auropalliata*	Geographic / group	(Wright and Wilkinson 2001)
Putty-nosed monkey	*Cercopithecus nictitans*	Geographic not encoded	(Price *et al.* 2009)
South-western Willow Flycatcher	*Empidonax Trailii Extimus*	Population variation across regions	(Fernandez-Juricic *et al.* 2009)

Appendix 2 Bespoke Howl Feature Extraction Matlab Code

This is the bespoke Matlab code used to extract howls, as written by Dr Martin Bencsik and Manfred Chebli.

```
% The purpose of this code is to extract the main parameters of a howling
% contained in a noisy recording, and save them.

% Clear Matlab's memory and close all the windows
clear all
close all

%First, we have to define where is the audio file
%and in which folder it is contained.
folder_name = 'C:\Users\Holly\Documents\Analysis\';
file_name = 'W Canis lupus R1 C3 st 1min 38s.wav';


% The name of the wolf is important as the parameters will be saved under
% its name.
wolf_name = 'RGF';


% This settings are the mean parameters of the recording that has been used to develop
```

```
% the software. They are considered as "standard" parameters and they will be used
% for the analysis of the first spectrum.

% The only unsure parameter is the frequency. If the analysis is no good,
% be sure that the first number is near the frequency of the fundamental,
% and modify it if needed.
params = [500 10 10 2 1.5];

% The p function will be used at the end of the code, when saving the data
% into a variable. So if the wolf has not changed, it will be incremented
% at the end of the code, given the analysis is sucessful.
display('Has the wolf changed ? (yes = 1 / no = 2)')


ant = input (' ');
if ant == 1
 p == 0;
end

% Read the wave file.
[sound_data, sampling_rate, Nbits] = wavread([folder_name file_name]);

% Get rid off the stereo part and keep only the
% left part of the sound.
mono_data = sound_data(:, 1);
```

```
% Here are the FFT's length and the duration of the howling in digits.
F = 2048; % FFT's length
T = ceil(length(mono_data)/F)-1; % Howling duration

% Generate a frequency axis, in Hertz, for the spectrogram:
frequency_axis = 0:((sampling_rate/2)/(F/2 - 1)):(sampling_rate/2);

% Generate a time axis, in seconds, for the spectrogram:
time_axis = 0:((T*F/sampling_rate)/(T - 1)):(T*F/sampling_rate);


% Cut the string of datas into :
% F parts vertically
% T parts horizontally
mono_data_reshaped = reshape(mono_data(1:T*F), F, T);

% At this point, the string of data are converted to a FxT Matrix.

% Compute the Fourier Transform :
f = fft(mono_data_reshaped);


% Time by 10*log(x) to get the log of the signal
```

```
% Take only the first F/2 points vertically to not get the symetrical part
% Realise a Brick Wall Filter for the spectrogram's 150 first points.
brick_wall_UL = 150;
[a BWUL] = min(abs(frequency_axis - brick_wall_UL));
f(1:BWUL, :) = 1e-3;

% At this point, the 150 first Hz of the spectrogram are set to 1e-3.

% Create the spectrogram by taking the logarithm of the modulus of the fft
% of the reshaped data. Note that only F/2 points vertically are used in
% order to get rid of the symetrical part of the fft.
spectrogram = 10*log(abs(f((1:F/2), (1:T))));



% Set the limits of the frequencies to be displayed:
F_min = 50;
% identify the corresponding index:
[a F_min_index] = min(abs(frequency_axis - F_min));

F_max = 5000;
% identify the corresponding index:
[a F_max_index] = min(abs(frequency_axis - F_max));

% Cleaning the variable
clear Fo CCF detected_frq data artificial_spectrogram m n howling_number
```

```
set(gcf, 'doublebuffer', 'on')

% Display the spectrogram for the user to choose the number of howlings he
% wants to analyse.
figure(1)
imagesc(time_axis, [frequency_axis(F_min_index) frequency_axis(F_max_index)],
spectrogram(F_min_index:F_max_index, :))
colorbar
set(gca, 'YDir', 'normal')
ylabel 'Frequency / Hz'
xlabel 'Time / s'
title 'Spectrogram'

% Number of howling
display 'how many howlings do you want to analyse ?'
howling_nb = input('number of howling: ');

% Default frequency limitation value
% The frequency limitation will be used in the cross-correlation part
frequency_lim = 220;

% This variable is used to end the while loop.
answer = 2;
```

```
% Variable for the changing of the beginning and ending points of the
% howling if the user is not satisfied.
emp = 1;

% Upper Frequency Limitation for the cross-correlation.
Upper_lim = 800;



% Here is the while loop. The code will run and the software will try to
% extract the main parameters of the howling. However, this will not work
% perfectly everytime. To obtain better results, we have decided to make
% more passes.

% At the end of the analysis, the results are displayed. The user is then
% asked is he or she is satisfied by the results obtained. If he is, the
% variable p is incremented and the parameters are saved. If he is not, he
% will be able to change several parameters to obtain a better result.

while answer ~= 1
 % Cleaning
 clear artificial_spectrogram data detected_frq

 % Display the spectrogram
 figure(1)
```

```
imagesc([], [frequency_axis(F_min_index) frequency_axis(F_max_index)],
spectrogram(F_min_index:F_max_index, :))
colorbar
set(gca, 'YDir', 'normal')
ylabel 'Frequency / Hz'
title 'Spectrogram'

% This for loop lets the user choose the beginning and the end of the
% each howling.
% On the second pass, it appears only if the user wants to change the
% beginning and the end of the howlings.
if emp == 1
for howling_number = 1:howling_nb
display(['select starting point of the howling n°' num2str(howling_number)])
tfn_LL = input('starting point = ');
display(['select ending point of the howling n°' num2str(howling_number)])
tfn_UL = input('ending point = ');

m(howling_number) = tfn_LL; % Store the startings points
n(howling_number) = tfn_UL; % Store the endings points
end
end

% The m and n variables are row of data which as many number as the
% number of howling analysed.
```

```
% m correspond to the starting point of each howling, and n correspond
% to the ending point.

close all

% The analysis is done for every howling separately
for howling_number = 1:howling_nb

UL = Upper_lim; % Defined above
LL = 100; % Lower limit of the cross-correlation
PP = m(howling_number); % Used for the loading bars

frequency_limitation = frequency_lim;

% Define the scanning strategy of the howling.
% The analysis start at the mid point of the howling, and goes
% forward until the end. Then, it return to the mid point and goes
% backward until the beginning.
scan_strategy = m(howling_number):n(howling_number);
mid_point = scan_strategy(round(length(scan_strategy)/2)-1);
scan_strategy = [scan_strategy(round(length(scan_strategy)/2)):scan_strategy(end)
scan_strategy(round(length(scan_strategy)/2)-1):(-1):scan_strategy(1)];

% This line change the set of parameters given at the beginning for
% some adapted to the mid point of the howling.
```

```
 params = fminsearch('difff_lorentz', params, [], exp(0.1*spectrogram(:,
scan_strategy(round(length(scan_strategy)/2)))), frequency_axis');

 for time_frame_nb = scan_strategy


 %%%%%%%%%%%%%%%%%%%%%%%%%%
 % CROSS CORRELATION PART %
 %%%%%%%%%%%%%%%%%%%%%%%%%%

 %Display the progress of the analysis with loading bars !
 if howling_nb > 1
 PP = PP + 1;
 else
 PP = PP + 1;
 end

 % Allocate the memory of the CCF_frequency_span variable
 CCF_frequency_span = zeros(1, UL);
 CCF_frequency_span(1, LL:UL) = LL:1:UL;
 Fo_index = 1;

 for Fo = CCF_frequency_span
 % For Fo going from LL to UL, realise a cross-correlation
 % to determine the frequency of the fundamental.
```

```
 % Plot the two first function of the Cross-Correlation :
 % the spectrum corresponding to the time frame number, and
 % the four generated Lorentzian peaks.

 % Decide which set of parameters will be used to generate the
 % four Lorentzian peaks, and calculate the cross-correlation
 % function.
 if time_frame_nb == mid_point;
 CCF(time_frame_nb, Fo_index) = (sum(exp(0.1*spectrogram(:, time_frame_nb))'.*
Lorentz(data(time_frame_nb+1, :), frequency_axis)));
 else
 CCF(time_frame_nb, Fo_index) = (sum(exp(0.1*spectrogram(:, time_frame_nb))'.*
Lorentz([Fo abs(params(2:5))], frequency_axis)));
 end

 Fo_index = Fo_index + 1;
 end

 % Find the maximum of the cross-correlation function.
 [a b] = max(CCF(time_frame_nb, 1:UL));


 if CCF_frequency_span(b) < frequency_limitation
 % The noise might be of higher amplitude than the howling,
 % so we check if the maximum is before or after the
```

```
 % frequency_limitation.
 % If it is before, it is considered as noise, so everything
 % from 1 to the frequency_limitation is forced to zero, and
 % the search for the maximum of the cross-correlation is
 % done once again.
 [a boundary] = min(abs(CCF_frequency_span - frequency_limitation));
 CCF(time_frame_nb, 1:boundary) = zeros;
 CCF(time_frame_nb, UL+1:end) = zeros;
 [a d] = max(CCF(time_frame_nb, :));

 detected_frq(time_frame_nb) = CCF_frequency_span(d);
 else
 % The Cross-Correlation worked.
 detected_frq(time_frame_nb) = CCF_frequency_span(b);
 end

 % End of the Cross-Correlation Part.

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%

 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
 % SEARCH OPTIMUM PARAMETERS PART %
 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
 % Fit the spectrum with generated Lorentzian peaks using the
 % fminsearch function.
 if time_frame_nb == mid_point
 params = fminsearch('difff_lorentz', abs(data(time_frame_nb+1, 1:5)), [],
exp(0.1*spectrogram(:, time_frame_nb)), frequency_axis');
 else
 params = fminsearch('difff_lorentz', [detected_frq(time_frame_nb) params(2:5)], [],
exp(0.1*spectrogram(:, time_frame_nb)), frequency_axis');
 end

 if params(1) > frequency_limitation

 % Save the data
 data(time_frame_nb, 1:5) = params;
 artificial_spectrogram(time_frame_nb, :) = Lorentz(params, frequency_axis);
 else
 % If the condition is not respected, it generates an array of
 % zeros instead of fitting the datas.
 % Save the data
 data(time_frame_nb, 1:5) = zeros(1, 5);
 artificial_spectrogram(time_frame_nb, :) = zeros(1, size(spectrogram, 1));
 end
 % Display the progress of the extraction so as to allow the
 % viewer to keep track of the quality of the extraction:
 imagesc(flipud(log(artificial_spectrogram')))
```

```
 pause(0.1)
 end
 end
 % close figure 7

 % End of the search for the optimal parameters

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%

 % At this point, we are in possession of the five main parameters which
 % defines the howling.

 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
 % RECONSTRUCTING THE SPECTROGRAM PART %
 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

 clear reconstructed_spectrogram howling_number params

 % Reconstruct the spectrogram from the variable 'data'.
 for howling_number = 1:howling_nb
 for time_frame_nb = m(howling_number):n(howling_number)
 params = [data(time_frame_nb, 1) data(time_frame_nb, 2) data(time_frame_nb, 3)
data(time_frame_nb, 4) data(time_frame_nb, 5)];
 reconstructed_spectrogram(time_frame_nb, :) = Lorentz(params, frequency_axis);
```

```
end
end

% If there is noise between the end of the last howling and the end of
% the recording, this space is set to zero.
reconstructed_spectrogram(n(howling_nb)+1:T, :) = zeros(T-n(howling_nb), F/2);

% Display the reconstructed spectrogram, in different boxes for each
% howling. Display the original howling too.
for howling_number = 1:howling_nb
figure(1)
subplot((ceil(howling_nb/3)+1), howling_nb, howling_number)
imagesc([], frequency_axis, 10*log(abs(reconstructed_spectrogram((m(howling_number) -
(round(0.01.*(n(howling_number)-m(howling_number))))):(n(howling_number) +
(round(0.01.*(n(howling_number)-m(howling_number))))), :)')), [-80 45])
set(gca, 'YDir', 'normal')
xlabel 'Digits'
axis([1 size(reconstructed_spectrogram((m(howling_number) -
(round(0.01.*(n(howling_number)-m(howling_number))))):(n(howling_number) +
(round(0.01.*(n(howling_number)-m(howling_number))))), :)', 2) 0 3500])

pause(1)

subplot((ceil(howling_nb/3)+1), howling_nb, howling_number+howling_nb)
```

```
 imagesc([], frequency_axis, spectrogram(:, (m(howling_number) -
(round(0.01.*(n(howling_number)-m(howling_number))))):(n(howling_number) +
(round(0.01.*(n(howling_number)-m(howling_number)))))), [-80 45])
 set(gca, 'YDir', 'normal')
 xlabel 'Digits'
 title 'Spectrogram'
 axis([1 size(spectrogram(:, (m(howling_number) - (round(0.01.*(n(howling_number)-
m(howling_number))))):(n(howling_number) + (round(0.01.*(n(howling_number)-
m(howling_number)))))), 2) 0 3500])

 pause(1)
 end

 % Ask the user if he is satisfied by the analysis.
 display 'Are you satisfied by this result ? (yes = 1; no = 2)'
 answer = input(' ');

 % If the user is not satisfied, he is asked to change settings for the
 % next pass.
 % This settings are :
 % - The frequency limitation
 % - The Upper frequency limitation
 % - The startings and endings points of the howlings
 if answer == 2
 figure
```

```
 plot(data(m(1):n(end), 1))
 display 'Choose the lowest frequency of the howlings. Previous value was :'
 frequency_limitation
 frequency_lim = input('frequency limitation = ');

 display 'Do you want to change the startings and ending points'
 display 'of each howling ? (yes = 1 / no = 2)'
 emp = input(' ');

 display 'Do you want to change the upper limit ? (yes = 1 / no = 2)'
 tamp = input(' ');
 if tamp == 1
 display 'Input new Upper Limit :'
 Upper_lim = input(' ');
 end
 end

end


% When the user is satisfied, the analysis will be labelled as successful,
% and the p variable will be incremented. Then, the five rows of data will
% be saved as *.mat files
p = p+1;
```

```
for howling_number = 1:howling_nb
 saved_data = data(m(howling_number):n(howling_number), :);
 save([wolf_name, '_', num2str(p), '_', num2str(howling_number)], 'saved_data')
end
```

Appendix 3 A Brief Description of Wolf Subspecies

This appendix consists of information on the different grey wolf subspecies here analysed.

9.1 Grey Wolves

Figure 9.1 shows the decreased range of the grey wolf in North America from the historic levels (Paquet and Carbyn 2003). Figure 9.2 shows the current distribution of wolf subspecies in North America with the currently accepted taxonomy of five main subspecies (Nowak and Federoff 1996). The importance of historic range is that once a particular subspecies of wolf has been extirpated, the same subspecies may not return when re-colonisation or reintroduction occurs and mixing may occur between subspecies if the origin population is of a different subspecies to the historic subspecies. Care must therefore be taken to check the current classification of recordings of subspecies which may be labelled '*Canis lupus crassodon*'.

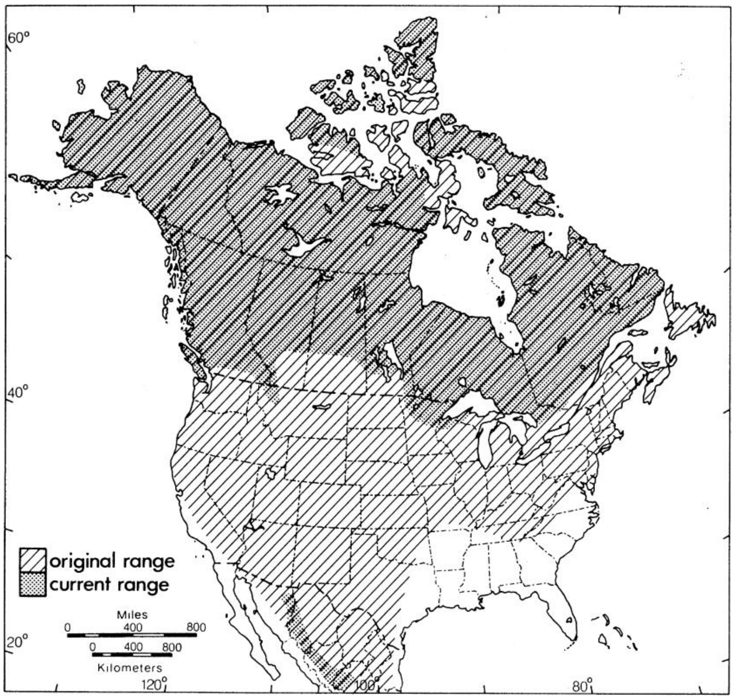


Figure 9.1 Original and Current Range of Grey wolf in North America (Paquet and Carbyn 2003)

Figure 9.2 Distribution of current wolf subspecies in North America (Nowak and Federoff 1996)

Subspecies of wolf are listed by Old World followed by New World. Much of the diet and behaviour of wolves is similar wherever they are found and interbreeding can occur between both Old and New World subspecies in any combination. Known examples of this interbreeding include Mackenzie Valley (*C.l. occidentalis*) and European wolf (*C.l. lupus*) crosses living in UK zoos, e.g. Torak at UK Wolf Conservation Trust. Differences between subspecies do not appear to stop pack bonding, with Europeans and Mackenzie Valley wolves living together at UK Wolf Trust and a Tibetan (*C.l. chanco*) wolf, which lived peacefully with European wolves in captivity in Bavaria in the 1970 s (Zimen 1981). Morphological differences exist but no genetic barriers to successful reproduction have been cited and the grey wolf is considered to be one continuously distributed species with distinct subspecies or races arising over geographic distance rather than a set of subspecies co-existing and undergoing diverging evolution in the same area (Nowak 2003; Agnarsson *et al.* 2010; Fain *et al.* 2010).

9.2 European wolf (*Canis lupus lupus*)

The European or Eurasian wolf is one of the most widely distributed subspecies of grey wolf, found from the wilds of far eastern Russia to Finland and as far south as Italy and Turkey (Vila *et al.* 1999). It has one of the largest variations in size within a subspecies with a length of 105-160cm and a weight of 32-50kg (Boitani 2000). Its diet, despite its reputation as a major predator of livestock, mostly depends on wild prey, with a preference for wild ungulates where available (Meriggi and Lovari 1996; Lanszki *et al.* 2012; Milanesi *et al.* 2012; Wagner *et al.* 2012). They have been extirpated from much of their former territory because of conflicts with humans and are still in continued conflict in many countries which prevent their further recolonisation (Mech 1970; Jedrzejewksi *et al.* 2005). The most recent summary of the legal status of wolves across Europe is shown in based on Salvatori & Linnell (2005). Table 9.1 shows Spain and Portugal to have European wolves, but these are considered as a separate subspecies (Iberian) by Palacios *et al.* (2007) and are therefore analysed as such.

Table 9.1 Legal Status, Population and Trend of Grey Wolves in Europe (Salvatori & Linnell 2005).

Country	No. of wolves	Trend	Legal status	Hunted?
Albania	450-600	Stable	Fully protected	Yes, illegally
Bosnia-Herzegovina	Unknown	Unknown	Partly protected	Yes, legally
Bulgaria*	1, 000	Stable	Not protected	Yes and legal bounty
Croatia	130-170	Stable	Partly protected	Yes, quota 15 per year
Czech Republic	5-17	Unknown	Fully protected	Unknown (probably)
Estonia	100-150	Stable	Partly protected	Yes, legally
Finland	185	Increasing	Fully protected	No, only lethal control
France	80-100	Increasing	Partly protected	Yes, only lethal control
Germany	c. 10	Stable	Fully protected	No
Greece	500-700	Stable	Partly protected	Yes, illegally
Hungary	3-6	Unknown	Fully protected	Yes, illegally
Poland	700	Stable	Fully protected	Yes, illegally
Romania	2, 000-4, 000	Stable	Partly protected	Yes, legally
Slovak Republic	500	Stable	Partly protected	Yes, legally
Slovenia	60-100	Stable	Fully protected	Yes, illegally
Italy	c. 500	Increasing	Fully protected	Yes, illegally
Latvia	300-500	Increasing	Partly protected	Yes, quota c. 140

Lithuania	400-500	Increasing	Not protected	Yes, legally
Macedonia	800-1, 000	Stable	Partly protected	Yes, quota c. 400
Norway	23-26	Increasing	Fully protected	No, only lethal control
Portugal	300	Stable	Fully protected	Yes, illegally
Spain	2, 000	Increasing	Partly protected	Yes, legally & illegally
Sweden	48-49	Increasing	Fully protected	No
Switzerland	3	Stable	Fully protected	Yes, legally & illegally
Turkey	5, 000-7, 000	Decreasing	Not protected	Yes legally encouraged
Ukraine	2, 000	Unknown	Not protected	Yes legally encouraged

Key to Table 9.1:

Not protected – no legal protection whatsoever in place and often refused to sign Bern Convention or did not allow it to be applied to wolves. *Bulgaria provides a significant bounty on each wolf, a policy shared previously by USSR and by Russia.

Partly protected – some legal protection with hunting seasons allowed or special quotas and licenses for hunting.

Fully protected – full legal protection with only lethal control or very low quotas for hunting wolves.

9.3 Iberian wolf (*Canis lupus signatus*)

The Iberian wolf is limited in its distribution to Spain and Portugal (Figure 9.3). It is a subspecies which is closely related to the European wolf and the Italian wolf, although it is smaller than the European wolf. Its name means ‘signed’ due to the black marks on the species’ forepaws, tails and cross and white marks on the upper lips (Palacios *et al.* 2007). Their weight is ~30kg for females and ~40kg for males, making them similar to red wolves and Mexican wolves and smaller than European wolves found in Russia and Asia (Palacios *et al.* 2007). Like all wolves, they are omnivorous with a varied diet including small mammals, roe deer (*Capreolus capreolus*), wild boar (Vos 2000) and domestic horses (Vos 2000; Barja 2009). Its howl has previously been described using fundamental frequency by Palacios *et al.* (2007), and the howls are the same collected and analysed by Vicente Palacios, with his kind permission.

Figure 9.3 Distribution of Iberian wolf in Spain and Portugal

Source: ‘Report on the conservation status and threats for wolf (*Canis lupus*) in Europe’ (Salvatori and Linnell 2005)

9.4 Golden Jackal (*Canis aureus*) and North African wolf (*Canis aureus lupaster*)

The golden jackal is found throughout North and East Africa, the Middle East, south-eastern Europe and central, southern and western Asia (Figure 9.4) (Rueness *et al.* 2011). It measures 70-106cm in body length and weighs 7-15kg with males 12% larger and heavier than females (Moehlman 1987). Their coats give them their 'golden' name as it shades from pale gold to brown-tipped, with a darker saddle across the back (Moehlman 1987). They are opportunistic omnivores, although they feed primarily on rodents (*Microtus spp.*) and occupy a range of habitats from deserts to evergreen forests (Moehlman 1987; Markov and Lanszki 2012). The longevity in the wild is eight to nine years and the oldest jackal in captivity died at eighteen (Moehlman 1987). They are monogamous cooperative breeders and young may stay with their parents as helpers to the succeeding year of pups (Moehlman 1987).

Following Rueness *et al.*'s (2011) reassessment of the phylogeny of the golden jackal, which was previously considered a monophyletic group, the North African wolf (*Canis aureus lupaster*) has recently been reclassified from a Golden Jackal. Its range was extended by Gaubert *et al.* (2012) to include Senegal, Mali and Algeria. The North African (or African) wolf is considered one of the four distinct lineages within the grey wolf clade, the other three being Holarctic wolves / dogs (*C. lupus / familiaris*), Tibetan or Himalayan wolves (*C.l. chanco*) and Indian wolves (*C.l. pallipes*) (Gaubert *et al.* 2012). Their distribution is shown in Figure **9.8**. Because it is impossible to know whether the sampled animals classified as golden jackal by the recorder at the time were in fact golden jackals or North African wolves, all are treated as *Canis aureus* spp. and included here as a monophyletic group, although possibly that of *Canis aureus lupaster*.

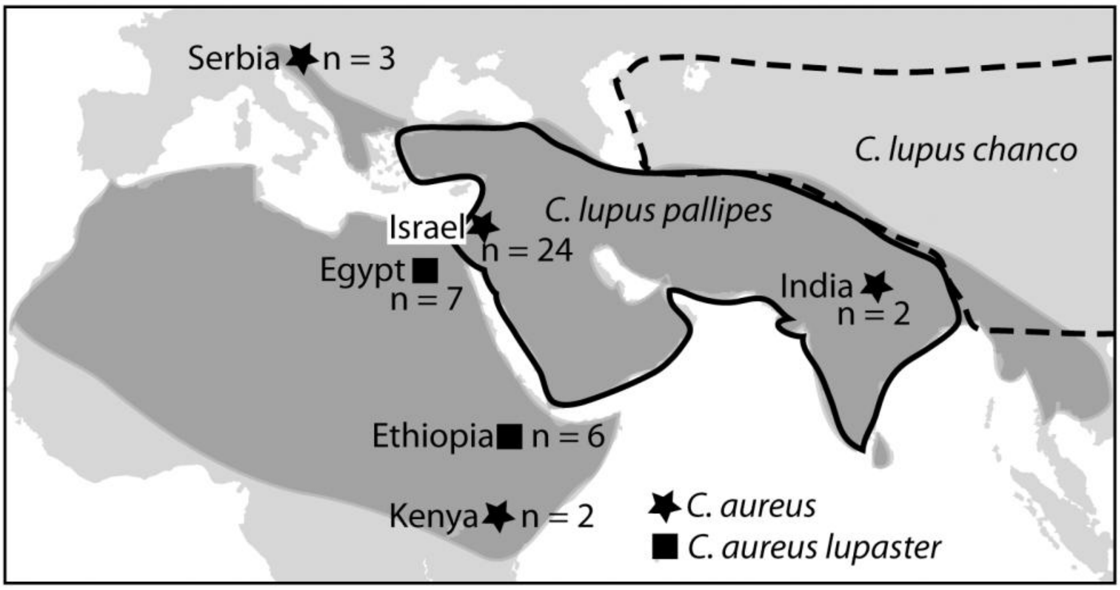


Figure 9.4 Distribution of Golden Jackal, including that of North African wolf in Ethiopia. Source: 'Figure 1' of Rueness *et al.* (2011).

NB: Numbers indicate the number of samples taken by Rueness *et al.* (2011) for their classification of the North African wolf, formerly the Golden Jackal.

9.5 Arctic wolf (*Canis lupus arctos*)

The Arctic wolf (*Canis lupus arctos*) is found throughout the Arctic Circle, including northern Canada and Greenland, and distribution is shown in Figure 9.5. They have the typical white coat of polar animals and feed primarily on Arctic hares (*Lepus arcticus*) (Mech 1997; Mech 2007). At the furthest most point of their range at Ellesmere Island, they rely on muskoxen (*Ovibos moschatus*) and live in larger than average packs of 20 or more adults (Mech and Cluff 2011). The territory size of the Arctic wolf is the largest of any known wolf, with one pack holding a range of 6, 640km^2 (Mech and Cluff 2011). Both recordings in the wild and in captivity are limited by opportunity – in the wild because of the harshness of conditions in the Arctic Circle and in captivity because only one pack is kept in the UK, at UK Wolf Conservation Trust.

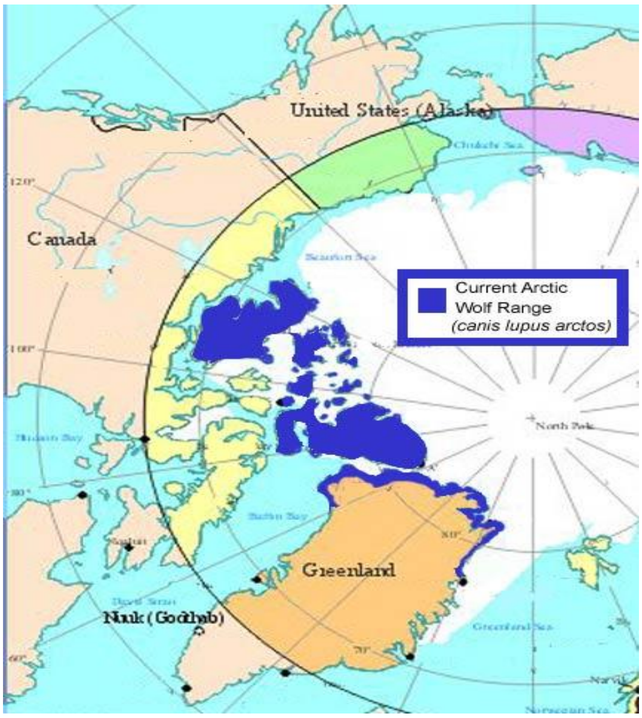


Figure 9.5 Distribution of Arctic wolf

Source: International wolf centre, downloaded 11/06/12

http://www.wolf.org/wolves/experience/field_notes/high_arctic/arctic_range.asp

9.6 North American subspecies

Table 9.2 shows wolf population estimates in Northern America.

Table 9.2 Legal Status, Population and Trend of Grey Wolves in North America taken from Boitani (2003) with updated figures from US Fish and Wildlife Service website (downloaded 25.06.12) (Anonymous 2012)

Area	**No. of wolves**	**Trend**	**Legal status**	**Hunted?**
Canada	52, 000-60, 000	Increasing	Only protected in 3% of Canada	As big game
Alaska	6, 000-7, 000	Increasing	Partially protected	As big game August-April
Minnesota	2, 900	Increasing	Protected but culled	Illegally
Wisconsin	600	Increasing	Fully protected	Illegally
Michigan	600	Increasing	Protected but culled	Illegally
Wyoming Idaho Montana	1, 700	Increasing	Protected but culled	Illegally
Oregon	24	Increasing	Protected but culled	Illegally
Arizona (Mexican subspecies)	42	Increasing	Fully protected	Illegally
North Carolina	70	Increasing	Fully protected	No

9.7 Eastern wolf (*Canis lupus lycaon*)

Eastern wolves weigh between 26-36kg, similar to Iberian wolves, with females about 5kg on average lighter than males (Mech and Paul 2008). They typically have silver or grey-brown coats, which may darken in winter, with a lighter undercoat (Nowak 2003). A more thorough discussion of their biology can be found in Chapter 2.

9.8 Mackenzie Valley wolf (*Canis lupus occidentalis*)

Otherwise known as the Canadian timber wolf, the Mackenzie Valley wolf is one of the largest subspecies of grey wolf weighing from 38-65kg and standing 81-95cm at the shoulder (Smith and Ferguson 2005). It has a pack size of typically six to twelve individuals, although the largest ever documented pack size was the Druid pack of Yellowstone Park which had 37 members (Smith and Ferguson 2005). Their diet is preferentially based around wild ungulates such as bison and elk but also includes salmon, rodents, vegetation and scavenged carrion, with local ecology affecting prey choice (Stahler *et al.* 2006; Garrott *et al.* 2007; Watts *et al.* 2010; Adams *et al.* 2010a). They are found across Canada and were the subspecies used to restore wolves to Yellowstone National Park (Smith and Ferguson 2005).

9.9 Great Plains wolf (*Canis lupus nubilus*)

Despite eradication from Wisconsin and Michigan in the 1960 s, the Great Plains wolf is still the most common subspecies in North America (Leonard *et al.* 2005). Its range is now mainly limited to Minnesota, with smaller populations on Isle Royale, and slowly returning to Wisconsin and Michigan (Leonard *et al.* 2005). They are considered to be a larger, broader headed, taxonomically distinct subspecies (Mech *et al.* 2011). Their diet is similar to the Mackenzie Valley wolf, with a preference for white tailed-deer (*Odocoileus virginianus*) and muskrats (*Ondatra zibethicus*) (Chavez and Gese 2005).

9.10 Mexican wolf (*Canis lupus baileyi*)

The Mexican wolf (*Canis lupus baileyi*) is the smallest of the North American subspecies of grey wolf, weighing 27-37kg (Servin 1997). Their diet mainly consists of elk (*Cervus elaphus*) but also includes domestic cattle (*Bos primigenius taurus*), deer (*Odocoileus spp.*), and small mammals (Merkle *et al.* 2009). Perhaps due to naivety of predation after the wolf's extirpation, Mexican wolves consume more large-sized prey than other North American grey wolves (Reed *et al.* 2006).

The Mexican wolf was extirpated from its historic range by the 1950 s and was declared endangered in 1976 and today is only kept in captivity and reintroduction zones in Blue Range, Arizona (Figure 9.6) (Brown and Parsons 2001). Despite all known Mexican wolves descended from seven founders that were captured from the wild and bred in captivity, they do not show inbreeding depression (Brown and Parsons 2001; Hedrick and Fredrickson 2008).

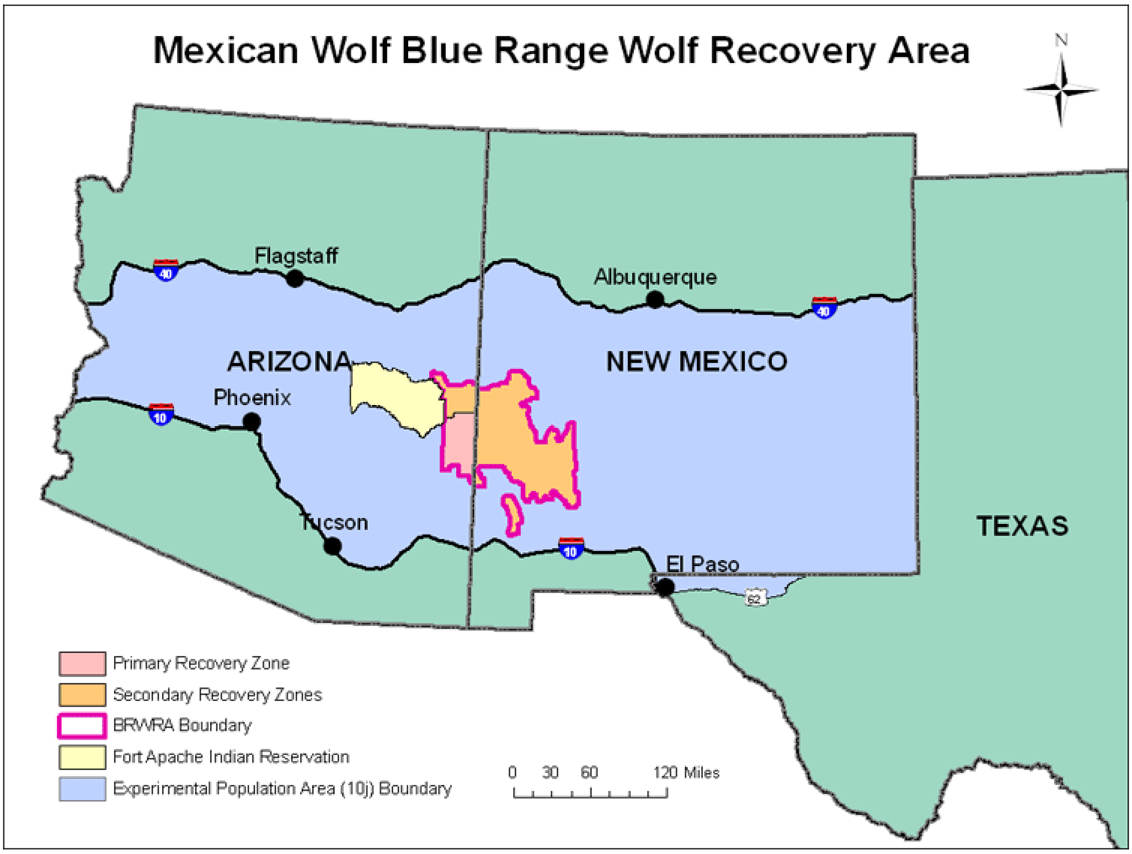


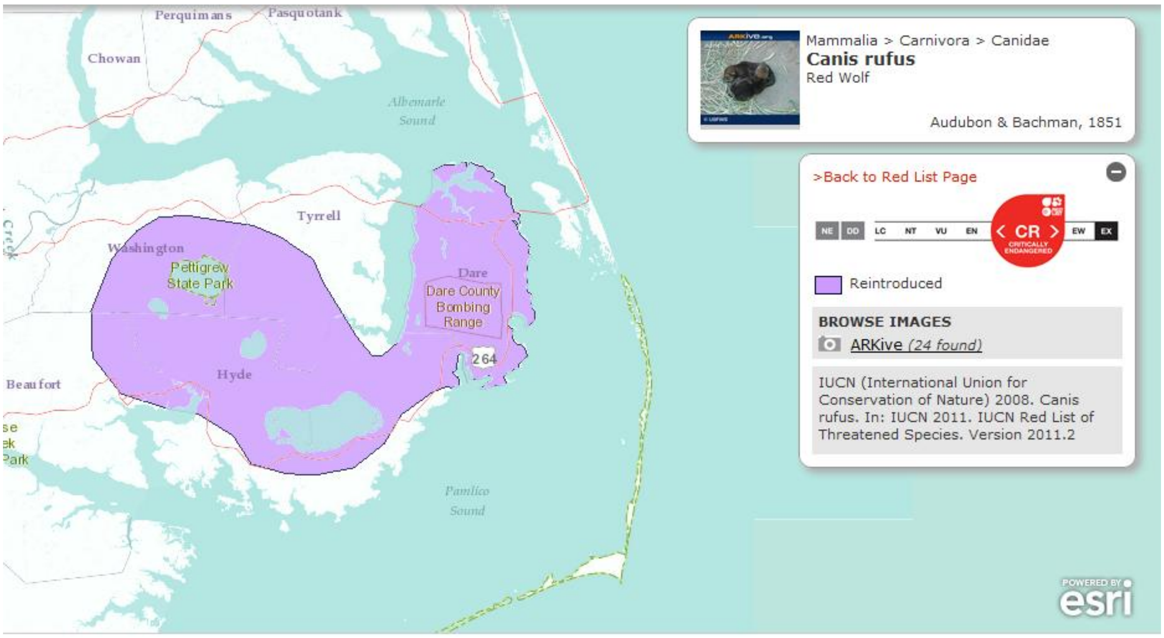
Figure 9.6 Current distribution of Mexican wolf

Source: U.S. Fish and Wildlife Service Mexican Wolf Recovery Program website downloaded 11/06/12. http://www.fws.gov/southwest/es/mexicanwolf/BRWRP_map.cfm

9.11 Red wolf (*Canis lupus rufus*)

The average red wolf weighs 23-28kg and is 135-165cm in length, notably smaller than other North American subspecies (Paradiso and Nowak 1972). They feed primarily on white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*) and marsh rabbits (*Sylvilagus palustris*) (Philips *et al.* 2003). A typical pack is five to eight individuals with offspring dispersing at one to three years of age (Karlin and Chadwick 2012).

Due to human persecution, the red wolf was almost completely extirpated during the 20th century, falling to an entire population of less than 100 individuals confined to a small area of coastal Texas and Louisiana in the 1970 s (Bohling and Waits 2011). Fourteen captured animals formed the base of the captive-breeding programme (Bohling and Waits 2011). Since 1987, red wolves have been released into north-eastern North Carolina (Figure 9.7) and in 1991 they were also released into Great Smoky Mountains National Park, Tennessee (Hedrick and Fredrickson 2008). Their numbers are slowly increasing but they are still listed as critically endangered by the IUCN (Bohling and Waits 2011).



Mammalia > Carnivora > Canidae
Canis rufus
Red Wolf
Audubon & Bachman, 1851

>Back to Red List Page

▨ Reintroduced

BROWSE IMAGES
ARKive *(24 found)*

IUCN (International Union for Conservation of Nature) 2008. Canis rufus. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2

Figure 9.7 Distribution of Red wolf (*Canis lupus rufus*) in North Carolina

Source: IUCN Red List website downloaded 11/06/12 http://maps.iucnredlist.org/map.html?id=3747

9.12 Grey wolves in Asia

Table 9.3 is taken from Boitani (2003) who used published figures and educated estimates provided by fellow members of the IUCN / SSC Wolf Specialist Group to compile the numbers. It remains the best estimate of wolf distribution across Asia.

Table 9.3 Status, Population and Trend of Grey Wolves in Asia (Boitani 2003)

Country	No. of wolves	Trend	Legal status	Hunted?
Syria	200?	?	Not protected	Yes
Lebanon	<50	?	Not protected	Yes
Israel	150	Stable	Protected	Yes
Jordan	200?	>	Not protected	Yes
Egypt (Sinai)	<50	Stable	Not protected	Yes
Saudi Arabia	300-600	Stable	Not protected	Yes
India	1, 000	Decr.	Not protected	Yes
China – Cheiludijang	599?	Decr.	Not protected	Yes
China – Xinjiang	10, 000	Decr.	Not protected	Yes
China – Tibet	2, 000	Decr.	Not protected	Yes
Mongolia	10-20, 000	Stable?	Not protected	Yes
Russia	25-30, 000	Increasing / stable	Not protected	Yes
Kazakhstan	30, 000	Stable	Not protected	Yes
Turkmenistan	1, 000	Stable	Not protected	Yes
Uzbekistan	2, 000	Stable	Not protected	Yes
Kirgizstan	4, 000	Stable	Not protected	Yes
Tadjikistan	3, 000	Stable	Not protected	Yes

Source: ‘Wolf Conservation and Reproduction, p.323, Table 13.1’ (Boitani 2003)

9.13 Tibetan wolf (*Canis lupus chanco*)

The Tibetan or ‘woolly’ wolf (*Canis lupus chanco*) is distributed throughout central Asia, Tibet, northern Mongolia, north China and the Himalayas (Figure **9.8**) (Srivastav and Nigam 2009). Sharma *et al.* (2004) suggest that it may even be a completely separate species of wolf to the wolf-dog clades. It measures 89-100cm long and around 25-30kg in weight (Srivastav and Nigam 2009). Like many other wolf subspecies, Tibetan wolf packs comprise two to twenty individuals, with a typical pack size of eight (Srivastav and Nigam 2009). The Tibetan wolf was not included in the analysis of individual identity as only a single captive individual from the subspecies has been recorded but can be considered for the pack and subspecies analysis.

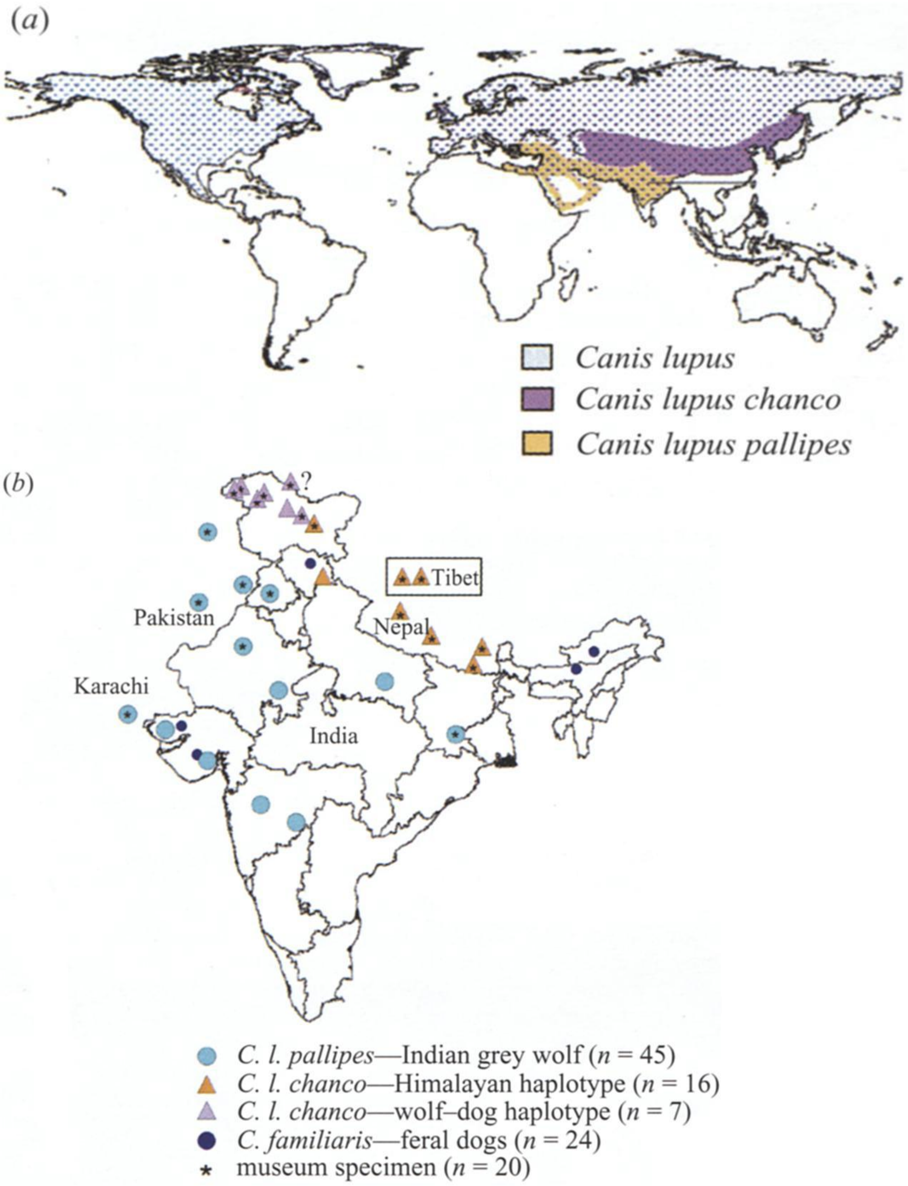


Figure 9.8 Wolf distribution in the Northern Hemisphere showing historical distributions of *Canis lupus*, and the subspecies Indian wolf *C. l. pallipes* and Tibetan woolly wolf *C. l. chanco*

“(a) Map of wolf distribution in the Northern Hemisphere showing historical distributions of *Canis lupus*, and the subspecies *C. 1. pallipes* and *C. 1. chanco*. (b) Map of the Indian subcontinent with study sampling localities indicated for wolves and dogs. No further locality data beyond country were available for two Tibet (in box) and one Nepal sample (below box). The question mark refers to a USNM sample that was of uncertain origin; field notes suggest it was collected in Ladakh, Kashmir.”

Source: Figure 1 from Sharma *et al.* 2011 (Sharma *et al.* 2004)

Appendix 4 Sources of Howls

1262 howls from 217 recordings were used in the database. This included howls from eleven subspecies. Table 10.1 lists all of the sources of recordings.

Table 10.1 List of all sources for howls used in Analyses 10 and 11, Chapter 3.

Subspecies	**Source**	**No. of howls**
Arctic	BBC Frozen Planet DVD	12
	Macaulay Sound Archive	1
	Personal recording	11
	Tigress Productions ‘In the Wild’ DVD	11
Eastern	Borror Laboratory	287
	British Library Sound Archive	111
	Fred Harrington (via PBS)	3
	‘Language & Music of Wolves’ CD	6
	Macaulay Sound Archive	186
	Wolf Park CD	162
European	Claudia Capitani	6
	‘The Voices of Wolves, Jackals and Dogs’ CD	17
	Macaulay Sound Archive	88
	Personal recording	46
	Museum für Naturkind Tierstimmen Archiv	13
	Wild Sweden (pers. rec.)	12
	Yorgos Iliopoulos (pers. rec.)	13
Great Lakes	Christine Anhalt (pers. rec.)	9
Great Plains	Tigress Productions ‘In the Wild’ DVD	7
Iberian	Vicente Palacios (pers. rec.)	25
Mackenzie Valley	Macaulay Sound Archive	9
	Personal recording	87

	Museum für Naturkind Tierstimmen Archiv	38
Mexican	Borror Laboratory	39
	John Theberge (pers. rec.)	3
North African	‘The Voices of Wolves, Jackals and Dogs’ CD	10
	Macaulay Sound Archive	1
	Museum für Naturkind Tierstimmen Archiv	33
Red	‘The Voices of Wolves, Jackals and Dogs’ CD	7
Tibetan	Macaulay Sound Archive	9