EXPLORATION OF THE ACOUSTIC STRUCTURE AND CONTEXTUAL OCCURRENCE OF AFFILIATIVE VOCALISATIONS IN RED WOLVES (CANIS RUFUS)

JENNIFER NICOLE SCHNEIDER

EXPLORATION OF THE ACOUSTIC STRUCTURE AND CONTEXTUAL OCCURRENCE OF AFFILIATIVE VOCALISATIONS IN RED WOLVES (*CANIS RUFUS*)

by

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Abstract

The purpose of this study was to determine the structure and nature of tonal vocalisations produced by red wolves. Video recordings of captive wolves from a breeding facility were analysed. Vocalisations were composed of 1-30 sound units. Linear units included squeaks (2600-9600 Hz) and wuhs (100-1600 Hz); non-linear units accounted for 22% of sounds and included between-type frequency jumps, harmonic and pure-tone biphonations, squeaks with sidebands, and squeak jumps. Five tonal vocalisation types were identified based on unit composition: squeaks 48.4%, wuhs 19.3%, and three mixed vocalisations 21.9%. Vocalisations occurred primarily during affiliative interactions and were relationally directed: squeaks were the most common vocalisation in individual activities and were distributed across relational states; wuhs were common in interactions, and directed toward pen-mates; and mixed vocalisations occurred at moderate rates across activity states, and were relationally directed. Tonal vocalisations were common, affiliative in nature, and differed in contextual occurrence by type.

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Chapter 1 Overview and Introduction

Overview

The purpose of this thesis is to examine the structure and contextual occurrence of red wolf (*Canis rufus*) affiliative vocalisations, and in so doing, to contribute to our knowledge base in two areas: red wolf life history and canid vocal communication. Although Bartram first described red wolves in 1791, little information was available about their ecology and behaviour until the mid-1900s (Phillips *et al.*, 2003). By that point the population was declining and focus quickly turned from field investigation to wildlife management, when the U.S. Fish and Wildlife Service began efforts to save the red wolf. However, by the late 1970s it was clear that extinction was imminent. U.S. Fish and Wildlife officials responded by establishing a captive-breeding program. In 1987, the first red wolves were reintroduced to Alligator River National Wildlife Refuge, North Carolina. Through careful management this program became the first to re-establish a carnivore considered extinct in its former range. Red wolf recovery is heralded as a conservation success story.

While the red wolves have been re-established in northeastern North Carolina, many of the threats that caused their extinction in the wild still exist. Knowledge about these animals is necessary for maintaining their well-being in the wild, and preventing further harm from humans. Red wolves have a number of physical and social needs. As members of a pack, they must cooperate to maintain territories, obtain food, rear young, and live amiably. Close-range, tonal vocalisations are associated with affiliative interactions between pack members, and therefore, contribute to the social bond between animals. Understanding the social bond can help managers make decisions that will enhance the well-being of the wolves.

The study of red wolf affiliative vocalisations also contributes to our understanding of canid communication. Recent studies reveal that many mammal vocalisations, including those of canids, contain non-linear sounds, sounds that are produced by means other than synchronous vocal fold vibrations. Inspection of published spectrograms of gray wolves (*Canis lupus*) (Harrington and Mech, 1978; Schassburger, 1993) and red wolf affiliative sounds (Schneider and Mace, 2005) reveal a biphonic structure: high-frequency and low-frequency vocalisations that occur separately or simultaneously. Similar biphonic structure has been documented in dhole (*Cuon alpinus*) whistles (Volodin and Volodina, 2002).

Dhole whistles differ in structure and function from what is known about wolf affiliative vocalisations. Gray wolf squeaking vocalisations, the only affiliative wolf vocalisation studied in detail, are composed of 1-44 individual squeak units arranged in 1-5 phrases. These units vary in frequency contour within a phrase, between wolves, and between social contexts (Weir, 1999). In contrast, dhole whistles demonstrate minimal variation within a phrase and are used primarily when hunting. Investigation of red wolf affiliative vocalisations extends our knowledge of canid communication by defining the structure and proportion of non-linear sounds produced by red wolves and examining the contexts in which these sounds are produced.

In the remainder of Chapter 1, I review the literature concerning red wolves in general and communication in particular. The red wolf review highlights important conservation issues, as well as life history information. Red wolves live in packs, therefore all aspects of their life history are relevant to an understanding of their communication. Their physical appearance shapes their visual communication; their habitat and food types influence their individual, as well as group behaviour; and their life cycle influences pack structure and the duration of their social bonds. The communication review discusses red wolf vocal communication in relation to general communication, vocalisations of other canids, and particularly vocalisations of gray wolves. Since vocalisations do not occur independently, a brief overview of the visual and behavioural cues used for interpreting social context is also included.

In Chapters 2 and 3, I report on the research of this thesis. Chapter 2 contains the analysis of the acoustic characteristics of red wolf affiliative vocalisations, including descriptions of the sounds, proportions of non-linearities, and individual differences in sound production. In Chapter 3 the contexts of the affiliative vocalisations, including vocalisation rates for each activity and relational state, contextual differences in production of different vocalisation types and rates of affiliative vocalisation in relation to other vocalisations are assessed. In Chapter 4, I draw some general conclusions and suggestions for further research.

The Red Wolf

Historical Range and Recent History

Prior to European settlement of North America, red wolves inhabited most of the eastern and southeastern United States. Their historical range spanned from the Atlantic Ocean to central Texas, and from the Gulf of Mexico through forested regions as far north as Maine (Riley and McBride, 1972; Carley, 1979; Nowak, 2002). This range shrank rapidly as settlers entered the area. In the early 1800s, three subspecies of red wolf were abundant in North America: *C. r. floridanus*, east of the Mississippi River; *C. r. gregoryi*, west of the Mississippi Valley; and *C .r. rufus* in the west. By the mid-1970s, two subspecies were extinct and *C. r. gregoryi,* whose range was limited to parts of Texas and Louisiana, was rapidly declining (Carley, 1979).

Several factors contributed to the demise of red wolves. Increases in human populations during the 1800s led to deforestation, effectively altering red wolf habitat. Changes in land use also led to conflict between wolves and humans. Predator control programs were put into effect during the first half of the twentieth century, killing thousands of wolves of all species (Gipson, 1972). These human intrusions, in combination with parasites such as heartworm, hookworm, tapeworm and mange mite, greatly reduced the number of all subspecies of red wolves (McCarley and Carley, 1978).

Land alterations that destroyed red wolf habitat proved to be beneficial for coyotes (*Canis latrans*). These medium-sized canids were once limited to western North America, but adapted well to human environments, expanding their range eastward in the 1940s. As the red wolf population decreased, it fragmented, thereby decreasing mate availability. Coyotes moved into vacated areas, and red wolves started hybridizing with coyotes. These hybrids began to dominate the red wolf population, furthering its decline (Carley, 1979; McCarley and Carley, 1978).

On March 11, 1967 red wolves were listed as a federally endangered species under the Endangered Species Preservation Act of 1966, and a limited Red Wolf

Recovery Program was initiated. This program was given priority with the passing of the Endangered Species Act in 1973 (Public Law 93-205; 87 Stat. 884). By late 1975, the U.S. Fish and Wildlife Service (USFWS) determined that it was no longer possible to maintain a pure red wolf population in the wild due to extensive hybridization. To prevent the species from going extinct, the remaining red wolves were captured and placed in captivity (Carley, 1979).

Taxomony

The origin of the red wolf has become a contentious issue, especially when considering their conservation. Since 1991, doubt has been raised concerning the validity of the red wolf as a species. Because hybrids are rarely protected by the Endangered Species Act, a summary of the relevant taxonomic issues is in order.

Three species of *Canis* occur in North America, gray wolf or timber wolf (*C. lupus*), red wolf (*C. rufus*), and coyote (*C. latrans*) (Nowak, 2003). There is some contention as to whether the red wolf should be classified as a separate species, because red wolves are intermediate in form to gray wolves and coyotes. Continued debate over wolf taxonomies has relied on both morphological and genetic evidence and has led to a variety of proposed relationships, from a shared common ancestor to hybridization to a North American canid line.

The common ancestor view is that red and gray wolves share a coyote-like ancestor (Nowak, 1992), and are indeed separate species. In this view, the red wolf is descended from an intermediate between this ancestor and the modern gray wolf. Support for this taxonomic view comes from morphological evidence such as a clear distinction between the skulls of the three species, and fossil evidence in North America showing little or no change in the form of the red wolf since the Rancholabrean age (Nowak, 1992). Besides morphological distinction, red wolves also have unique ecology and behavior (Phillips and Henry, 1992).

A hybrid view and a crossbreed view stem from the proposal that red wolves are hybrids of gray wolves and coyotes and are supported by molecular comparisons between the three canids. Roy *et al.* (1994) demonstrated that red wolf mitochondrial DNA is similar to that of gray wolf populations that have hybridized with coyotes and different than that of non-hybridizing gray wolf populations and coyote populations. The proposed hybrid origin of the red wolf raises doubts about its identity as a separate species (Brownlow, 1996). If the red wolf has a historical hybrid origin, then it is a subspecies of the gray wolf (Nowak, 1992). However if the red wolf has continually hybridized with the coyote and gray wolf, than it is a crossbreed, not even a distinct subspecies (Brownlow, 1996).

An Eurasian-North American divergence view grows out of further study of mtDNA between coyotes, red wolves, and different subspecies of gray wolf. Wilson *et al.* (2000) found that the similarities between the hybridizing gray wolves and red wolves did not correspond with the coyote portion of the genome, making a hybrid origin unlikely. Instead, they proposed that the eastern Canadian wolf (*C. l. lycaon*) (e.g. Algonquin wolf) is not a subspecies of gray wolf, but either a fourth distinct species or a subspecies of red wolf. The differences in mtDNA sequences among canids suggest a divergence of eastern Canadian wolves and red wolves from coyotes 150,000-300,000 years ago and a divergence from a Eurasian ancestor of gray wolves 1-2 million years ago (Wilson *et al.*, 2000). Examination of the histocompatibility complex, a genetic system for infectious disease resistance, also revealed that red wolf alleles are more similar to coyotes alleles than to gray wolf alleles (Hedrick *et al.*, 2002). In this proposed taxonomy coyotes, red wolves, and eastern Canadian wolves developed in North America, while gray wolves developed in Eurasia.

The proposed taxonomic classification of the eastern Canadian wolf as a red wolf is in dispute. In addition to the study by Wilson *et al.* (2000), a DNA study of eastern wolves from historically coyote-free areas also indicates a genetic divergence from gray wolves (Wilson *et al.*, 2003). In contrast, morphological study reveals that eastern Canadian wolves are intermediate to red wolves and western gray wolves, indicating the possibility of historical hybridization (Nowak, 2002).

These debates expanded beyond scientific discussion, resulting in petitions to delist the red wolf from ESA protection. Public awareness of the debate led to a movement by organisations opposed to wolf restoration to stop the Red Wolf Recovery Program. All proposals to abort the program were rejected. Red wolves are currently being treated as a separate species by the Endangered Species Act, and will be treated as such in this study.

The Red Wolf Recovery Program

The Red Wolf Recovery Program has two components: captive and wild populations. In 1973 the U.S. Fish and Wildlife Service (USFWS) in collaboration with Point Defiance Zoo and Aquarium in Tacoma, Washington established the Red Wolf

Captive Breeding Program. The goals of the breeding program were to separate pure red wolves from hybrids, and through breeding, increase the number of pure red wolves in captivity. Initially, animals were carefully selected based on the best morphological and taxonomic criteria available to distinguish hybrids from pure red wolves. The captive-breeding process provided final proof of the genetic integrity of the red wolves, resulting in 14 animals becoming the founding stock of today's red wolf population (USFWS, 1989). The long-term goal of this program is to maintain the gene pool for reestablishment of populations in the wild, and for distribution of individuals to approved zoos (Carley, 1979; Waddell, 1996).

Since 1984 the breeding program has been managed under the American Zoo and Aquarium Association's Species Survival Plan® (Waddell, 1996). The population grew steadily from the early 1970s, until experiencing a slight decline in the mid-1990s. As of June 2003, 167 red wolves resided in 37 facilities across North America. Due to the small founding population, managers form breeding pairs to minimise inbreeding depression, maximise founder representation and gene diversity, and increase total population size. Management techniques include artificial insemination and fostering of pups by non-birth mothers (Long and Waddell, 2003).

The second part of the Red Wolf Recovery Program involves the establishment of stable wild red wolf populations. Minimum recovery goals include establishing 220 red wolves in three mainland locations (Phillips *et al.*, 2003). To date wildlife officials have attempted to establish populations in two locations, one successfully and the other unsuccessfully.

According to the USFWS (n.d.) the first red wolves were released into Alligator River National Wildlife Refuge, North Carolina on September 14, 1987. During the first five years of the program, 42 wolves were released into the wild (19 adults, 1 yearling, and 22 pups) on 15 occasions. Initial death rates were high, 17 deaths in five years. An affinity for people led to many recaptures. During the same time period, 11 adults produced 8 litters totalling 22 births. Wild born wolves showed less affinity toward people than did their captive-raised parents. Successful rearing and dispersal of young demonstrated that the red wolves were capable of surviving in the wild. By fall 1992, the population was predominately wild born and growing.

The Alligator River National Wildlife Refuge population was a numerical and management success. Wildlife officials were able to manage problems with minimal injury to animals, without changing established land use practices, and with the backing of local hunters and trappers. Since the implementation of the program, the red wolf recovery area has been extended to include Pocosin Lakes and Mattamuskeet National Wildlife Refuges, as well as private lands in five counties in North Carolina (Dare, Hyde, Tyrrell, Washington, and Beaufort). Currently over 100 wild wolves inhabit 1.5 million acres of northeastern North Carolina.

After successfully establishing a wild wolf population in North Carolina, the USFWS initiated reintroduction efforts in Great Smoky Mountains National Park in 1991. Initially biologists released a family of two adults and two pups into the Great Smoky Mountains National Park in Tennessee for a one-year trial to monitor both the wolves' potential for survival and the public's reaction to the wolf project. Initial results were positive and two families of wolves were released to inhabit the park in 1992 and 1993 respectively (Baron, 2002).

Although both packs produced pups in 1993, seven of the 16 wolves had died by the end of the year. Both packs continued to struggle for several years. One pack failed to establish a home range within the park and had to be repeatedly removed from private lands. Pup survival was low in both groups and wolves were malnourished and susceptible to disease, probably due to a lack of food availability. The USFWS cancelled the recovery program in October 1998, relocating the remaining wolves to Alligator River, North Carolina (Baron, 2002).

Although the program failed to re-establish red wolves in Tennessee, it was successful in educating the public about the plight of the red wolf. Most importantly, it provided researchers and management personnel with valuable data on habitat use, movements, disease, and behaviour in red wolves as well as practical experience in managing wolves in areas with high human and livestock use (Baron, 2002).

Management issues that face wildlife officials concerning wild red wolf populations are biological and social-political. From a biological perspective, increasing the wolf population through natural breeding and population supplementation are essential for managing inbreeding in wolves and hybridization with coyotes. Introducing captive wolves into the population must be done periodically to increase genetic diversity. The small population of wolves in northeastern North Carolina is susceptible to stochastic events, such as disease and parasite outbreaks, as well as natural disasters, such as hurricanes. Although currently thriving, intensive management is required to maintain the population.

Wolves used to supplement the wild population come from two primary sources: island propagation sites and captive born pups. The recovery program currently includes two island propagation sites. St. Vincent National Wildlife Refuge in northwest Florida and Bulls Island, part of South Carolina's Cape Romaine National Wildlife Refuge, are currently being used as an intermediate step between zoo facilities and reintroduction on the mainland. Each island is capable of supporting a pack of wolves, allowing animals to learn necessary survival skills without the pressure of intra- and inter-specific competition and human land development. Captive born pups have been fostered in small wild wolf litters with pups of similar age. The red wolves willingness to foster pups from other litters has aided the captive breeding program and has potential for further use in the reintroduction program (USFWS, 2004).

The most important challenge wildlife personnel face is hybrid management. At a red wolf conference in April 1999, experts determined that hybridization with coyotes was a serious threat to the wild wolf population in North Carolina (Phillips *et al*., 2003). Eleven out of 64 wild-born litters were hybrids. A model of the population revealed that if these animals had not been removed, the red wolf population would be unrecognisable in 3-6 generations. An adaptive management plan was developed to protect the wild population northeastern North Carolina and to gain an understanding of the hybridization process.

This plan continues to rely on a large range of experts in biology, ecology, and behaviour from numerous organisations and universities. Key research includes means of identifying wolves, hybrids, and coyotes; the study of habitat use; and the monitoring of wolf-coyote interactions. Since little was known about hybridization, the management plan for northeastern North Carolina wolves was designed to be flexible, easily adapted to changes in the situation and knowledge base (Phillips *et al.*, 2003). The main steps taken over the last few years as reported in Red Wolf News (USFWS, 1999; 2000b, 2002) include:

1999	Radio collaring of known breeding pairs/families prior to the new breeding season
	Identification of new breeders and their mates (wolf, hybrid, or coyote)
2000	Capture and removal of coyotes
	Establishment of a coyote-free zone comprising 20% of the recovery area
2001	Establishment of three zones:
	Zone 1 coyote and hybrid free (peninsula)
	Zone 2 all hybrids sterilized
	Zone 3 minimal hybridization on edges
2002	Expansion of zones 1 and 2
	Removal of sterile hybrids and replacement with island-born wolves

Miller *et al.* (2003) developed a pedigree-based assignment test using DNA for identifying hybrids in the red wolf population. These tests are most accurate for first and second generation hybrids, highlighting the importance of early detection. Adams *et al.* (2003) developed a non-invasive method for identifying individuals and detecting the presence of coyotes and hybrids in the three zones. They collected scat samples from throughout the refuge, marking the position of the scats using global positioning system (GPS) co-ordinates. Using faecal mitochondrial DNA sequence analysis, they were able to identify individuals with coyote maternity and locate those individuals based on where the scat was collected. This method is more accurate than morphological observations, can be used in a wide variety of contexts, and poses no danger to the animals.

All plans for future mainland release sites have been put on hold pending the outcome of the hybrid management plan in northeastern North Carolina. Genetic integrity must be maintainable for reintroduction efforts to continue. Only one hybrid litter was born on the edge of Zone 3 in 2002, indicating early success of current management techniques. To be self-sustaining, red wolves must also be able to compete with and displace other canid species. Preliminary observations indicate that wolves can displace coyotes (USFWS, 2002). Further documentation is required before major management decisions can be made.

Morphology/Physical Description

Red wolves are intermediate between coyotes and gray wolves in size. Red wolves weigh less than gray wolves, but more then coyotes; red wolves weigh between 18-36 kg, with most individuals in the 23-30 kg range. They are taller than coyotes, and are known for their long legs. Their legs average between 610-760 mm from toe to shoulder—almost the same as the gray wolf (Riley and McBride, 1972). Red wolves average a total length of 1403-1650 mm, a tail length of 343-420 mm, a hind foot length (including longest claw) of 210-254 mm, and condylobasal length of 187.5-233.7 mm (Hall and Kelson, 1959). All of these morphological features are intermediate in size between gray wolves and coyotes. As with other canids, females tend to weigh less and be shorter than the males, accounting for the wide range in sizes.

The shape of their facial features distinguishes red wolves from other canids. Red wolves have broader heads and muzzles than the coyotes, with wide nose pads. Red wolves have small heads than gray wolves, and their ears are tilted to the side rather than straight upright, giving their faces a unique triangular look. Red wolf ears are equal in length to those of the gray wolf, even though their heads are smaller (Riley and McBride, 1972).

Like all canids, red wolves display a variety of colorations. They can be gray, black, yellow, cinnamon, cinnamon-buff, or tawny. Typically the dorsal half of the wolf is overlaid in black, while their undersides are whitish to pinkish buff. Muzzles tend to be light-coloured, especially on the sides, and light or tan spots surround their almond shaped eyes. These colours vary with location, and change in climate and season (Riley and McBride, 1972; Carley, 1979).

Habitat and Home Range

Red wolves historically were abundant in forested regions, but by the mid-1900s as their range diminished, they were restricted to the coastal plains and marshes of Louisiana and Texas. The climate in these areas is primarily subtropical, with high humidity. The habitat consisted of heavy vegetation (Carley, 1979). Vegetation common in coastal plains and marshes includes various species of tall bunchgrasses, loblolly pine, oaks, magnolia, and sweet gum (Riley and McBride, 1972). Alligator River National Wildlife Refuge is largely made up of swamps and clearcuts (Phillips, 1993).

Reintroduced red wolves did not adapt well in the mountainous terrain of the Great Smoky Mountains National Park (Baron, 2002).

According to pre-reintroduction reports, the average home range of a red wolf was dependent on its sex and age. Reported home range of an adult male averaged 117 km^2, while the home range of an adult female was 65-78 km^2 (Riley and McBride, 1972; Carley, 1979). Individual and pack home ranges of three Alligator River packs average 88.5 ± 18.3 SD km^2 and 123.4 ± 53.5 SD km^2. Current pack home ranges vary with habitat type, the smallest home ranges occurring in prey rich agricultural areas (Phillips *et al.*, 2003).

Food Types

Red wolves are predators, but like other canids will feed on carrion. Food consumption data pre- and post-reintroduction differ. In the 1970s, the red wolves' main food source was rabbits (*Sylvilagus aquaticus* and *S. floridanus*) and nutria (*Myocastor coypus*). Their diet also included muskrat (*Ondatra zibethicus*), raccoon (*Procyon lotor*), fox squirrels (*Sciurus niger*), and various rat and bird species (Riley and McBride, 1972; Carley, 1979). Larger prey included feral pigs (*Sus scrofa*), white-tailed deer (*Odocoileus virginianus*), and young domestic calves when other food sources were not available or were difficult to find. Red wolves were not known for attacking adult cattle, unless the cattle were sick or injured; however, they attacked smaller barnyard animals such as hogs and poultry (Carley, 1979).

In contrast, scat studies of current wolves in northeastern North Carolina show that white-tailed deer made up 43% of the biomass consumed by wolves followed by racoons (31%), lagomorphs (13%), rodents (11%), and domestic ungulates (2%). Prey consumption differed by pack according to habitat. Packs in prey rich areas displayed resource partitioning, with younger wolves eating higher proportions of rodents (Phillips *et al.*, 2003).

Social Structure

Red wolf packs have similar social structure to gray wolf packs, although on average they are smaller in size (Phillips and Henry, 1992; Phillips, 1993). Red wolf packs range between 2-7 individuals (Riley and McBride, 1972; Carley, 1979), while gray wolf packs number between 2-42 individuals (reported mean pack sizes ranging from 3.4-9.4 wolves) (Mech and Boitani, 2003). Like gray wolf packs, red wolf packs are composed primarily of related individuals with a breeding pair, offspring of 1-2 years of age, and pups. All adults assist in pup-rearing and territory defence. Like gray wolves and unlike coyotes, red wolves are territorial with non-overlapping home ranges (Gier, 1975; Phillips and Henry, 1992).

The nature of the pair bond in red wolves is not fully understood. Red wolves tend to stay together in the wild year after year, even when translocated to a new area. However, in captivity wolves will quickly accept a new mate (Carley, 1979). This ambiguity is consistent with observations of gray wolf pack structure. While once thought to be strictly monogamous, observational data suggest that wolves will mate outside of their pair bonds if given the opportunity (Harrington *et al.*, 1982). When resources are abundant packs will sometimes produce two litters of pups; the paternity of the second litter in wild packs has not been determined (Mech and Boitani, 2003).

Additional breeding individuals occur in an estimated 6-8% of all gray wolf packs (Mech, 2000).

Likewise, what was once thought to be a strict dominance hierarchy in gray wolves is now thought to be a flexible web of relationships centred around the leading pair (Moran, 1987; Mech, 1999; Peterson *et al.*, 2002). Breeding status, relative body posture, and scent marking activities distinguish dominant animals from other pack members. The breeding pair demonstrates leadership of the pack. Throughout the year, this pair does the majority of the leading during pack travel and activity initiation. During the whelping season, the male leads in travel and hunting activities, while the female allocates time and energy to pup-rearing. Social structures and roles vary between packs and individuals (Peterson *et al.*, 2002). The breeding pair controls access to food, deciding who has feeding priority within the pack. This pack structure develops from natural divisions of age, sex, and reproductive structure and is rarely contested (Mech, 1999). Little is known of the social dynamics within a wild red wolf pack.

Wolf social structure and composition are dynamic, adapting to environmental and social constraints. For example, pack and litter sizes in many canid species, including wolves, increase and decrease with changes in prey availability (Geffen *et al.*, 1996). Large packs have been known to split territories and divide into smaller packs as their numbers increase (Mech and Boitani, 2003). Packs often replace a breeding vacancy with a dispersing wolf. For example, wildlife officials observed the acceptance of a dispersing neighbour into a pack in Yellowstone National Park after both adult males in the pack had been killed, despite the fact that the two packs had a prior history of fatal conflicts

(Stahler *et al.*, 2002). Young dispersing males have also been temporarily adopted into packs (Mech and Bointani, 2003) While broad generalisations can be made about pack structure, adaptability and individual variation make it impossible to apply strict rules.

Reproduction, Dispersal, and Mortality

Red wolves have a gestation period of 60-63 days. Breeding occurs in late January through March; pups are born between late March and May (Riley and McBride, 1972; Carley, 1979). Captive litter sizes range from 2 to 6 pups, but up to 12 pups have been seen in wild litters. Historically red wolves den in a number of different natural and artificial substrates. Dens can be found in hollow tree trunks, stream banks, and sand knolls. Artificial locations that have been adapted into dens include drainpipes, culverts, banks of irrigation ditches, and brush piles (Carley, 1979). Red wolves dig dens that average 2.4 m long and 1.0 m deep, with a 0.6-0.8 m entrance (Riley and McBride, 1972). Wolves that dig dens tend to return to the same den site in subsequent years, while wolves that use above ground nests do not (Phillips *et al.*, 2003).

As with gray wolves, both male and female red wolves have been observed dispersing from their natal home range when they reach sexual maturity. Male red wolves disperse at 27 ± 9 SD months and females at 23 ± 10 SD months (Phillips *et al.*, 2003), with the youngest dispersing at seven months after the death of a parent (USFWS, n.d.). Red wolves in northeastern North Carolina have been known to disperse up to 192 km from their natal habitat with averages of 36 ± 22 SD km for males and 45 ± 58 SD km for females. Dispersal occurs between September and March, most occurring between November and February (Phillips *et al.*, 2003). Post-dispersal home ranges of young red wolves tend to be larger than home ranges of adults. These individuals tend to stay in small areas for 7-10 days, move a few miles to a new area for another 7-10 days, and after several moves, usually return to their original area (Carley, 1979).

Wild red wolves live an average of 6-7 years, while captive wolves live up to 15 years. In comparison, wild gray wolves usually live 4-5 years, but can live much longer. Recorded causes of mortality (N) of wild red wolves in Alligator River National Park from the time of reintroduction through 1999 included natural causes (37) (parasites, disease, intra-specific strife, etc.), collisions with vehicles (28), unknown (20), suspicious and/or illegal killings (18), and legal killings (7) totalling approximately 112 deaths. In the same time period, 191 wolves were born in the wild (USFWS, 2000).

Communication

Before discussing wolf vocal communication, it is necessary to understand communication on a general level. Communication is a sharing of information between individuals. It requires a sender, a medium for conveying information, and a recipient. Anything in the environment that an individual can sense is informative (Smith, 1977). Therefore any attribute or behaviour of an organism that can be sensed is a potential medium for communication. Media of communication can dissipate quickly (e.g. vocalisations and facial expressions), or linger in the environment (e.g. scent marking and coat patterns). Hauser (1996) divides media of communication into three categories: signals, cues, and signs. Signals and cues are designed to convey information, while signs are designed for another purpose but convey information to unintended observers in certain situations (e.g. howling might alert prey to a wolf's presence). Signals are temporally constrained and have immediate energetic costs (e.g. warning vocalisations), while cues are permanent and have no immediate cost (e.g. coat pattern) (Hauser, 1996). The medium used should be appropriate to the type of information being exchanged. Wolf vocalisations are temporary, hence the information being conveyed have immediate relevance.

Information must be coded in the medium in such a way that it can be easily understood. Both stereotyped displays and continua provide systems for understanding the nature of the information being shared. Some animals use stereotyped signals or displays when communicating, with little variation in the message, therefore little ambiguity (Smith, 1977). Morton's (1977) motivation-structural model is an example of the second type of system, the graded continuum. According to this model, most short-range animal vocalisations fit on a continuum with tonal, affiliative sounds on one end and noisy, agonistic sounds on the other, providing a nearly universal system of interpretation. Wolf vocalisations fit into this continuum (Schassburger, 1993; Schneider and Mace, 2005).

Senders and recipients are also constrained. Most forms of communication entail an energetic cost for the sender, and therefore should be beneficial (Smith, 1977). For communication to be beneficial, recipients must be able to understand the information given to them and react predictably. Recipients must ascribe meaning to the information that they receive. The interpretation of the message is dependent on the individual recipient. Genetics, sex, age, location, season, time of day, past experiences, and familiarity with the sender influence how a recipient will interpret and act on information received from the sender. Effective communication necessitates the recipient's familiarity with the sender when variation within a communication medium extends beyond the fixed action patterns and universal continua. The differential use of various characteristics of vocalisations (e.g. individual variation in the timing or frequency of a vocalization) and other media allows for individual communication between pack members (Theberge and Falls, 1967). The meaning of the message for the sender is also dependent on temporal, environmental, and genetic factors. Communication is a complex and dynamic interaction among sender, medium, and recipient.

Just as there is a balance of common perceptions and individual experiences between sender and recipients, there is also a measure of both involvement and separation between the study animals and the observer. Behavioural categories created by observers are products of not only the actual behaviour of the study organism, but also the methods and criteria used by observers to define categories (Fentress, 1990). As human observers, it is difficult to determine if wolves classify vocalisations and other signals and cues by the same criterion as do the researchers. A variable, such as change in frequency, can be measured on a spectrogram, but may not be perceived by the wolf, may be perceived and ignored, or perceived and acted upon. Observers need to categorize behaviour to make sense of it. However, observers need to be ready to modify their behavioural categories as more is learned about the study organism.

Howling in Canids

Vocal communication can be divided into two categories: long-range and short range. Long-range vocalisations are used to communicate between animals that are typically not in visual range of each other. These sounds are high in amplitude and carry over great distances. The primary long-range vocalisation of the wolf is the howl.

Howls are the best known and most studied of the canid vocalisations. Wolves, coyotes, jackals, and dingoes are known for their howling. These canids demonstrate similar patterns of diurnal, lunar, and seasonal variation in howl production. On a diurnal cycle, howling peaks around or shortly after sunset with a secondary peak before or shortly after sunrise (Skead, 1973; Harrington and Mech, 1978; Laundre, 1981; Corbett, 1995; Gazzola *et al.*, 2002). Jaeger *et al.* (1996) noted an exception in the wild golden jackal (*Canis aureus*). In the breeding season, howling followed the typical late evening, early morning pattern. However, during denning, howling peaked in the middle of the night, possibly due to increased late night human activity in rural areas during spring.

Bender *et al.*, 1996 address the common myth that canids bay at the moon. They found that spontaneous howling in coyotes followed a lunar cycle, coyotes howled most during a new moon, when light levels are lowest. Although no such study has been conducted with wolves, the coyote data suggest that howling is optimal when visual communication is most limited, and therefore wolves should follow a similar howling pattern. Harrington and Asa (2003) proposed that canids lift their muzzles toward the sky when they howl to maximise the distance between the source of vocal production and the ground, increasing the range of distribution of the sound. The ground acts as a dampener for high-frequency sound waves produced less than 1 m above the surface (Marten and Marler, 1977).

Annual howling rates in response to simulated howls vary with changes in the social and reproductive status of canids, peaking during breeding and dispersal and declining during the denning period (Skead, 1973; Harrington and Mech, 1978; Laundre, 1981; Walsh and Inglis, 1989; Corbett, 1995; Gese and Ruff, 1998; Gazzola et al., 2002). Howling peaks during late winter, corresponding to the time of intense territoriality. Nikol'skii and Frommolt (1986) found that gray wolf howling peaked immediately before and after breeding, while aggressive vocalisations (i.e. growls, barks, yelps) dominated during rut. Howling peaks again during late summer and autumn, when pups are leaving the home site and becoming active members of the pack. Canids are least likely to howl during late spring and early summer when pups are in the den and most vulnerable to intruders. Breeding animals, which defend territories or den sites, are most likely to howl, while transient animals rarely, if ever, howl (Harrington and Mech, 1979; Gese and Ruff, 1998).

Seasonal approach responses to simulated howls differ by species. Corbett (1995) found that dingoes (*Canis lupus dingo*) approach howlers during the breeding season and flee during all other seasons. This response indicates dingoes may approach howlers when looking for mates. Jaeger *et al.* (1996) found that responses of wild golden jackals to simulated howls varied by type of response and season; approach responses declined during the breeding season and increased during denning, while howling responses increased during breeding and declined during denning. This seasonal difference in behaviour decreases the chances of an intruder learning the location of the den when pups are most vulnerable. These patterns are consistent with the assumed function that howling serves as a form of territory defence.

While howling behaviour is similar between species, the structure and types of howls differ allowing for species identification. Howls have been used to distinguish between gray wolf, red wolf, and coyotes in the wild (McCarley, 1978; Riley and McBride, 1972). Howls of New Guinea singing dogs (*Canis hallstromi*, Troughton 1957) are distinct from howls of dingoes, gray wolves, and coyotes (Koler-Matznick *et al.*, 2003). Canids produce a variety of howl types. For example, wild dingoes produce four types of howls with varying structure and function: plateau, inflexion, chorus, and bark-howls (Corbett, 1995). Red wolf howls include flat howls, barking howls, combination howls, yip howls, and choruses. Choruses are described as two or more wolves producing a combination of various howls in succession (McCarley, 1978).

Howls differ in structure between individuals and contexts. Gray wolf howls differ acoustically between individuals and may function as a means of individual identification during intra-specific communication (Tooze *et al.*, 1990). However, distance from the pack and chorus howling may mask not only individual identity, but also group numbers during inter-specific communication (Harrington, 1989). Adult gray wolf howls are distinguishable from pup howls (Harrington, 1986). Maximum fundamental frequencies are higher in spontaneous than stimulated gray wolf howls (Theberge and Falls, 1967). Similarly, analyses of red wolf howls suggest that there is a difference between provoked howls, those in response to stimuli presented by the researcher, and spontaneous howls (Riley and McBride, 1972). McCarly and Carly (1976) observed a continuum of sound types in a single howling session of a red wolf x coyote hybrid, demonstrating a high capacity of variability which could potentially be used for exchanging information.

Howling serves both inter-pack and intra-pack functions, aiding in maintaining distance between packs and group cohesion respectively (Harrington and Mech, 1978). The use of howling in territory defence can be seen in the spatial differential in howling rates and in the structure of howls. Coyotes howl more often along the border of their territory than in the core (Gese and Ruff, 1998). Similarly, dingo chorus howls are primarily given along territorial borders as a response to howling by other dingoes. Dingo bark-howls are used exclusively in alarm situations, often when pups are being threatened (Corbett, 1995). Gray wolf howls decrease in fundamental frequency when approaching simulated howls, possibly indicating an increase in aggression (Harrington, 1987).

Howling often differs structurally between inter- and intra-group communication. Differential responses of adult and pup coyotes to the playback of group howls and group yip-howls suggest that yip-howls are used primarily in territory identification, while group howls are used for location identification (Lehner, 1982). Dingo plateau howls are the primary howl type used in locating individuals and territory defence. Males use inflexion howls primarily during the breeding season, attracting mates or pack members (Corbett, 1995). Nikol'skii and Poyarkov (1979) found that an individual golden jackal's howls during spontaneous group howling were gradually modulated such that by the end of the howling session, frequency and time characteristics of the group were nearly synchronised. They suggest that mutual imitation of howls reinforces the group social bonds.

Short-Range Vocal Communication in Canids

The focus of this thesis is on the short-range vocalisations of wolves. Short-range vocalisations are used by animals in close proximity to one another and occur in a variety of social contexts. In general, canids produce a continuum of graded vocalisations ranging from high-frequency, tonal harmonic sounds to low-frequency harsh/noisy sounds. The complexity of the sounds on each end of the continuum reflects the species' social structure. Solitary species tend to produce a small number of stereotyped sounds, while social species produce a larger number of individually variable sounds (Fox, 1975; Brady, 1981; Robbins and McCreery, 2003). Wolves are among the more social canids. A brief overview of canid non-howling vocal communication follows.

South American Canids

South American canids provide a good example of how vocal repertoires reflect social structure. Bush dogs (*Speothos venaticus*), crab-eating foxes (*Cerdocyon thous*), and maned wolves (*Chrysocyon brachyurus*) have similar basic vocal repertoires, which vary in complexity relative to their in social structures. All three canids whine in affiliative contexts. Bush dogs, the most social of the three, produce the greatest variety of whines, increasing whine duration, number, and rate with increased level of arousal. Bush dogs and crab-eating foxes produce pulsed vocalisations and siren howls, respectively, when separated from familiar individuals. All three species produce growls and barks in agonistic situations. Maned wolves, a solitary species, produce the greatest variety of noisy agonistic vocalisations, including hums, screams, and roar-barks used in medium and long-distance communication to prevent hostile encounters. In contrast, Bush dogs, the most social of the three, produce the highest variety of tonal/affiliative vocalisations that are involved in forming and maintaining social bonds (Brady, 1981).

Foxes

Foxes are small canids that live in a variety of habitats and social organisations. As with other canids, fox vocal repertoires reflect aspects of their social organisation, such as territoriality. Newton-Fisher *et al.* (1993) examined the acoustic structure of vocalisations of the territorial red fox (*Vulpes vulpes*) and divided the repertoire into 20 distinct classes based primarily on frequency differences. Adult vocal classes include barks, yell barks, shrieks, whines, ratchet calls, staccato barks, wow-wow barks, yodel barks, growls, coughs, screams, and yell whines. Cub vocal classes include murmurs, warbles, whines, ratchet calls, two types of wow-wow barks, and two types of growls. Barks are the most varied and most common vocalisations. Like other territorial canids, the vocalisation rate peaked during winter—the dispersal and breeding season. In contrast, the non-territorial southern bat-eared foxes (*Otocyon m. megalotis*) produce mostly low amplitude affliliative sounds. They rarely use high-amplitude barks (Nel and Bester, 1983).

The potential for individual identification in serial barking has been noted in the arctic fox (*Alopex lagopus*) and swift fox (*Vulpes velox*). Frommolt *et al.* (1997) found that territorial barks of individual arctic foxes differed significantly in frequency, but not temporal variables. However, Kruchenkova *et al.* (2003) subsequently found that temporal variables (duration of barks and inter-bark intervals) differed by age and sex. Both temporal variables gradually increased as the series progressed. Adult barks were shorter in bark number and duration than yearling barks. Females produced the shortest barks and longest pauses, while yearlings produced the longest barks and shortest pauses. Darden *et al.* (2003) found that the mean cycle (time between the start of one bark and the start of the next), duty cycle (ratio of sound to silence in a series), and mean element centre frequency discriminated between the territorial bark series of individual swift foxes. Barking in both cases is used for establishing and maintaining territorial boundaries.

African Wild Dogs

African Wild Dogs (*Lycaon pictus*) are among the most social of the Canidae. As expected, they have a complex vocal repertoire. Robbins (2000) divided the vocal repertoire into 11 classes and 18 subclasses: twitter (social, spar, mob, attack), begging cry (gurgle), yelp/squeal (yelp, whistle, begging), whimper, whine, moan (full, buzz), rumble, growl (social, alarm), bark (alarm, threat, howl, attack, clear, yelp), hoo, and pack call. Vocal types in pups emerge at different ages. Unlike adults, pups produce purr sounds, often when resting or nursing (Robbins and McCreery, 2003). Most of the vocal classes correspond to classes observed in other canids. The twitter, a “bird-like” sound, is found only in African wild dogs and dholes (see also Koler-Matznick *et al.*, 2003 for similar vocalisations in New Guinea singing dogs). Begging cries are unique to African wild dogs. Unlike most canids, wild dogs do not howl and pack calls are rare (Robbins, 2000).

As with other social canid vocalisations, these sounds are not stereotyped. They are highly variable, occur successively, and sometimes are superimposed on one another

(Robbins, 2000). Subclasses of twitters are a result of biphonation, the simultaneous production of two harmonically unrelated sounds. Twitters are high-frequency whistles (3-16 kHz) that can occur with or without lower frequency cries (below 2 kHz) (Wilden *et al.*, 1998). Although the system as a whole is consistent with Morton's motivation-structural model, his model does not hold when examining variation between subclasses of twitters and barks. Robbins (2000) suggests that the low level of aggressive behaviour in the social system may account for these differences.

Asiatic Wild Dogs (Dholes)

Volodin *et al.* (2001) identify nine dhole vocalisations: howl, bark, cry, wail, weeping cry, whistle, blather, long blather, and whistle-blather (roughly translated from Russian by K. Smart, personal communication). The most remarkable are the whistles, which have given them the name the whistling hunters. These medium distance vocalisations are used to coordinate group hunting in thick vegetation where visual contact with pack members and prey may not be possible (Fox, 1984). The whistles of pack members differ in both frequency and temporal components, particularly fundamental and maximum frequency and cycle duration (time from start of one whistle to the start of the next), allowing for individual recognition of pack members (Durbin, 1998). Individual recognition may be very important if individuals play different roles in hunting.

Spectral analysis of dhole vocalisations reveals a biphonic structure. Dhole whistles are composed of two harmonically unrelated frequencies that occur in four variations: high component only, low component only, high component followed immediately by low component, and high and low components simultaneously. These components may serve separate functions. High-frequency sounds are easier to hear over background noise, may contain information on individual identity, and can be difficult to locate. Low-frequency sounds are easier to locate. Both would aid in the hunting process (Volodin and Volodina, 2002).

Dingoes

Corbett (1995) reports three major vocalisation in wild dingoes: howls (plateau, inflexion, chorus, and bark-howls), moans, and snuffs. Moans are described as a soft howl that is given when dingoes of different packs approach a common resource such as a watering howl. Snuffs, rapid exhalations of air, indicate mild alarm.

Coyotes

As their name implies—*Canis latrans* means barking dog—coyotes are vocal animals. Like vocalisations of other social canids, coyote vocalisations form a continuum of sounds and are not stereotyped. Lehner (1978) divided coyote vocalisations into 11 categories based on structural and contextual differences: growl, huff, woof, bark, bark-howl, whine, wow-oo-wow, yelp, lone howl, group howl, and group yip-howl. All 11 categories correspond to descriptions of gray wolf vocalisations; however none of them structurally resembles wolf squeaks (described in the following section).

Short Range Vocal Communication in Wolves

Much of the research on wolf vocalisations has been limited to descriptions based on field observations, leading to inconsistencies in nomenclature and classifications. The gray wolf literature identifies anywhere from four to eleven classes of vocalisations, the most common classes being howls, whimpers, growls, and barks. Using spectral analysis, Schassburger (1993) divided the latter two into snarls, growls, barks, and woofs. The term whimper has been used to describe all high-frequency, tonal vocalisations (Joslin 1967; Mech, 1970; Harrington and Mech, 1978) including a variety of whines, whimpers, squeaks, and yelps (Peters, 1980; Theberge and Falls, 1967; Schassburger, 1993). Squeaks have been identified as distinct vocalisations (Fentress, 1967; Fentress *et al.*, 1978; Field, 1979; Coscia *et al.*, 1991, Weir, 1999). Schassburger adds three additional categories: whine-moan, moan, and growl moan.

The few early studies of the vocal repertoire of red wolves have categorised the vocalisations as howls, choruses, barks, growls, and whimpers (Riley and McBride, 1972; Shaw, 1975; McCarley, 1978). As with gray wolves, the term whimpers refers to a number of high-frequency, tonal vocalisations. Schneider and Mace (submitted) subdivided high-frequency, tonal tone vocalisations into yelps (mid-frequency, high-amplitude), wuhs (low-frequency), squeaks (high-frequency), and transitional vocalisations.

There appear to be two main reasons for the variety of classifications among high-frequency, tonal wolf vocalisations. First, these vocalisations are detectable only at close proximity. High-frequency vocalisations do not propagate far and are generally of low amplitude. Because field encounters and audio recordings are rare, and descriptions are vague, it has been difficult to compare observations between researchers. All detailed studies of these vocalisations have been conducted with captive animals.

Second, wolf vocalisations are not stereotyped and can be highly variable within each category (Theberge and Falls, 1967; Fentress *et al.*, 1978; Schassburger, 1993). Field (1979) described the problem as looking through a microscope. At a low level of magnification there are four categories of vocalisations. At higher levels of magnification, one notices more and more differences. The variability of each class led Schassburger (1993) to examine relationships among classes. The eleven classes of vocalisations that he identified could be plotted on a continuum based on their fundamental frequencies. This continuum was consistent with Morton's (1977) motivation-structural model for short-range vocalisations.

Focusing closer, Field (1979) and Fentress *et al.* (1978) found that variability within classes is context-dependent. They reported that squeak structure varied between contexts more than it varied between individuals in a single context. For example, squeaks produced during howling sessions were longer in duration with greater frequency variation than squeaks produced in other contexts.

At yet a closer level of inspection, Schassburger (1993) identified gradations and transitions between categories, demonstrating the difficulty of identifying discrete classes. Examination of some transitional vocalisations reveals the simultaneous production of two vocal classes. These vocalisations contain biphonations, the simultaneous production of two distinct frequency contours, and other non-linear sounds (i.e. those exhibiting subharmonics, frequency jumps, and/or chaos) (Nikol'skii and Frommholt, 1989; Wilden *et al.*, 1998). Inconsistent nomenclature regarding non-linear phenomena (see Harrington and Mech, 1978; Schassburger, 1993) has added confusion to the classification process. To date, no study has examined the acoustical structure or possible function of biphonations and other non-linearities in wolf vocalisations, with the exception of Tooze *et al.* (1990) who used the presence of subharmonics (misidentified as two component harmonics) for individual identification of wolf howls.

Of the high-frequency, tonal vocalisations, only the gray wolf squeak has been studied beyond basic description. Squeaks occur in trains of 1-44 units separated into 1-5 phrases (Field, 1979; Weir, 1999). The fundamental frequency of squeak units ranges from 1805-5974 Hz, with varying contour. Squeaks are brief, lasting only 0.03-2.40 seconds (Weir, 1999). Cristler (1958) first described the "social" squeak as a "mouse squeak" uttered by hand-raised wild wolves when they had an unexpected encounter with their human pack members. Fentress's (1967) hand-raised captive wolf uttered "high squeaks" when greeting familiar people and new dogs.

Squeaks are uttered in a number of social situations, such as in greeting other wolves (and familiar people and dogs) after a time of separation (Fentress, 1967), when approaching other wolves at food, during play, prior to and during howling choruses (Weir, 1999), and in pup rearing. Squeaks are the most common vocalisation used in pup rearing (Goldman *et al.*, 1995) and are often uttered by an adult before entering the den. Pups begin squeaking as early as 15 days of age (Coscia *et al.*, 1991). Squeak structure and use differ between contexts and individuals (Weir, 1999).

The Context of Tonal Vocalisations

Wolves, like all pack-living animals, must be able to communicate in order to work cooperatively and live amiably. Both red wolves (*Canis rufus*) and gray wolves (*Canis lupus*) live in family groups that work together to maintain territories, hunt, and raise young. Pack life provides a number of situations where wolves can benefit from a vocal exchange of information. Vocalisations may serve a practical function in a specific context such as aiding wolves to maintain contact during hunting or to alert other wolves to the vocalising wolf's presence when approaching from behind. They can be used to call pups to return to or come out of the den. Vocalisations may be used to express a desire to interact. They may help distinguish between agonistic and play intent. Given the wide range of contexts in which vocal behaviour may occur, it is necessary to identify the social situations in which wolves vocalise to understand the function of the vocalisations.

During social interactions wolves use their whole bodies to communicate. They utilise elements of olfactory, tactile, visual, and auditory communication. These elements rarely occur in isolation and are always changing as the social situation changes. A thorough understanding of these social interactions must be holistic, looking at the fluid behaviour of the wolf and its context. A discussion of some of the visual and behavioural cues used in interpreting the social situation is in order.

Wolf Hearing

Little research has been done on the auditory system of wolves. It has been assumed that the auditory system of the wolf is similar to that of domestic dogs. Canids have a low-frequency threshold similar to humans and a high-frequency threshold (60-80 kHz) between that of humans and domestic cats. Dogs can detect a change in frequency of 8-10 Hz at around 1 kHz with decreasing discrimination at durations less than 100-200 msec. Humans can perceive a 3 Hz change at the same frequency level. Estimated peaks for best frequency detection in dogs vary between researchers and include 2, 4, and 8 kHz. Dogs have a spatial acuity of 4-8° while humans can localise sound within 1° of accuracy (Harrington and Asa, 2003).

Wolf Visual and Dynamic Communication

Wolves are expressive animals, using their faces, ears, tails, legs, body position, hackles, and so on, to communicate a variety of social messages such as social position, mood, intent, and degree of arousal. These expressions can be relative (e.g. the body posture of one wolf in relation to that of the group) or used in combination with movement/action patterns directed at one or more individuals. Expression and action patterns of wolves are complex and fluid, and should not be confused with fixed action patterns and displays reported for many insects and birds (Barlow, 1977). Interpretation of a given expression (e.g. high tail) should not be made in isolation of other expressions. Small changes in the combinations of expressions can communicate subtle changes in mood or intent. The fluidity and multivariate structure of this communication system produce a variety of combinations, gradations, and transitions allowing for a dynamic range of communication.

Schenkel (1947/1999) produced the foundational work on wolf expression and visual communication. Although his descriptions focus on the expressions of gray wolves, he observed both gray wolves and red wolves. Others have added to his observations. Following is a list of some of the key features of wolf postural and dynamic communication. This list in not meant to be comprehensive or interpretative, but rather to provide an overview, highlighting some areas that should be examined when interpreting wolf communication.

Schenkel (1947/1999) stated that the head was the most important area of communication in the wolf. The orientation of the head and gaze are essential aspects of social communication. Coat coloration patterns enhance the visual effect of facial expressions. Areas of the head that are particularly important are the mouth, forehead, and ears. The mouth region of the wolf is used for both visual and kinetic communication. In addition to opening the mouth to various degrees, the wolf can retract its lips to bare its teeth or pull back the corners of its mouth to grin. The wolf also uses its muzzle to make contact with other wolves on the head or the body, as well as to lick them. Pups use muzzle to muzzle contact when soliciting food, which probably develops into muzzle-related social contact in adults. Wolves have broad foreheads that allow for a range of expressions from fully contracting, or wrinkling the skin in a threat posture to stretching the skin flat as a show of insecurity. Connected to the forehead expressions are the shapes of the eyes, which change as the forehead is contracted or stretched. Fox (1970) noted that the amount of variation in the facial expression of wolves was greater than the more stereotyped expressions of solitary foxes. Wolves move their ears to express a number of subtle changes in moods. Ears can range from pointed upward to flat against the head. The openings can face forward, outward, or downward. Goldman *et al*. (1990) found that mother wolves often orient with only their ears in response to pup vocalizations.

The tail plays an important role in communication in the wolf pack. In social situations wolves express confidence or aggression by raising their tails above the relaxed position either at the base or end of the tail and insecurity by tucking their tails. Tail movement can also communicate intent, such as wagging the tail during play encounters. Schenkel (1947/1999) felt that the tail was the most dynamic of the wolves' postural attributes.

The guard hairs on the ridge of the backs of wolves are longer than other hairs and stand on end during periods of excitement, especially aggression. The position of the wolf's body relative to other wolves communicates a level of confidence. Havkin and Fentress (1985) examined the pitch of the wolf's body relative to the ground during social interaction to monitor changes in mood and intensity.

Social interactions are more than the sum of postures and expressions given during the encounter. These interactions are active, fluid, and flexible—reflecting a dynamic communication. This type of dynamic communication can involve elements of tactile communication and ritualised movements. Two important areas of tactile communication are anal-genital sniffing and licking and muzzle-muzzle contact (Schenkel, 1947/1999). The former also includes elements of olfactory communication. Ritualised movements are non-stereotyped behaviours that are used to communicate a mutually understood, unambiguous message. These behaviours can be key elements in understanding the nature of a social interaction. Examples of ritualised behaviours include the threat gape or the play bow (Moran, 1987). Many wolf behavioural patterns have been identified in a number of contexts. This type of dynamic communication can also be spontaneous and innovative.

When examining interactions between wolves it is important to realise that the behaviour of one wolf is dependent on the behaviour of the other. Moran (1987) points out that a wolf's position and movement with respect to the other wolf is more important to the interaction than the wolf's position with respect to its physical location. For example, if two wolves are circling they may stay in the same position relative to each other although they move over a large area of ground. Socially, their position has not changed. There are three areas to examine when looking at an interaction: the relative distance between wolves in wolf-lengths, the mutual orientation of the wolves along the main body axis, and the point of opposition or nearest point on each wolf to the other wolf (Havkin and Fentress, 1985; Moran 1987).

Wolf Vision

Wolf vision has not been studied in great deal, but differs from humans and probably from domestic dogs. Wolf vision is designed to function in low light levels. They have higher densities of rods in the centre of their retinas than humans and a tapetum lucidum on the lower part of their retina that reflects light inward. Together these physical characteristics increase the wolves' sensitivity to light, especially below the horizon. Canids have cones sensitive to blue and green, but are green-red colour-blind. Wolves have a wider lateral field than humans, but a smaller binocular field. This difference allows them to see more without shifting their gaze (a potential benefit for coordinating hunting), but limits their ability to focus on objects near their head. Bands of ganglion cells give them sharp vision, aiding their vision across the lateral field. While canids do not have the sharp spatial acuity of humans, dogs are more sensitive to movement and shades of gray. Dogs have better temporal acuity than humans (Harrington and Asa, 2003).

Summary

Red wolves, like gray wolves, are social animals that live in family groups and work together to defend territories, capture prey, and rear young. Social cooperation of this magnitude requires a bond between pack-mates. This bond stems from genetic relationships between most pack-mates, but must be reinforced by amiable social interactions between all pack members. Previous studies suggest that tonal vocalisations are produced during social interactions and may play a part in reinforcing the pack social bond.

Red wolves, like many canids, produce high frequency vocalizations. Preliminary examination of these and other tonal vocalizations show evidence of non-linear sounds, particularly biphonations. The production of non-linear sounds maybe under the direct control of the vocalising animal, and may have a social function in the life of the red wolf. This thesis is an exploratory study of the acoustical structure and behavioural contexts of tonal vocalisations of red wolves. Since red wolves are endangered, any insight into their ecology and behaviour has potential for aiding in conservation efforts.

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Chapter 2 Structure and frequency of occurrence of linear and non-linear sounds in red wolf (*Canis rufus*) tonal vocalisations

Introduction

A growing body of evidence suggests that animals produce non-linear sounds as a normal part of their vocal repertoire. When oscillators, such as the vocal folds, are synchronously coupled, they produce harmonic sounds that can be modelled linearly and are referred to as limit cycles. When coupling is not 1:1, non-linear modelling is required. Examination of sonograms of some tonal red wolf vocalizations reveals evidence of non-linear sounds (Schneider and Mace, 2005).

The vocal apparatus contains several paired oscillators. Studies on excised larynges demonstrate that all common types of non-linearities can be created by desynchronising the vocal folds (Ouaknine *et al.*, 2003). In addition, in vivo studies demonstrate desynchonisation of the vocal folds during the production of non-linear vocalisations in humans (Tigges *et al.*, 1997). Other anatomical oscillators that may contribute to the production of non-linearities include the vocal tract, arytenoid cartilages, ventricular folds, and epiglottis (Fitch *et al.*, 2002).

Paired oscillators can become desynchronised when the vocal apparatus is asymmetrical, each part having its own natural oscillating frequency. Causes of asymmetry in vocal folds include differences in size, tension, and structural abnormalities (Fitch *et al.*, 2002). Although all some degree of asymmetry is present in all vocal apparatuses, these paired oscillators normally influence each other, creating a natural vibratory frequency for the system that is different than that for either part, rather than non-linear sounds. The nervous system or physiological mechanisms such as vocal fold tension or subglottal pressure can control synchronisation of the oscillator system. These controls work together to produce harmonic sounds. Every system is limited by its physical composition and therefore every control parameter has a functional threshold. When any one of these thresholds is surpassed, non-linearities occur (Fitch *et al.*, 2002). Systems with greater asymmetry will have lower thresholds.

Non-linear sounds also occur when normal vocal fold vibration and a secondary sound source, such as a nasal or glottal whistle, produce sounds simultaneously. Some canid vocalisations are significantly higher in frequency than sounds typically produced by normal mammalian vocal fold vibrations. These vocalizations are often characterised by a lack of harmonics. A secondary production mechanism has been suggested for these vocalisations (Solomon *et al.*, 1995; Wilden *et al.*, 1998; Volodin and Volodina, 2002). Normal vocal fold oscillations result from the rapid, forced opening and closing of the vocal folds caused by air pressure from the lungs. This pulsing of the airflow is amplified by the resonant cavity (the larynx, pharynx, and mouth) (Sundberg, 1977). High frequency vibrations can also be caused by turbulence in the airflow. When air flows through a constricted space, such as the mouth of a bottle, vortexes are formed (Anagnostopoulos, 2002). When these vortexes are shed at a high rate, they produce acoustic vibrations (Howe, 2003). Vortex-shedding has been identified as a possible source of whistle vocalisations in canids. Researchers hypothesise that in the vocal apparatus flow-induced vibrations can be transferred to the vocal folds, causing high-frequency or "true" whistles. (Solomon *et al.*, 1995; Wilden *et al.*, 1998; Volodin and

Volodina, 2002).

Four major types of non-linear phenomena have been discussed in the literature: subharmonics, biphonations, chaos, and frequency jumps (Riede *et al.*, 1997; Wilden *et al.*, 1998; Fitch *et al.*, 2002). Transitions between types of cycles within a single vocalisation are called bifurcations.

Subharmonics: In spectral analysis, subharmonics appear as bands above or below the fundamental frequency and its corresponding harmonics at ratios of 2:1 or 3:1. Subharmonics occur when one oscillator is vibrating at two or three times the frequency of the other oscillator. The oscillators are still coupled, likely through contact with one another, but the period of oscillation of the whole system is doubled or tripled.

Biphonations: In spectral analysis, biphonations appear as nonparallel frequency bands that occur simultaneously or as parallel frequency bands that are not in integer ratios. Biphonations occur when oscillators vibrate independently of each other, producing two fundamental frequencies (f_0 and g_0). Sidebands, a series of parallel frequency bands, are often produced as by-products of biphonation, resulting from linear combinations of the two fundamental frequencies (e.g. g_0+f_0, g_0-f_0, g_0-2f_0).

Chaos: In spectral analysis, chaos appears similar to turbulent noise, but contains some evidence of periodic energy. Perceptually these sounds are harmonic with a harsh quality. Chaos occurs when two oscillators vibrate independently of each other and with irregular vibrations (multiple limit cycles).

Frequency jumps: In spectral analysis, a frequency jump appears as a sudden jump, a pause or a noisy section between two distinct fundamental frequencies within the same vocal unit. Frequency jumps occur when physiological constraints of the biological system cause a sudden change from one limit cycle to another. Vocal folds have multiple vibratory patterns or "regions" that are produced by a combination of elongation, tension, and subglottal pressure. Gradual adjustments of these factors cause sudden transitions from one vibratory pattern to another (Berry *et al.*, 1996). For example, gradual change in vocal fold tension, which alters membrane thickness, is responsible for the chest-falsetto transition in human voice (Švec *et al.*, 1999). Non-linear frequency jumps should not be confused with abrupt linear changes in frequency caused by gross changes in elongation, tension, or subglottal pressure.

Non-linear sounds are rare in human speech, but do occur during laughter, during specialised singing or ethnic practices, and in infant vocalisations—especially cries (Michelsson *et al.*, 1977; Robb and Saxman, 1988; Wilden, 1989; Bachorowski *et al.*, 2001). Non-linear vocalisations in humans, commonly referred to as irregular vocalisations, have also been associated with pathology in adults and infants (Michelsson, 1971; Michelsson *et al.*, 1977; Juntunen *et al.*, 1978; Robb and Saxman, 1988; Titze, 1994).

Non-linearities are common in non-human mammal vocalisations and have potential for adaptive significance (Wilden *et al.*, 1998: Fitch *et al.*, 2002). Non-linearities have been observed in vocalisations of Sykes's monkey (*Cercopithecus albogularis*) (Brown and Cannito, 1995), chacma baboons (*Papio cynephalus ursinus*) (Fischer *et al.*, 1999 cited in Riede et al., 1997), 30% of a rhesus macaque's (*Macaca mulatta)* calls (Fitch *et al.*, 2002), 3.5-45% of infant Japanese macaques' (*Macaca* *fuscata*) calls (Riede *et al.*, 1997), adult Japanese macaques (*Macaca fuscata*), domestic piglets (*Sus scrofa*) (Tokuda *et al.*, 2002), common dormice (*Muscardinus avellanarius*), pumas (*Puma concolor*), gorillas (*Gorilla gorilla*), marbled cats (*Pardofelis marmorata*) (Wilden *et al.*, 1998), domestic cats (*Felis catus*) (Riede and Stolle-Malorny, 1999), red deer (*Cervus elaphus bactrianus*) (Nikolskij, 1975 cited in Wilden *et al.*, 1998), pinnepeds (Tyack and Miller, 2002), and cetaceans (Killebrew *et al*., 2001). In most species, occurrences of non-linearities are highly variable between individuals and tend to be most frequent in younger animals.

All types of non-linearities have been observed among canid vocalizations. Tokuda *et al.* (2002) found that non-linearity in dog barks (*Canis lupus familiaris*) was correlated with a high harmonic to noise ratio, indicating that these phenomena are products of harmonic as opposed to noisy sound production. Riede *et al.* (2000) examined the vocal folds of a wolf-dog mix that produced all non-linearities except frequency jumps in her howls. Three out of four additional wolf-dogs produced non-linearities, but in smaller proportions (3-24%) and durations. The animal's vocal folds showed vocal lip structures not seen in other members of her pack, indicating that unique physiology can contribute to, but is not solely responsible for non-linear sound production. Whoops of spotted hyenas (*Crocuta crocuta*), used for medium to long distance communication, contain subharmonics (Wilden *et al.*, 1998). Wilden *et al.* (1998) pointed out non-linearities in the gray wolf literature: subharmonics have been used to discriminate individual gray wolf howls (*Canis lupus*) (Tooze *et al.*, 1990); howls contain frequency jumps (Harrington and Mech, 1978; Tooze *et al.*, 1990); and neonatal wolf vocalizations also show non-linearities (Coscia *et al.*, 1991).

Unlike most biphonations reported in non-canid mammals, which result from desynchronization of the vocal system, biphonations in high-frequency canid vocalizations possibly result from the simultaneous production of vocal fold vibrations and glottal whistles. These vocalizations are characterized by their whistle-like quality. African wild dogs (*Lycaon pictus*) produce a cry, a higher frequency twitter, and 60% of the time, a combination twitter-cry. The fundamental frequencies of cries (2 kHz) and twitters (3 kHz) are not related (Wilden *et al.*, 1998 Robbins, 2000). Dhole (*Cuan alpinus*) whistles exhibit biphonations with more extreme differences in f_0 and g_0, the two fundamentals differing by several kilohertz. These vocalizations can occur with only the high-fundamental, only the low-fundamental, with frequency jumps from high to low, or with both fundamentals simultaneously (Volodin and Volodina, 2002).

Nikol'skii and Frommolt (1989) first noticed that whistle-like vocalizations produced by gray wolves occur with or without secondary non-parallel frequency bands of lower frequency. Wilden *et al.* (1998) pointed out that the high-frequency components in gray wolf howls, whines and whimpers are often identified as harmonics or overtones as can be seen in spectrographs presented by Harrington and Mech (1978) and Schassburger (1993). Like dhole vocalizations, these sonograms of gray wolf affiliative vocalizations demonstrate a biphonic structure between lower and higher frequency short-range vocalizations. While the presence of biphonations in wolf vocalizations has been acknowledged (Wilden *et al.*, 1998), the extent of occurrence and possible significance of these nonlinear phenomena have not been studied.

Wolf vocalizations are not stereotyped, showing variation within a vocalization type, as well as gradations and transitions between types. Wolf vocalizations can be organized on a structural continuum ranging from tonal, high-frequency vocalizations on one end to harsh, low-frequency vocalizations on the other (Schassburger, 1993; Schneider and Mace, 2005). The tonal end of the continuum includes very high-frequency (3-9 kHz), pure-tone whistle-like sounds and high-frequency (0.3-2 kHz), harmonic sounds. The harsh end of the continuum includes low frequency (80-120 Hz) growls and barks. Although vocalizations on the tonal end of the continuum are primarily tonal in quality, they can contain harsh elements, while vocalizations on the harsh end are primarily harsh, but can contain tonal elements.

Of the tonal vocalisations produced by wolves, only the gray wolf squeak has been studied beyond basic description. Squeaks occur in trains of 1-44 units separated into 1-5 phrases (Field, 1979; Weir, 1999). The fundamental frequency of squeak units ranges from 1805-5974 Hz with varying contours. Squeaks are short, lasting only 0.03-2.40 seconds (Weir, 1999). Squeaks are uttered in a number of social situations such as in greeting other wolves (and familiar people and dogs) after a time of separation (Fentress, 1967), when approaching other wolves in a gathering area or at food, during play, prior to and during howling choruses (Weir, 1999), and in pup rearing (Goldman *et al.* 1995). Squeak structure and use differ between contexts and individuals (Weir, 1999).

In this study I examined the acoustic structure of tonal vocalisations produced by captive red wolves. While some information is available on gray wolf squeaks, little is known about the structure and function of red wolf tonal vocalization and how these compare with those of gray wolves. In addition, the red wolf repertoire contains both lower-frequency wuhs (130-890 Hz) and higher-frequency squeaks (4000-5500 Hz) with transitions and gradations between types (Schneider and Mace, 2005). Preliminary inspections of transitional vocalisations revealed a potential biphonic structure similar to that of the dhole whistle, making red wolf vocalizations ideal for the study of non-linear phenomena. The aim of this study was to gain further insight into the importance of non-linear vocal production in canid communication by: (1) describing the physical characteristics of the sounds produced by red wolves, (2) determining the frequency of occurrence of non-linear sounds in relation to linear sounds, and (3) examining the structural variation of linear and non-linear sounds within and between individual wolves to determine the potential for acoustic based individual identification..

Methods

Study Site

I carried out this study using individuals from the Red Wolf Captive Breeding Program housed at a breeding facility in Graham, WA associated with Point Defiance Zoo and Aquarium (PDZA). Red wolves at PDZA are housed in 18 enclosures, each enclosure measuring 465 or 929 m^2. Large enclosures are square, with access corridors between each enclosure so that wolves in adjacent enclosures do not have physical contact with each other. Small enclosures are half of a large enclosure with a chainlink fence dividing the larger unit into two equal rectangles. Wolves in side A can physically contact wolves in side B through the fence. Wolves in all pens have visual, audio, and olfactory contact with wolves in neighbouring enclosures.

Most enclosures contained two pair-bonded wolves, and occasionally their offspring. These arrangements were consistent with pack make-up in wild wolves. Enclosures had dirt and river rock substrate with scattered conifers, low shrubs, and patches of tall grasses. Each enclosure had an artificial den that was approximately 1.2 m wide, 2.4 m long, and 1.5 m high. The wolves were fed 0.9 kg each of Mazuri Exotic Canine Diet (dry dog chow), six days a week on a varying time schedule. They fasted one day a week, except during cold weather. In addition they were given bones for nutritional enrichment. Wolves were only fed immediately prior to or during an observation session on a few occasions.

Thirteen wolves from four enclosures (Pen 7 = 1 adult female, 3 male pups and 2 female pups; Pen 8b = breeding pair; Pen 11a = breeding pair; Pen 12 = breeding pair and 1 juvenile male) were observed from 16 June – 26 August, 2003 (Table 2.1). These enclosures were situated in a square grid, allowing the observer to change observation pens with minimal changes in set-up. The time period was chosen to ensure the least disturbance to the wolves and management staff. Vocalisations were not recorded during breeding or early pup rearing to ensure that there was no interference with rearing success. With the exception of sexual behaviour and pup rearing, there is no evidence of seasonal effects on short-range sound production (Weir, 1999). Wolves were distinguishable by sex and markings. Two housing changes took place during the course of the study. On 17 June, female 1123 was introduced to male 687 in Pen 11a. On 7 July, two adults were removed from Pen 7 and replaced with female 1009 and her five pups. Wolves will be referenced to by pen number and sex or age (i.e. F12 is the female in pen

Table 2.1 Age and housing information for red wolves observed At the Point Defiance Zoo and Aquarium breeding facility during June-August 2003.

Wolf*	Age (years)	Month moved to current pen**	Physical contact with neighbouring wolves?
F7***	4	Jul-03	No
F8	9	Jan-02	Yes
M8	12	Apr-02	Yes
F11	2	Jun-03	Yes
M11	9	Feb-01	Yes
J12	1	May-02	No
F12	11	Dec-97	No
M12	10	Mar-01	No

*Wolves labelled by gender or age and pen number in which they are housed F = female, M = male, and J = juvenile male.

** F8 moved out Jan-Apr, 2003, F12 moved out a few months in 2000

*** Housed with her 5 pups born April 28, 2003

12 and J12 is the juvenile in pen 12). Pup vocalisations were not included in the study.

Data Collection

Observation sessions were conducted five days a week in early morning or late evening, when captive wolves are most active (Schneider and Mace, 2005; Weir, 1999). In general, wolves showed most activity prior to sunset, so the majority of recordings were made in the evenings. To insure the highest range of activity types, morning sessions were conducted at least once a week. Because wolves are not active in the evening if daytime temperatures are high, morning sessions were conducted during the warmest parts of the summer.

Two blinds were used for observations during the course of the study. Management staff constructed a camouflaged wooden blind on the path at the intersection of enclosures 12, 11a, 8b, and 7. This blind had little mobility and poor visibility due to the angle of the window and the fence meshing. Hence, a nylon tent was used for the majority of the study. This blind was placed on the path between two pens for the optimal viewing of both pens (Appendix 2). The blind was moved once a week at the end of an observation session, so that wolves in pens 11a and 12 were observed one week and those in pens 7 and 8b were observed the next. The majority of each enclosure could be seen through the camera's wide-angled lens where the maximum distance between camera and wolf was 43 m. The slope of the enclosure, vegetation, dens, and fence meshing obscured the view of the wolves during some parts of filming.

Video recordings were made using a Sony Digital-8 Camcorder (Model DCR-TRV240) with an external Audio-Technica AT835b shotgun microphone mounded on a tripod such that the camera and microphone were always pointed toward the target wolves. Previous research has demonstrated that the audio tracks from analog video recorders are sufficient for analysing close-range wolf vocalisations (Weir, 1999). However, the digital camcorder used in this study did not have manual audio gain-control; as a result faint structural details of the vocalizations may not have been adequately recorded (see Appendix 1).

Wolves were given 5-10 minutes after I entered the blind to adjust to my presence before the beginning of each recording session. Recordings were made of one pen a day. Sessions lasted until one hour of video had been collected or wolves had been observed for two hours. The camera was turned off whenever wolves were inactive for more than one minute and sessions terminated when inactivity surpassed 20 minutes. To ensure the behaviour of the vocalising animal and the recipient could be analysed, the camera was situated so both wolves were in the video field whenever possible. If both wolves could not be kept in the video field, the camera was centred on the most active wolf. The camera was zoomed in on wolves when they were interacting and zoomed out when they were less active. Approximately 40 hours of video was collected.

Data Analysis

Video Processing

Video was downloaded from the camera onto a computer using Pinnacle® Systems Studio 7. Raw video was converted to MPEG format using Studio 7 and Adobe® Premier® Pro 7.0. MPEG videos were burned onto DVD for analysis and storage. Videos were viewed using Windows Media Player 9.0. Each video was viewed in its entirety and the time of occurrence of each short-range vocalisation and identity of the vocalising wolf was recorded when known.

When possible, identification of the vocalising animal was determined using field notes indicating from what direction/wolf the sound was coming, amplitude of the signal in relation to the wolves' proximity to the camera, and visual identification of wolf movement associated with vocalising. When producing affiliative vocalisations, wolves sometimes bellow their diaphragm-rib cage and/or their nose-snout region in a fashion similar to when they are sniffing. These visual cues, however, are difficult to observe during brief vocalisations.

Audio Analysis

Vocalisation structure was quantified by spectrogram analysis using Wavesurfer 1.6.0 (Sjölander and Beskow, 2003, http://www.speech.kth.se/wavesurfer/index.html) for sounds greater than 2 kHz and Raven 1.0 (Cornell Lab of Ornithology) for sounds less than 2 kHz. AVI video clips containing affiliative vocalisations were copied from raw videos and analysed in Wavesurfer using both wideband (FFT window length 256, Hamming analysis, bandwidth 375) and narrowband (FFT window length 1024, Hamming analysis bandwidth 58) settings. For low-frequency vocalisations, AVI clips were converted to WAV files and analysed in Raven using both wideband (FFT window length 512, Hamming analysis, 3dB bandwidth 122) and narrowband (FFT window length 2048, Hamming analysis, 3dB bandwidth 30.5) settings.

Spectograms were made for each tonal vocalisation. The term vocalization is used to refer to the complete utterance produced by the wolf. Unit refers to the continuous tracing of sound on the spectrogram. Linear units have one component, while non-linear units may have multiple components. Groups of units within the vocalization are referred to as phrases. Figure 2.1 shows an example of the vocalisation structure and the measured acoustical parameters. New vocalisations were determined primarily by changes in context or sometimes arbitrarily by inter-unit intervals of greater than 10 seconds. Phrase identification within the vocalisations was determined by a plot of the log frequency of the inter-unit intervals (Sibley *et al.*, 1990); phrases were separated by inter-unit intervals greater than 500 msec (Appendix 3).

Tonal vocalisations were quantified by fundamental frequency at the start and the end of each unit (or unit component) (Hz), maximum frequency (Hz), minimum frequency (Hz), and average frequency (Hz). Duration of each unit (msec), inter-unit interval (msec), cycle duration (duration of unit and following interval) (msec), and number of units per vocalisation were also recorded. Rate (units/total cycle durations) and duty cycles (total unit durations/total duration) were calculated for each vocal phrase (Fig. 2.1). The presence or absence of biphonations, frequency jumps, harmonics, and subharmonics was noted.

Reliability of Audio Analysis

Measurement reliability was tested by making new measurements on a random sample of the first 411 sound units that were analyzed, totalling 35/411 sound units and 41 unit components. The two sets of measurements were significantly correlated for both temporal and frequency variables (correlation range = 0.989-1.000, $p < 0.001$) and there was no significant differences between sets for temporal or frequency variables (t range =

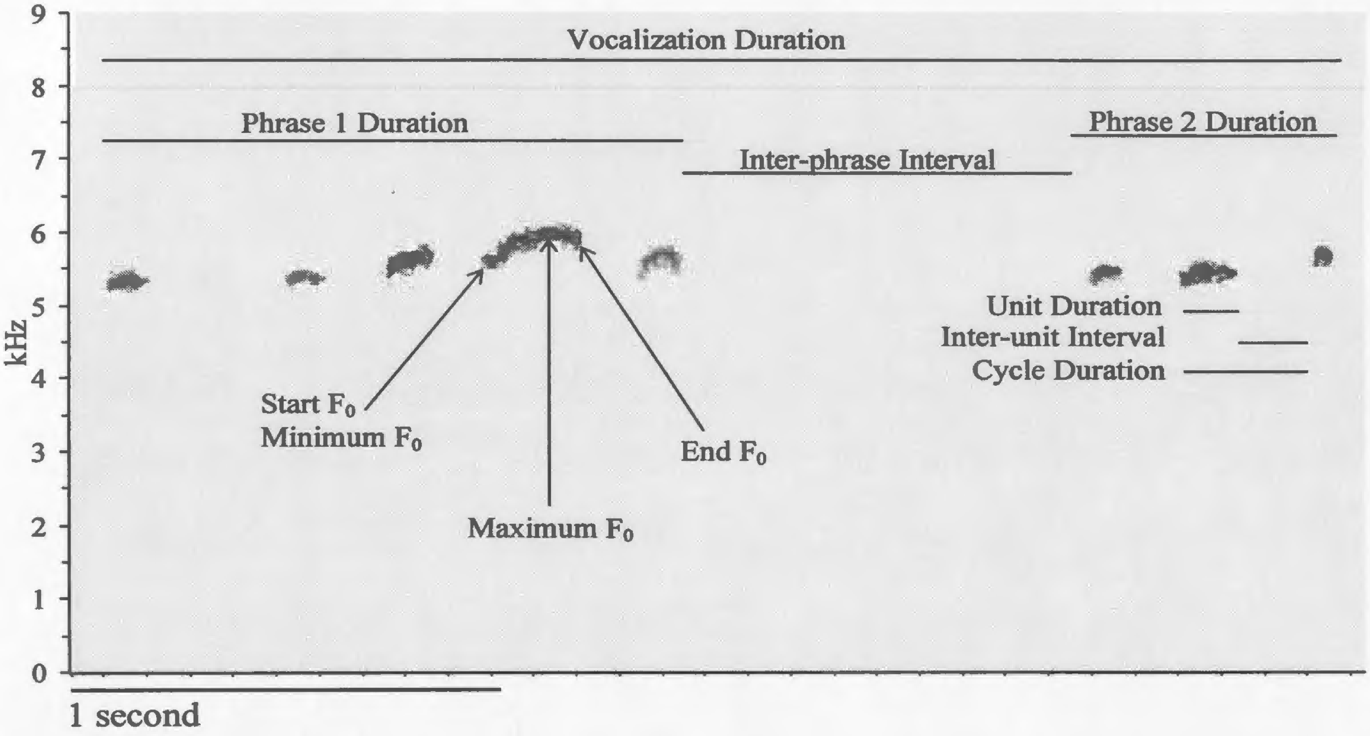

Figure 2.1 Example of a squeak vocalisation (SV) composed of two phrases and eight units, produced by a captive red wolf, demonstrating the structural composition of tonal vocalisations. Other tonal vocalisation types have similar structure and include other unit types. Temporal and frequency measurements used to characterise tonal vocalisations are shown.

-1.19 to 0.71, $p > 0.05$).

Classification

All tonal sounds that were of sufficient clarity on the spectrograph were measured and classified. Sound unit types were identified and classified based on their overall acoustic structure, the presence and nature of non-linear phenomena, and the similarity to previously published vocal categories. Vocalization types were determined according to their unit compositions.

Statistics

All statistical tests were performed using SPSS 12.0 unless otherwise noted. Correlation analysis was used to determine if acoustic measurements were independent. Frequency variables were highly correlated and only start frequency was included in analyses. Auto-correlation function analysis was performed on a random sample of all categorized squeak and wuh vocalizations to determine if the structural characteristics of a unit in a vocalization are independent of the characteristics of the two units preceding it.

Sequential analysis, the analysis of the order of events in a behavioural sequence (Bakeman and Quera, 1995), was performed to determine if there was a predictable order of units in mixed vocalisations, vocalizations that contained more than one type of unit. All classifiable vocalizations were used for this analysis. Z-scores were calculated to determine if unit types were more likely than chance to occur at the first or terminal position in the sequence. ANOVA was used to determine if a unit type was more likely to be found in the beginning, middle, or end of the sequence. GSEQ 4.1.2 (Bakeman and Quera, 2002, http://www2.gsu.edu/~psyrab/sg/sg_e_programs.html) was used to examine the order of units. Chi-square values were calculated for Lag 1, Lag 2, and Lag 3 to determine if the order of units were random. Two-tailed p-values were used to determine the probable transitions between unit types.

Preliminary exploratory analysis was used to determine if vocal structure differed by wolf. Only wolves producing two or more vocalisations of a given type were included in the analysis. For each vocalisation type, ANOVA was used to determine which acoustic variables potentially discriminated between individuals at the phrase and unit level. If only one variable differed and $p<0.05$, this variable was graphed by wolf and visually examined for degree of overlap between animals.

If two or more variables differed by $p < 0.15$ they were used in discriminate function analysis (DFA) to explore if and how these variables distinguish between wolves. An alpha of 0.15 was chosen to ensure that all variables that may contribute to discrimination when in combination with other variables were included. Discriminate functions were generated and used to categorise sounds by wolf, and the proportions of vocalisations correctly categorised were compared to chance. When group covariance matrixes were equal, cross-validation was used to categorise sounds (i.e. the sound being categorised was not included in the discriminate function). When group covariance matrixes were not equal, sounds were categorised using separate-group covariance matrixes and categorisation was validated using repeated random sampling and categorising. A wolf was considered distinguishable from others if vocalisations for that wolf were correctly categorised more often than chance and few vocalisations from other wolves were mis-categorised as belonging to that wolf.

Results

General Behavioural Observations

Since pups could not be individually identified, their behaviour and vocalisations were not analysed. The seven adults and one juvenile demonstrated individually distinctive activity and vocal behaviour patterns (see Chapter 3 for detailed analysis). During the study, they spent most of their time in individual activities such as sleeping, resting, standing, or walking. They interacted with pen-mates through socialising and play and with neighbouring wolves though play. M11 and F12 often oriented and vocalised toward the pen where the pups were housed. Agonistic interactions were rare; most occurred between the newly introduced wolves in Pen 11a. Some wolves, like F7, kept their distance from the observer, while other wolves, like M12, directed investigative or play behaviour toward the observer. Activity levels were highest during howling sessions when wolves would produce a variety of vocalisations, socialise, and play.

A total of 295 short-range tonal red wolf vocalisations composed of 1570 sound units were of suitable quality for quantification using spectral analysis. These vocalisations were composed of 1-30 (median = 3) units of continuous sound clustered in 1-7 (median = 1) phrases. Eight known individuals produced 111 of these vocalisations and 649 sound units.

Description of Sound Units

Seven types of units were identified based on differences in fundamental frequency and the presence of non-linear phenomena. Quantitative descriptions can be found in Table 2.2 and Appendix 4, and spectrographic examples in Figure 2.2. Linearsound types included high-frequency squeaks (S) (Fig. 2.2a) and lower-frequency wuhs (W) (Fig. 2.2b). Squeaks ranged in frequency from 2630-8840 Hz, with bi-modal frequency peaks around 5000 and 8500 Hz. Squeaks were pure-tone sounds and rarely displayed visible harmonics. To the human ear, they sound like a soft bird-like whistle. Wuhs had a median frequency of 450 Hz. They were also pure-tone sounds, but sometimes occurred with harmonic bands. [Note: Lower-frequency, harmonic vocalisations in canids are often called whimpers. This term implies begging and suggests function without contextual evidence. The term wuh was selected to be a phonetic representation of the sound that does not imply a presumed function.] Wuhs are variable (Fig. 2.3), sometimes sounding like a pulsing hollow whistle; longer wuhs often do not occur in series and have a moan-like quality. Approximately 60% of all units identified were squeaks, while 20% were wuhs (Fig. 2.4a).

Five non-linear sound unit types were identified. Three percent of the total identified units contained frequency jumps within the frequency range of the linear unit. Squeak jumps (SJ) (Fig 2.2c) contained up to four high-frequency components, most containing two. The first portion was often lower in frequency than the second. Because only two wuh jumps were identified, they were lumped with wuh units for all additional analyses. Four sound unit types displayed non-linear phenomena resulting from the combination of squeaks and wuhs in a single unit: frequency jumps between squeaks and wuhs (FJ) (Fig. 2.2e), squeak-wuh biphonations (i.e. squeaks and wuhs superimposed on one another) with (BpH) (Fig. 2.2g) and without (BpP) (Fig. 2.2f) sidebands associated

Table 2.2 Acoustic characteristics of seven unit types appearing in tonal vocalisations produced by captive red wolves (*Canis rufus*). (n = 1570)

Unit	Abb.	N	Acoustic Structure*	Duration (msec)	Frequency Range (Hz)	Visible Harmonics (%)
Squeak	S	919	F_0	10-1400	2630-8840**	5
Squeak Jump	SJ	45	F_0 with a frequency jump	30-965	2940-9390**	11
Squeak Band	SB	153	F_0 with sidebands (no visible G_0 component)	30-800	3800-8740**	8
Squeak-Wuh Frequency	FJ	63	Frequency jump between F_0 and G_0	30-700	3840-9620**	8
Jump					260-980	86
Squeak-Wuh Pure	BpP	20	Biphonation of F_0 and G_0 without sidebands	50-1260	3840-9520**	11
Biphonation					230-1570	0
Squeak-Wuh Harmonic	BpH	58	Biphonation of F_0 and G_0 with sidebands	40-1020	3850-9170**	2
Biphonation					235-1440	100
Wuh	W	312	G_0	10-1980	110-1530	26

* F_0 = high frequency component, G_0 = low frequency component

**Frequency characteristics of high-frequency sounds are unevenly distributed with bimodal occurrence peaks around 5000 and 8500 Hz (see Appendix 5)

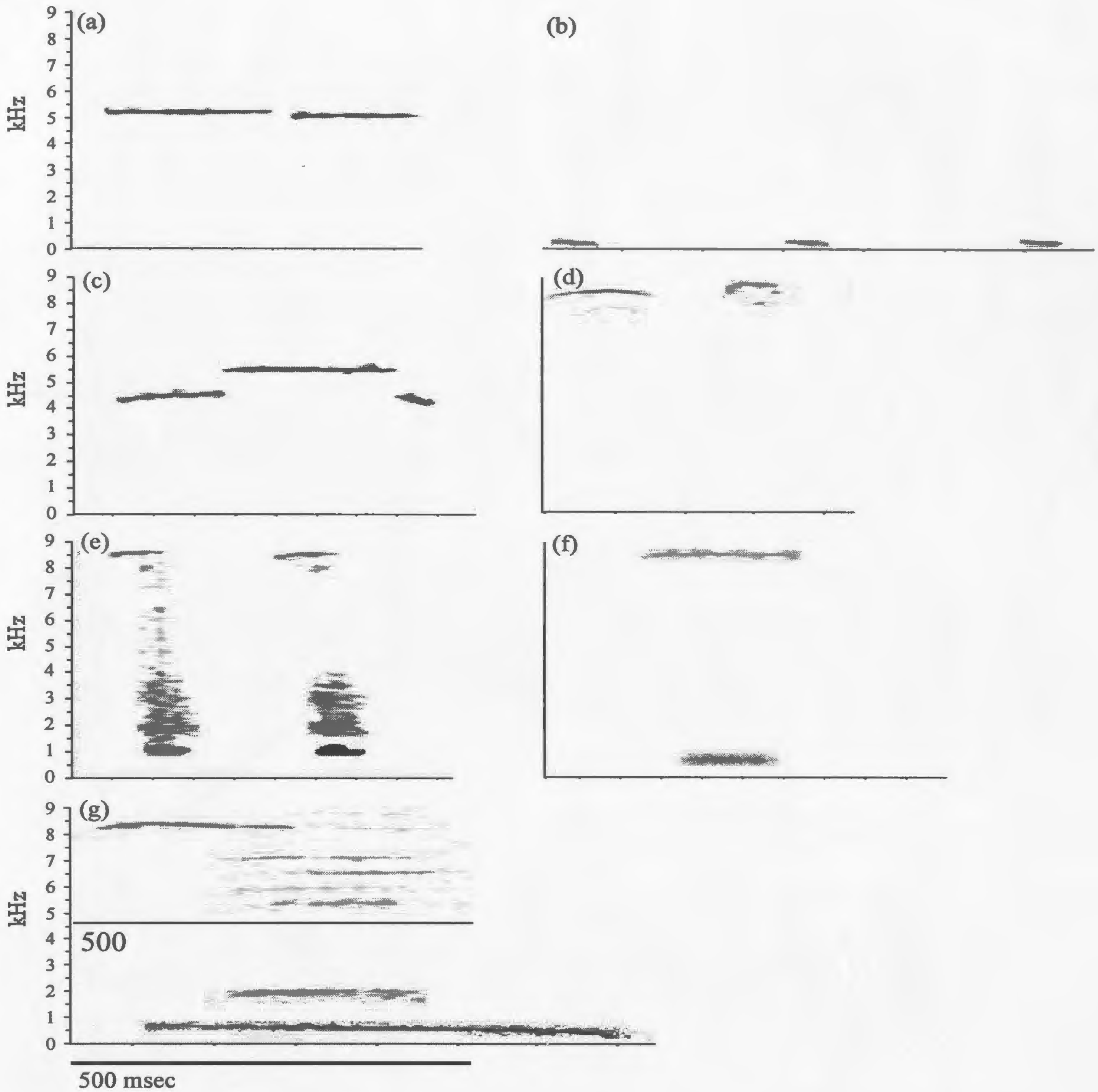

Figure 2.2 Spectrograms representing acoustic structure of (a) two squeak units (b) three wuh units (c) one squeak jump unit consisting of three components and two frequency jump bifurcations (d) two sideband units with 1-2 faint sidebands below the fundamental (e) two units: one biphonation-harmonic unit with minimum temporal overlap (> 10 msec) and one frequency jump unit (note harsh quality sidebands on these units) (f) one biphonation-pure unit and (g) one biphonation-harmonic unit with obvious temporal overlap and clear sidebands. Spectrograms have been cleaned up; however, immediately surrounding the sound, background noise may appear as light grey smudges.

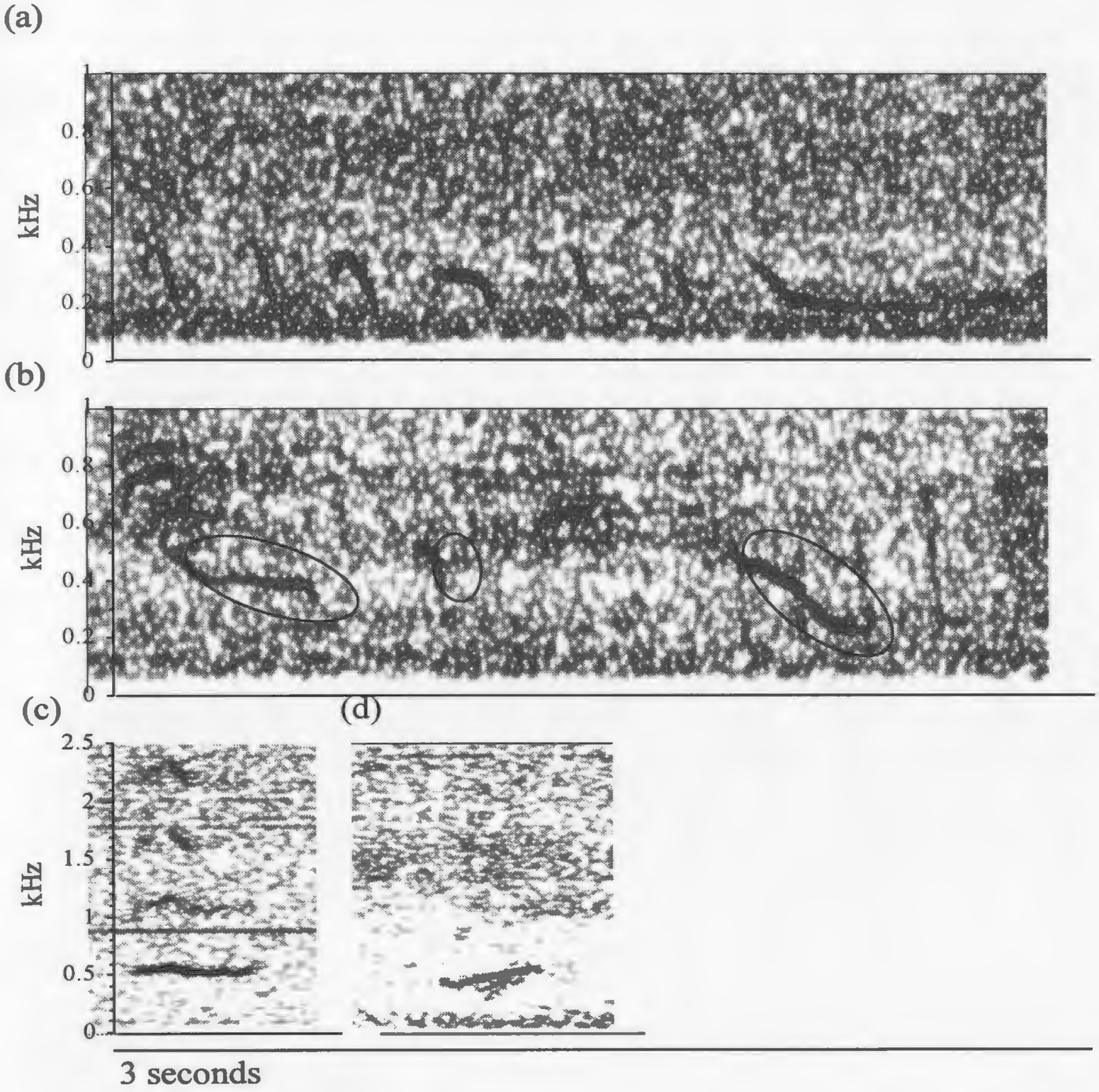

Figure 2.3 Wuhs occur in (a) periodic and (b) sporadic series (wuhs circled in black), (c) with and (d) without sidebands, and have a variety of durations and frequency contours (75% net down-sweeps).

with either fundamental, and squeaks with sidebands (SB) (Fig. 2.2d) but no visible lower fundamental. Together, these non-linear sound unit types make up nearly 20% of the 1570 units identified (Fig. 2.4a).

FJs usually transitioned from the high-frequency component to the low-frequency component, but could be more complex. For instance, FJs were identified with jumps from low to high, jumps from high to an intermediate frequency then to the lower fundamental, and with multiple jumps between high and low components. Frequency jumps were sometimes difficult to distinguish from biphonations. At the onset of the lower component, the higher component often faded making it difficult to determine temporal overlap. In most Bps, the lower frequency component began at the middle or end of the high-frequency component and continued on when the latter had ceased. SBs contained sidebands above or below the fundamental, starting near the beginning, middle, or end and sometimes continuing when the fundamental faded. Normally only one or two distinct sidebands were identified.

Sound units were short in duration: 95% of all units were less than or equal to 300 msec, with a median of 70 msec. Units could be as long as 1980 msec. Over half (54.8%) of long units, those over 300 msec, occurred as single unit vocalizations ($n = 13$), single unit phrases ($n = 21$), and/or as the last unit in a multi-unit vocalization ($n = 28$, $n = 3$ both last and single unit phrase). Long squeaks and wuhs occurred significantly more often than chance in single unit vocalizations (all: $z = 4.85$, $p < 0.001$, $n = 71$; squeaks: $z = 4.53$, $p < 0.001$, $n = 45$; wuhs: $z = 2.77$, $p = 0.003$, $n = 23$), and long wuhs occurred significantly more often in single unit phrases (all types: $z = 2.05$, $p = 0.020$, $n = 75$,

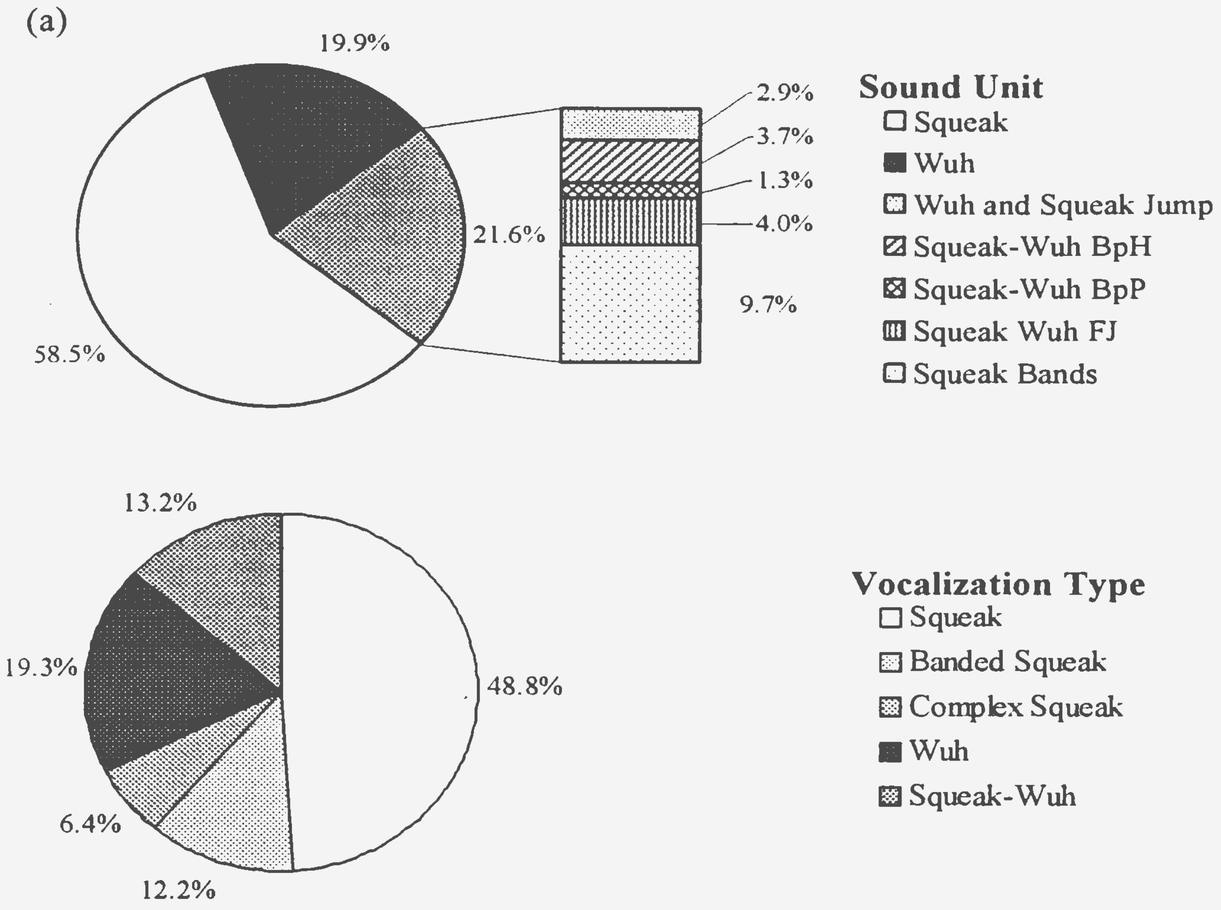

Figure 2.4 Relative occurrence of linear and non-linear phenomena produced by captive red wolves (*Canis rufus*): (a) Black and white portions represent linear sound units. Stacked bar represents types of non-linear sound units based on 1570 analysed sound units. (b) Black and white portions represent vocalisation types containing only linear sound units, and grey portions represent vocalisation types containing non-linear units based on 295 analysed tonal vocalisations.

wuhs: $z = 3.39$, $p = 0.001$, $n = 29$); non-linear units were rare in both cases. Long units occurred as the last unit in multi-unit vocalizations significantly more often than chance ($z = 4.76$, $p < 0.001$, $n = 224$), independent of sound type (squeak: $z = 3.02$, $p = 0.001$, $n = 137$; wuh: $z = 3.41$, $p < 0.001$, $n = 46$; non-linear: $z = 1.89$, $p = 0.029$, $n = 41$). Multi-unit vocalizations were significantly more likely to begin with short squeaks (all types: $z = 1.78$, $p = 0.038$, $n = 224$; squeaks: $z = 1.91$, $p = 0.028$, $n = 158$).

Description of Vocalisation Types and Composition

Five types of vocalisations were identified based on their unit composition (Table 2.3). Almost half of these were squeak vocalisations (SV), which contained only squeak units. Banded squeak vocalisations (BSV) were composed of squeaks and squeak bands, while complex squeak vocalisations (CSV) contained squeaks, squeak jumps, and squeak bands. BSVs and CSVs comprised nearly 20% of the vocalisations. Wuh vocalisations (WV) contained only wuhs and comprised another 20% of the vocalisations. The remainder of vocalisations were squeak-wuh vocalisations (SWV) containing combinations of all seven sound unit types (Fig. 2.4b).

The composition of mixed vocalizations, BSVs, CSVs, and SWVs, varied between and within wolves. Absolute ranges of the proportional contribution of each sound unit, for all classified vocalizations, are shown in Table 2.3. SWVs were the most complex and therefore demonstrated the most variability. Figure 2.5a demonstrates the variability between SWVs of five wolves. SWVs of individual wolves contained 4-7 sound unit types, the overall proportions of sound unit types per vocalisation varying substantially across wolves. For example, SWVs of F7 and J12 contained over 50%

Table 2.3 Composition and structure of five tonal vocalisation types produced by captive red wolves (*Canis rufus*). (n = 295)

Type	Abb.	N	Number of Phrases	Number of Units	Unit Composition* (Percent of Vocalization)
Squeak Vocalization	SV	144	1-4	1-19	S (100)
Banded Squeak Vocalization	BSV	36	1-7	1-30	S (0-92) SB (8-100)
Complex Squeak Vocalization	CSB	19	1-5	2-26	S (0-92) SJ (8-67) SB (0-67)
Wuh Vocalization	WV	57	1-7	1-19	W (100)
Squeak-Wuh Vocalization	SWV	39	1-5	1-30	S (0-80) W (0-83) FJ (0-100) BpP (0-100) BpH (0-100) SB (0-75) SJ (0-20)

* See text and Table 2.2 for description of sound units

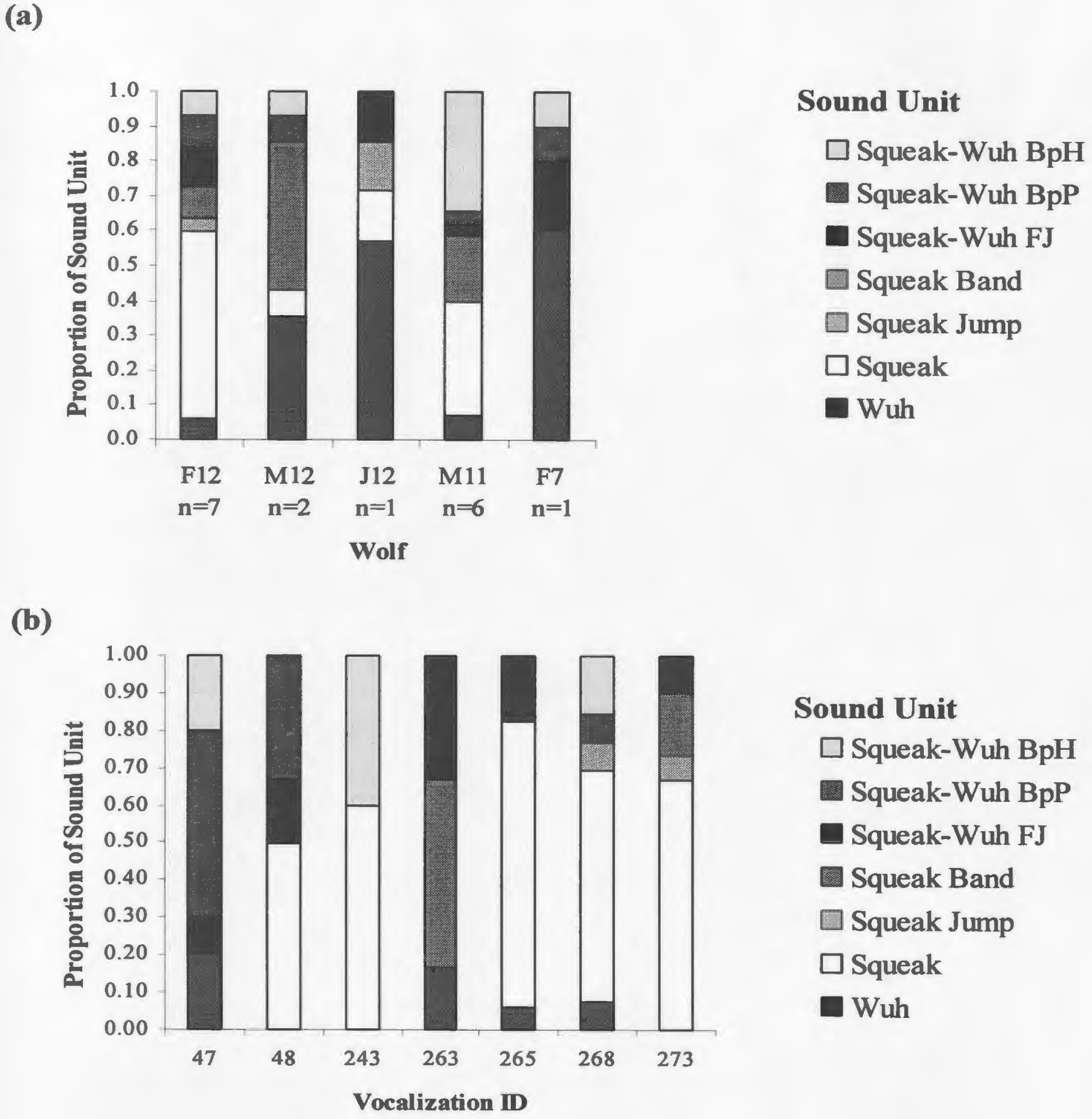

Figure 2.5 Variation in unit composition of squeak-wuh vocalisations produced by five captive red wolves (*Canis rufus*), (a) combined over vocalisations and (b) produced by F12, across seven vocalisations.

wuhs, while SWVs of F12 contained over 50% squeaks. The composition of SWVs also varied within a wolf, as demonstrated in Figure 2.5b. The seven SWVs produced by F12 contained 2-5 sound unit types. While the majority of sound units in five of these vocalisations were squeaks, no squeaks occurred in the other two vocalisations.

Sequential Analysis

Auto-correlation function analysis (ACF) on 10% of SVs and WVs revealed only the occasional correlation of time (14% SV, 0% WV) and frequency variables (21% SV, 17% WV) between units at Lag 1 and Lag 2, but no overall pattern (Appendix 7).

Sequential analysis was performed to determine if there was a predictable order of units in mixed vocalisations. No predictable order could be determined for BSVs or CSVs, except that units were likely to be followed by 1-3 units of the same type.

More extensive analysis was performed on SWVs. Squeaks were significantly more likely than chance to be the first unit in the vocalisation ($z = 2.07$, $p < 0.019$) and BpHs had a high probability of being the last unit ($z = 1.66$, $p < 0.049$). FJs had a low probability of being the first unit ($z = -1.75$, $p < 0.040$) and squeaks had a low probability of being the last unit ($z = -1.72$, $p < 0.043$). In a subsequent analysis, SWVs were divided into three parts: beginning, middle, and end. Squeak units occurred significantly more often in the beginning of the vocalisations than in the middle or end (ANOVA $F_{2,68} = 5.275$, $p = 0.007$), while all other units had equal probability of occurring in each section.

Figure 2.6 displays transitions between unit types in squeak-wuh vocalisations. The order of units was not random at Lag 1, adjacent units, ($X^2 = 312.88$, $p < 0.001$) or Lag 2, units separated by one unit ($X^2 = 79.06$, $p < 0.001$). There was insufficient sample

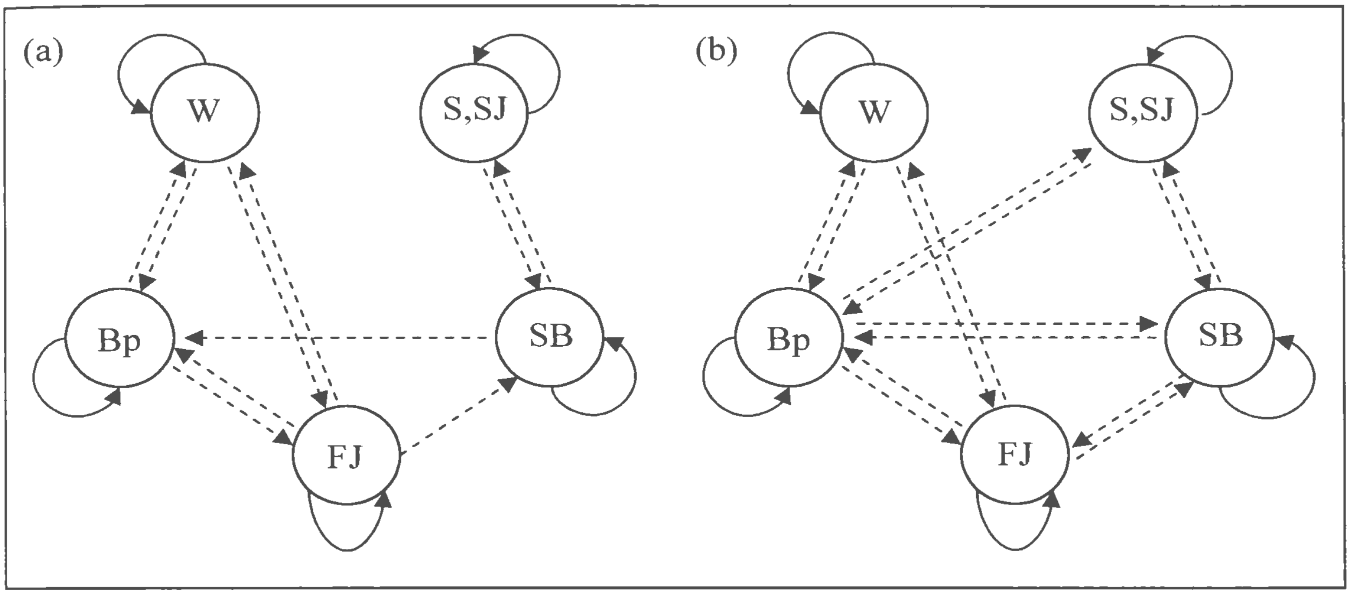

Figure 2.6 Transitions between units* in squeak-wuh vocalisations were non-random at: (a) Lag 1, no intervening unit, and (b) Lag 2 one intervening unit. Solid lines represent significant transitions ($p < 0.05$), dashed lines represent transitions that are not significantly different than chance, and the absence of lines represents transitions that occur significantly less often than chance ($p < 0.05$).

*S = squeak, SJ = squeak jump, SB = squeak band, FJ = squeak-wuh frequency jump, Bp = squeak-wuh biphonation with and without harmonics, and W = wuh. Squeak jumps were lumped with squeaks and pure biphonations with harmonic biphonations due to insufficient sample sizes of these units.

size to analyse Lag 3. Observed transition rates were compared to those calculated by chance and two-tailed p-values were obtained using GSEQ. All unit types were followed significantly more often by one or two units of the same type than by other types of units ($p < 0.001$). In general, Lag 1 transitions between units containing only high components and units containing low components occurred significantly less often than chance ($p < 0.05$) (e.g. wuhs and squeak bands do not transition into each other). At Lag 2 more transitions occurred at chance levels, but wuh units and high-frequency units are still significantly less likely to transition into each other ($p < 0.05$).

Individual Variation

Wolves demonstrate individual variation in number and type of vocalisations produced (Table 2.4). Three wolves produced only 2-3 vocalisations during the observation period, while other wolves produced almost 30. All wolves produced at least one squeak vocalization, and 7 out of 8 wolves produced linear vocalisations (squeaks and wuhs) at least 50% of the time. The proportion of mixed vocalisations varied between animals. Wolves in Pen 8b did not produce mixed vocalisations, while 62% of M11's vocalisations were mixed. Four to five wolves produced each of the vocalisation types. Vocal output was not associated with age.

Exploratory analysis was used to determine if vocal structure differed by wolf at the phrase and units levels, using scatter plots for single variables and discriminate function analysis for multiple variables. Phrase level variables did not differ between vocalisation types, and hence vocalisation types were not considered at the phrase level. J12 was distinguishable (50% categorised correctly) from M8, M11, M12 and F12 on the

Table 2.4 Production of tonal vocalisation types by individual captive red wolves (*Canis rufus*) approximately 40 hours of observations.

Vocalisation Type	F7	M8	F8	M11	F11	M12	F12	J12	Total
Squeak	1	4	1	11	2	5	15	11	50
Wuh		6	1			7	1	1	16
Combination	1			6		2	7	1	17
Squeak Jump				1		1	4	8	14
Non-linear Squeak				11	1		1	1	14
Total	2	10	2	29	3	15	28	22	111

basis of rate, duty cycle, and number of units.

At the unit level, WVs did not differ between wolves. SVs produced by M12 (100% categorised correctly), M11 (77.8% categorised correctly), and J12 (70% categorised correctly) differed from each other on the basis of inter-unit intervals and unit durations, but overlapped considerably with SVs produced by F12 (47% categorised correctly). In CSVs, F12 differed from J12 on the basis of the start frequency of squeaks (11% overlap) and squeak bands (0% overlap). Similarly, in SWVs, wolf F12 differed from M11 on the basis of start frequency of squeaks (25% overlap) and other high-frequency components (0% overlap). M12 differed from F12 and M11 in start frequency of low-frequency components of SWVs (0% overlap) (Table 2.5). Only one wolf produced more than 1 BSV so no analysis was done on this vocalisation type. With the exception of M8, all wolves included in the analysis were distinguishable from other wolves by at least one vocalisation type.

Table 2.5 Structural distinctions in tonal vocalisation at the phrase and unit levels between individual captive red wolves (*Canis rufus*) determined by Discriminate Function Analysis, and plots of variables for wolves producing 2 or more vocalisations per category.

Structure Level	Distinguishable	Not-Distinguishable	Distinguishing Variables
All Phrases	J12	M8, M11, M12, F12	rate, duty cycle, number of units
SV units	M11, M12, J12	F12	inter-unit interval, unit duration
BSV units	-	-	-
CSV units	F12, J12	-	start frequency (high components)
WV units	none	M8, M12	-
SWV units	M11, F12	M12	start frequency (high components)
	M12	M11, F12	start frequency (low components)

Discussion

Description and Production of Red Wolf Sounds

This study of non-linear phenomena has led to the identification of several red wolf sounds that have not been previously described. I found that red wolves produce tonal vocalizations with linear and non-linear sounds similar to those reported in dhole whistles (Volodin and Volodina, 2002). These red wolf sounds contained high and low-frequency components and occurred as separate units or in combination. The separate units have previously been identified as squeaks and wuhs respectively (Schneider and Mace, 2005). Component combinations included frequency jumps, primarily from high to low components, and biphonations, the simultaneous production of high and low components. The parallel sidebands present in squeak bands indicate the presence of biphonations, even though no lower component was identified. Together, these sounds represent a structural continuum, transitioning from high-frequency squeaks on one end to low-frequency wuhs on the other.

Red wolf squeaks are similar in structure to those of gray wolves, but have a higher fundamental frequency (including a second higher-frequency occurrence peak), have narrower frequency ranges within each sound unit, and have lower maximum numbers of units per vocalisation (Weir, 1999). The higher frequency squeaks are potentially a product of shorter vocal apparatus, since red wolves are smaller then gray wolves. Similar to dhole whistles and consistent with the proposed glottal whistle mechanism for high-frequency canid vocalisations, red wolf squeaks rarely demonstrated visible harmonics (Solomon *et al.*, 1995; Wilden *et al.*, 1998; Volodin and Volodina, 2002).

In addition, red wolves produced frequency jumps within their high-frequency squeaks that may be similar to a register jump in the human voice. Such jumps have not been reported in other short range canid vocalizations. It has yet to be determined whether squeak jumps are a result of non-linear vocal output, or whether they are sudden linear changes in frequency production. All adults that produced squeaks showed a bimodal distribution of start frequencies. All squeak jumps produced by adults involved a jump from one node to the other. However, sample sizes for most adults were small, and the juvenile did not show the same bimodal pattern. Further research into the development and functional boundaries of the red wolf vocal apparatus is needed.

Low-frequency, pure-tone vocalisations have not been reported for other canids (see Robbins and McCreery, 2003 for a possible exception in African wild dog pup vocalisations). Unlike the lower component of dhole vocalisations, red wolf wuhs and the low-frequency components that occurred in squeak-wuh vocalisations often did not have visible harmonic bands. Wuhs were often low in amplitude and difficult to distinguish from background noise, indicating that low amplitude harmonics may have been present but not detectable. However, wuhs without harmonic bands have a pure-tone quality distinguishable by the human ear from those with harmonic bands and are unlikely to be by-products of the recording equipment.

I examined the sequence of unit types in squeak-wuh vocalisations to determine if any pattern could be identified. There was insufficient sample size to compare differences in sequences between wolves or contexts. Examination of the relative probability of units in the beginning, middle, and end of the vocalisation and the transitional probabilities between unit types revealed a non-random order to the units. In general, squeak-wuh vocalisations began with high-frequency components and moved toward mixed-frequency components. Like unit types often appeared in succession. Direct transitions between squeaks and wuhs were rare. These constraints follow the structural continuum of the unit types mentioned above, indicating that non-linear phenomena may be a physiological by-product of transitioning between squeaks and wuhs. A proposed structural order of sound types is presented in Fig. 2.7. An examination of potential context differences in sequence is necessary to determine any potential meaning of the sequential constraints.

While most units were short in duration, 5% were longer than 300 msec and were most likely to occur as single unit vocalisations or phrases, and as terminating units in multi-unit vocalisations. Single unit vocalisations may be longer in duration to increase the likelihood of detection (Brumm *et al.*, 2004). Only long wuhs occurred as single unit phrases, indicating a possible contextual relevance. Long units may communicate termination of vocalization.

Observations of the distribution of sound intensity within the sound unit also indicated a physiological link between high and low frequency components. Although the intensity of the vocalisations was not measured in this study, differences in intensity were distinguishable to the human ear. The onset of a second frequency component was often accompanied by a decrease in the intensity of the first frequency component, suggesting that the production of frequency components is energetically interdependent. During

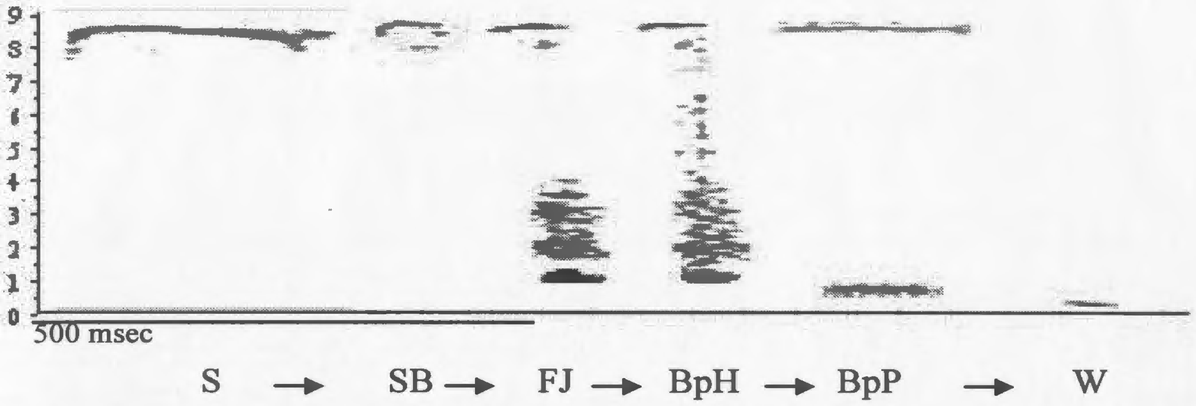

Figure 2.7 Tonal sounds produced by red wolves can be represented as a structural continuum transitioning from high-frequency squeaks, through four types of non-linear sounds, to low-frequency wuhs. Note: The representation does not reflect the actual structure of any vocalization observed in this study.

S = squeak, SB = squeak band, FJ = squeak-wuh frequency jump, BpH = squeak-wuh biphonation (harmonic), BpP = squeak-wuh biphonation (pure), W = wuh biphonation, high-frequency components were often faint. However, as is the case with squeak bands, low-frequency components did not always dominate.

Potential Adaptive Value of Non-linear Sounds

Direct transitions between squeaks and wuhs were rare, but not impossible, indicating that the production of non-linear sounds is not necessary and may be adaptive. Additionally, no obvious relationship between the production on non-linear sounds and age of the wolves was found. Non-linear phenomena occurred in 22% of all vocal units analysed and were produced by 6 of 8 individuals. These results are a minimum estimation of occurrence of non-linear sounds. A comparison between digital video and DAT audio indicates that analysis of digital video recordings (used in this study) may result in an underestimate of the abundance of non-linear vocal phenomena (Appendix 1). DAT recorders are more sensitive to low-amplitude sounds, including sidebands and faint secondary frequency bands. Non-linear vocal sounds are common in red wolves and many other mammal vocalisations, indicating they are a normal aspect of vocal production (Wilden *et al.*, 1998).

Several explanations for non-linear phenomena exist. The structural shift from squeak to wuh could indicate a gradation in motivation (Wilden *et al.*, 1998). However, this structural transition is primarily uni-directional, implying a uni-directional change of motivation, which seems unlikely. Interpreting this gradation according to Morton's (1977) structural-motivation rules, the change in frequency indicates a transition toward an aggressive motivation. Behavioural data are needed to determine if wuhs occur in less affiliative contexts than squeaks, and if the use of squeak-wuh vocalizations corresponds to a change in context and/or motivation.

The presence of non-linear phenomena may be indicative of stress. Caldwell and Caldwell (1979) suggest that dolphins have less muscle control over their sound production systems during times of stress, which can lead to the production of biphonations and/or chaos (Killebrew *et al.*, 2001). Many squeak-wuh vocalizations began with squeaks and ended with mixed sound units indicating a potential increase of excitement. A comparison of the overall behaviour patterns of wolves in this study indicated a potential association between excitement and the production of non-linear sounds. Two of eight wolves did not produce non-linear sounds. While these wolves may have physiological differences from the other wolves, it is more likely that their lack of non-linear sound production was contextual. Both wolves were housed in the same pen, displayed similar activity patterns, and produced minimal vocal output. A larger sample of vocalizations from these animals may yield non-linear phenomena. These two wolves demonstrated less interaction with wolves in neighbouring pens, and less overall excitement than other wolves.

The nature of the breeding facility, where wolves could see but not interact with other wolves, may increase the social stress of some wolves and increase the likelihood that they will produce non-linear sounds. Weir (personal communication) did not observe sidebands when examining gray wolf squeaks, and recording quality did not allow for the examination of sounds under 1 kHz. There are contextual and methodological explanations for this lack of non-linear phenomena. The study population lived in a single familial pack in a 3.8 hectare forested enclosure. This situation differs greatly from the small, adjacent pen set-up of the red wolf breeding facility. In addition, Weir's recordings were made from a greater distance (up to 60 m) with an analog video camera, making detection of low amplitude non-linear sounds unlikely.

The production of non-linear sounds may also be linked to features of the environment. Wiley (1991) proposed the sidebands in bird songs (produced by rapid amplitude fluctuations) attenuate faster in forested rather than open environments. He proposed that birds in forested environments could judge distance to a singer based on the level of attenuation. Volodin and Volodina (2002) proposed that dholes use high-frequency components for individual identification and low-frequency components for localization. While most red wolf tonal sounds are produced when wolves are within visual contact, non-linear sounds may provide additional information when wolves are not in visual contact. Non-linear phenomena may also provide variation which could attract attention when receipts are not responding to normal squeaks (Fitch *et al.*, 2002).

Canid Tonal Vocalizations

Identification of non-linear phenomena has led to several potential new descriptive categories for red wolves, dholes, and African wild dogs, suggesting that canids have richer vocal repertoires than previously suspected (Wilden *et al.*, 1998; Volodin and Volodina, 2002). Currently, these categories are structurally, not functionally defined. It remains to be determined whether non-linear sound types occur in addition to previously identified linear sound types, or if non-linearities are variations of current vocal categories. All studies of non-linear vocal productions reported to date occur in social canids, suggesting that these vocalisations serve a social function. In contrast, solitary canids are thought to produce more stereotyped sounds, vocalizations within a category showing little variation (Fox, 1975), and probably do not produce non-linear sounds. An in-depth comparison of vocal structure and contextual usage across canids is necessary for understanding the adaptive use of non-linear vocalisations in canids.

Non-linear vocal production in dholes, African wild dogs, and wolves have a similar structure—a combination of high and low-frequency vocalisations. While this structure can be seen in published sonograms of gray wolf vocalisations (Harrington and Mech, 1978; Nikol'skii and Frommolt, 1989; Schassburger, 1993), further study is needed to determine the extent of this phenomenon in gray wolves. A similar structure to red wolf vocalisations is expected. Wuh vocalisations have not been described in the gray wolf, but potentially exist given the common biphonic structure of social canid vocalisations. Further study of the gray wolf vocal repertoire is needed.

In addition, the presence or absence of non-linear sounds in the closely related, but more solitary coyote (*Canis latrans*) should be investigated. The taxonomic relationships between gray wolves, red wolves, and coyotes are controversial (Nowak, 1992; Roy *et al.*, 1994; Wilson *et al.*, 2000). Vocalization data may provide further behavioural distinctions between species. To date, neither squeaking nor non-linear sounds have been reported in coyotes. The presence or absence of whistle-like sounds in coyote vocalizations may aid in an understanding of the ecological function of these sounds.

Individual Identification

A preliminary investigation of the variation in sound structure was conducted to determine if sufficient variation was present between red wolves to yield the potential for individual recognition. Because sample sizes were small, conclusions were drawn with caution. At the phrase level, only the vocalizations of the juvenile wolf could be distinguished from others. These differences could be due to age, but definite conclusions are not possible since he was the only yearling in the study.

At the unit level, squeak (SVs), complex squeak (CSVs), and squeak-wuh (SWVs) vocalizations contained information that wolves could potentially use for individual identification. All four wolves used in these analyses were distinguishable from each other in at least one vocalisation type. In CSVs and SWVs, the start frequency of high and low-frequency components differed between individuals. In contrast, during SVs temporal variables, inter-unit interval and unit duration, played a greater role in distinguishing between wolves than frequency variables. Wuh vocalisations did not differ between the two wolves that produced an adequate sample.

While individuals were distinguishable, vocalisations produced by all individuals overlapped other individuals in temporal and frequency variables. Categorisation was rarely 100% accurate. Variability exists in all sound unit types that was not explained by individual variation and may be a result of contextual differences between vocalisations, as is the case with gray wolf squeaks (Weir, 1999). Unlike dhole whistles (Durbin, 1998) and swift fox (*Vulpes velox*) barks (Darden *et al*., 2003), which are used as contact calls, wolf tonal vocalisations occur in a variety of contexts (Weir, 1999; Schneider and Mace, 2005). The contextual occurrence of red wolf tonal vocalisations is discussed elsewhere (Chapter 3).

Individuals were not distinguishable by composition of squeak-wuh vocalizations. Although no squeak-wuh vocalisations contained all unit types, some contained as many as five unit types. The proportion of unit types varied greatly between wolves and between vocalisations of a single wolf. From this level of variation, I infer that vocalisation composition is not correlated with the physical structure of the vocal mechanism of an individual wolf.

Summary

The study of non-linear phenomena in tonal vocalisations produced by captive red wolves has led to greater understanding of the richness of their vocal repertoire. At the production level, three sound types are apparent: high-frequency squeaks, lower-frequency wuhs, and sound types containing both squeaks and wuhs. It remains to be determined if production of the latter category is under the wolves' control, or whether the production of non-linear phenomena is a physiological by-product of switching between linear sound types. Furthermore, although squeak bands, frequency jumps, pure-biphonations, and harmonic-biphonations differ from each other structurally, I do not know if they differ functionally. Likewise, we do not know if squeak jumps function differently from squeaks. The functional validity of the new vocal categories can be strengthened by assessing their contextual occurrence and variation.

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Chapter 3 Production rate and behavioural context of affiliative vocalisations in red wolves (*Canis rufus*)

Introduction

Wolves, like all pack-living animals, must be able to communicate in order to work cooperatively and live amiably. Both red wolves (*Canis rufus*) and gray wolves (*Canis lupus*) live in family groups that work together to maintain territories, hunt, and raise young (Phillips *et al*., 2003; Mech and Bointani, 2003). Pack life provides a number of situations where wolves can benefit from a vocal exchange of information. Vocalisations may serve a practical function in a specific context such as aiding wolves in maintaining contact during hunting (see Volodin and Volodina, 2002 concerning dholes) or alerting other wolves to the vocalising wolf's presence when approaching from behind (Weir, 1999). They can be used to call pups to return to or come out of the den (Coscia *et al*., 1991). Vocalisations may be used to express a desire to interact. Like play bows, they may help distinguish between agonistic and play intent (Bekoff, 1995). It is therefore necessary to identify the social situation in which vocalisations occur (Fentress, 1978; Bekoff, 1995; Weir 1999).

Identifying the functions of short-range vocalisations requires understanding how short-range vocalisations are utilised during social interactions. Wolves use their whole bodies to communicate. In addition to auditory communication, they utilise elements of olfactory, tactile, and visual communication. These elements rarely occur in isolation and are always changing as the social situation changes (Schenkel, 1947/1999; Harrington and Asa, 2003). A thorough understanding of the importance of vocalisations in these social interactions must be holistic, looking at the fluid behaviour of the wolf and its context.

Short-range wolf vocalisations vary from tonal, high frequency sounds, such as squeaks and wuhs, to harsh low-frequency sounds, such as barks and growls, with gradations and transitions between basic types (Schassburger, 1993; Schneider and Mace, 2005). Tonal vocalizations are primarily pure tone or harmonic, but may also contain harsh elements. Of the tonal vocalisations, only the gray wolf squeak has been studied beyond basic description. Red wolf squeaks are similar in structure to gray wolf squeaks. They are composed of 1-19 short sound units (10-1400 msec) clustered in 1-4 phrases (Chapter 2). Gray wolf squeaks are similar, but have a lower fundamental frequency, have wider frequency ranges within each sound unit, and can contain more units per vocalisation (Weir, 1999).

Gray wolves squeak in a number of social situations. Cristler (1958) first described the "social" squeak of the gray wolf as a "mouse squeak" uttered by hand-raised wild wolves when they had an unexpected encounter with their human pack members. Squeaking has been documented when wolves are greeting other wolves (and familiar people and dogs if hand-raised) after a time of separation (Fentress, 1967; Fentress *et al.*, 1978), when approaching other wolves at food of in a clearing, during play, and prior to and during howling choruses (Weir, 1999). Squeaks are the most common vocalisation used in pup rearing (Goldman *et al.*, 1995), often uttered by an adult before entering the den (Coscia *et al.*, 1991). Pups begin squeaking as early as 15 days. Squeak structure differs between individuals and contexts providing the potential for individual recognition and contextual specificity (Weir, 1999).

Recent investigation into the tonal vocalisations of the red wolf (*Canis rufus*) (see Chapter 2) revealed that red wolves produce non-linear vocal sounds. Non-linear vocal sounds are common in many mammal vocalisations. Humans control their vocal system in such a manner that non-linear sounds are rare; occurring most often during laughter, in pathological voices, and infant vocalisations—especially cries (Michelsson *et al.*, 1977; Robb and Saxman, 1988; Bachorowski *et al.*, 2001). However, these vocal phenomena are common in many mammal vocalisations, including canid vocalisations, and their production may be under the direct control of the vocalising animal (Riede, 1997; Wilden, 1998).

When the vocal system works as a network of coupled oscillators operating within a set of physical parameters, it produces normal vocal-fold vibration or linear sound. Linear sound is represented on a spectrogram as a fundamental frequency band and its corresponding harmonic bands, which appear at integer ratios of the fundamental. Non-linear sounds are produced when the vocal system is adjusted beyond its normal parameters and the system becomes uncoupled disrupting the normal sound production or when sound is produced by a secondary source such as nasal or glottal whistles (Fitch *et al.*, 2002). Non-linear sound is often represented on a spectrogram as sidebands that do not appear at integer ratios of the apparent fundamental.

Four non-linear phenomena are commonly identified. Sub-harmonics are characterized by harmonic bands at 1/2 or 1/3 the normal harmonic interval. Frequency jumps, abrupt shifts from one fundamental frequency to another, are caused by minor adjustments to the vocal apparatus and should not be confused with frequency shifts that have a linear relationship to adjustments to the vocal apparatus. Biphonations, the simultaneous production of two independent fundamental frequencies, are often accompanied by sidebands resulting from linear combinations of both fundaments. Chaos is characterized by sections of periodic noise (Riede *et al*., 1997; Wilden *et al.*, 1998; Fitch *et al.*, 2002).

As described in Chapter 2, red wolf tonal vocalisations are composed of high-frequency (2600-9600 Hz) and low-frequency (100-1600 Hz) sounds that occur as distinct sound units, called squeaks and wuhs respectively, or as components in combination sound units. Combinations of components include squeak-wuh frequency jumps (FJ), sudden jumps from squeaks to wuhs; squeak-wuh biphonations, squeaks and wuhs superimposed on one another, with (BpH) and without (BpP) sidebands associated with either fundamental; and squeak bands, squeaks with sidebands (SB), but no visible lower fundamental. Red wolves also produce frequency jumps within their high-frequency components, called squeak jumps (SJ), which are similar to a register jump in the human voice. Non-linear sounds composed 22% of the tonal sound units produced by red wolves.

In Chapter 2, five types of red wolf tonal vocalisations were identified based on their unit composition. Almost half of these were squeak vocalisations (SV), which contained only squeak units. Banded squeak vocalisations (BSV) were composed of squeaks and squeak bands, while complex squeak vocalisations (CSV) contained squeaks, squeak jumps, and squeak bands. BSVs and CSVs comprised nearly 20% of the vocalisations. Wuh vocalisations (WV) contained only wuhs and comprised another 20% of the vocalisations. The final vocalisation type was a squeak-wuh vocalisation (SWV), containing combinations of all seven unit types (SWV).

The biphonic structure of red wolf tonal vocalizations is similar to those reported in the hunting whistles of dholes (Volodin and Volodina, 2002). A review of published sonograms of gray wolf tonal vocalisations (Harrington and Mech, 1978; Nikol'skii and Frommolt, 1989; Schassburger, 1993) reveals a similar biphonic structure between low and high-frequency vocalisations. The biphonic structure may be common to social canids. Studies of the vocal repertoires of social canids reveal a graded system, with transitions that often involve two call types superimposed (Lehner, 1978; Brady, 1981; Schassburger, 1993; Robbins, 2000). These transitions are likely products of biphonation.

Wolf squeaks differ from the hunting whistles of dholes both structurally and contextually. Dhole whistles occur in strings of similar sound units and are used for mid-distance communication during hunting. The sound units in a vocalisation produced by a single dhole contain minimal variation (Durbin, 1998). In contrast, wolf tonal vocalisations are used for short-range communication and can be highly variable within each category and within a single wolf (Fentress *et al.*, 1978, Schassburger, 1993, Weir, 1999; Schneider and Mace, 2005). Field (1979), Fentress *et al.* (1978), and Weir (1999) found that squeak structure varies with social context.

Since non-linearities are occur frequently in non-human mammal vocalisations, especially among canids, there is potential for adaptive significance (Wilden *et al.*, 1998). No study has examined the possible function of biphonations and other non-linearities in tonal wolf vocalisations. Several possible functions have been suggested for non-linear non-human mammal sounds. Since many irregularities are dependent on the structure of the vocal apparatus, the possibility of individual recognition is present (Fitch *et al.*, 2002). For example, subharmonics (identified as two component harmonics) occur in wolf howls and could be a cue for individual identification (Tooze *et al.*, 1990). Subharmonics and chaos lower the perceptual pitch of a vocalisation, perhaps indicating the size and/or status of the animal (Fitch *et al.*, 2002). Such phenomena can be seen in spectrograms showing howl responses produced when wolves approached the source of simulated howls, possibly indicating an increase in aggression (Harrington, 1987). Variations may add an element of unpredictability that prevents acclimation to commonly used vocalisations (Fitch *et al.*, 2002). Finally, it has been suggested that biphonations indicate the vocaliser's dual message (Wilden *et al.*, 1998). For example, Volodin and Volodina (2002) proposed that the high-frequency component of dhole whistles facilitates individual identification during hunting, while the low component facilitates localisation. Wolf tonal vocalizations are lower in amplitude than dhole hunting whistles, being used in short rather than mid-range communication.

In this study I use audio and video analysis to examine the contexts in which captive red wolves (*Canis rufus*) produce all types of tonal vocalisations and to compare the frequency of occurrence of these types of vocalisations to those of other common vocalisations. Objectives include developing a general description of the activity and relational contexts in which tonal vocalizations are produced and comparing these contexts to those reported for squeaking vocalizations in gray wolves. In addition, I examine the behavioural context of squeak vocalizations, wuh vocalizations, and vocalizations containing non-linear units.

Methods

Study Site

I carried out this study using individuals from the Red Wolf Captive Breeding Program housed at a breeding facility in Graham, WA associated with Point Defiance Zoo and Aquarium (PDZA). Red wolves at PDZA are housed in 18 enclosures, each enclosure measuring 465 or 929 m^2. Large enclosures are square, with access corridors between each enclosure so that wolves in adjacent enclosures do not have physical contact with each other. Small enclosures are half of a large enclosure with a chainlink fence dividing the larger unit into two equal rectangles. Wolves in side A can physically contact wolves in side B through the fence. Wolves in all pens have visual, audio, and olfactory contact with wolves in neighbouring enclosures.

Most enclosures contained two pair-bonded wolves, and occasionally their offspring. These arrangements were consistent with pack make-up in wild wolves. Enclosures had dirt and river rock substrate with scattered conifers, low shrubs, and patches of tall grasses. Each enclosure had an artificial den that was approximately 1.2 m wide, 2.4 m long, and 1.5 m high. The wolves were fed 0.9 kg each of Mazuri Exotic Canine Diet (dry dog chow), six days a week on a varying time schedule. They fasted one day a week, except during cold weather. In addition they were given bones for nutritional enrichment. Wolves were only fed immediately prior to or during an observation session on a few occasions.

Thirteen wolves from four enclosures (Pen 7 = 1 adult female, 3 male pups and 2 female pups; Pen 8b = breeding pair; Pen 11a = breeding pair; Pen 12 = breeding pair and 1 juvenile male) were observed from 16 June – 26 August, 2003 (Table 2.1). These enclosures were situated in a square grid, allowing the observer to change observation pens with minimal changes in set-up. The time period was chosen to ensure the least disturbance to the wolves and management staff. Vocalisations were not recorded during breeding or early pup rearing to ensure that there was no interference with rearing success. With the exception of sexual behaviour and pup rearing, there is no evidence of seasonal effects on short-range sound production (Weir, 1999). Wolves were distinguishable by sex and markings. Two housing changes took place during the course of the study. On 17 June, female 1123 was introduced to male 687 in Pen 11a. On 7 July, two adults were removed from Pen 7 and replaced with female 1009 and her five pups. Wolves will be referenced to by pen number and sex or age (i.e. F12 is the female in pen 12 and J12 is the juvenile in pen 12). Pup vocalisations were not included in the study.

Data Collection

Observation sessions were conducted five days a week in early morning or late evening, when captive wolves are most active (Schneider and Mace, 2005; Weir, 1999). In general, wolves showed most activity prior to sunset, so the majority of recordings were made in the evenings. To insure the highest range of activity types, morning sessions were conducted at least once a week. Because wolves are not active in the evening if daytime temperatures are high, morning sessions were conducted during the warmest parts of the summer.

Table 3.1 Age and housing information for red wolves observed At the Point Defiance Zoo and Aquarium breeding facility during June-August 2003.

Wolf*	Age (years)	Month moved to current pen**	Physical contact with neighbouring wolves?
F7***	4	Jul-03	No
F8	9	Jan-02	Yes
M8	12	Apr-02	Yes
F11	2	Jun-03	Yes
M11	9	Feb-01	Yes
J12	1	May-02	No
F12	11	Dec-97	No
M12	10	Mar-01	No

*Wolves labelled by gender or age and pen number in which they are housed F = female, M = male, and J = juvenile male.

** F8 moved out Jan-Apr, 2003, F12 moved out a few months in 2000

*** Housed with her 5 pups born April 28, 2003

Two blinds were used for observations during the course of the study. Management staff constructed a camouflaged wooden blind on the path at the intersection of enclosures 12, 11a, 8b, and 7. This blind had little mobility and poor visibility due to the angle of the window and the fence meshing. Hence, a nylon tent was used for the majority of the study. This blind was placed on the path between two pens for the optimal viewing of both pens (Appendix 2). The blind was moved once a week at the end of an observation session, so that wolves in pens 11a and 12 were observed one week and those in pens 7 and 8b were observed the next. The majority of each enclosure could be seen through the camera's wide-angled lens where the maximum distance between camera and wolf was 43 m. The slope of the enclosure, vegetation, dens, and fence meshing obscured the view of the wolves during some parts of filming.

Video recordings were made using a Sony Digital-8 Camcorder (Model DCR-TRV240) with an external Audio-Technica AT835b shotgun microphone mounded on a tripod such that the camera and microphone were always pointed toward the target wolves. Previous research has demonstrated that the audio tracks from analog video recorders are sufficient for analysing close-range wolf vocalisations (Weir, 1999). However, the digital camcorder used in this study did not have manual audio gain-control; as a result faint structural details of the vocalizations may not have been adequately recorded (see Appendix 1).

Wolves were given 5-10 minutes after I entered the blind to adjust to my presence before the beginning of each recording session. Recordings were made of one pen a day. Sessions lasted until one hour of video had been collected or wolves had been observed for two hours. The camera was turned off whenever wolves were inactive for more than one minute and sessions terminated when inactivity surpassed 20 minutes. To ensure the behaviour of the vocalising animal and the recipient could be analysed, the camera was situated so both wolves were in the video field whenever possible. If both wolves could not be kept in the video field, the camera was centred on the most active wolf. The camera was zoomed in on wolves when they were interacting and zoomed out when they were less active. Approximately 40 hours of video was collected.

Data Analysis

Video Processing

Video was downloaded from the camera onto a computer using Pinnacle® Systems Studio 7. Raw video was converted to MPEG format using Studio 7 and Adobe® Premier® Pro 7.0. MPEG videos were burned onto DVD for analysis and storage. Videos were viewed using Windows Media Player 9.0. Each video was viewed in its entirety and the time of occurrence of each short-range vocalisation and identity of the vocalising wolf was recorded when known.

When possible, identification of the vocalising animal was determined using field notes indicating from what direction/wolf the sound was coming, amplitude of the signal in relation to the wolves' proximity to the camera, and visual identification of wolf movement associated with vocalising. When producing affiliative vocalisations, wolves sometimes bellow their diaphragm-rib cage and/or their nose-snout region in a fashion similar to when they are sniffing. These visual cues, however, are difficult to observe during brief vocalisations.

Audio Analysis

Vocalisation structure was quantified by spectrogram analysis using Wavesurfer 1.6.0 (Sjölander and Beskow, 2003, http://www.speech.kth.se/wavesurfer/index.html) for sounds greater than 2 kHz and Raven 1.0 (Cornell Lab of Ornithology) for sounds less than 2 kHz. AVI video clips containing affiliative vocalisations were copied from raw videos and analysed in Wavesurfer using both wideband (FFT window length 256, Hamming analysis, bandwidth 375) and narrowband (FFT window length 1024, Hamming analysis bandwidth 58) settings. For low-frequency vocalisations, AVI clips were converted to WAV files and analysed in Raven using both wideband (FFT window length 512, Hamming analysis, 3dB bandwidth 122) and narrowband (FFT window length 2048, Hamming analysis, 3dB bandwidth 30.5) settings.

Spectograms were made for each tonal vocalisation. The term vocalization is used to refer to the complete utterance produced by the wolf. Unit refers to the continuous tracing of sound on the spectrogram. Linear units have one component, while non-linear units may have multiple components. Groups of units within the vocalization are referred to as phrases. Figure 2.1 shows an example of the vocalisation structure and the measured acoustical parameters. New vocalisations were determined primarily by changes in context or sometimes arbitrarily by inter-unit intervals of greater than 10 seconds. Phrase identification within the vocalisations was determined by a plot of the log frequency of the inter-unit intervals (Sibley *et al.*, 1990); phrases were separated by inter-unit intervals greater than 500 msec (Appendix 3).

Tonal vocalisations were quantified by fundamental frequency at the start and the end of each unit (or unit component) (Hz), maximum frequency (Hz), minimum frequency (Hz), and average frequency (Hz). Duration of each unit (msec), inter-unit interval (msec), cycle duration (duration of unit and following interval) (msec), and number of units per vocalisation were also recorded. Rate (units/total cycle durations) and duty cycles (total unit durations/total duration) were calculated for each vocal phrase (Fig. 2.1). The presence or absence of biphonations, frequency jumps, harmonics, and subharmonics was noted.

Reliability of Audio Analysis

Measurement reliability was tested by making new measurements on a random sample of the first 411 sound units that were analyzed, totalling 35/411 sound units and 41 unit components. The two sets of measurements were significantly correlated for both temporal and frequency variables (correlation range = 0.989-1.000, $p < 0.001$) and there was no significant differences between sets for temporal or frequency variables (t range = -1.19 to 0.71, $p > 0.05$).

Classification

All tonal sounds that were of sufficient clarity on the spectrograph were measured and classified. Sound unit types were identified and classified based on their overall acoustic structure, the presence and nature of non-linear phenomena, and the similarity to previously published vocal categories. Vocalization types were determined according to their unit compositions.

Video Analysis-Activity Budgets

In total 65.8 hours of video were coded to estimate the activity budgets of each wolf and of the group: 8.0 hours per wolf from Pen 8b, 8.4 hours per wolf from Pen 11a, and 11.0 hours per wolf from Pen 12. These activity budgets were used to examine individual differences in activity allocation, the frequency of tonal vocalisations in comparison to other vocalisations (i.e. barks, growls, howls, and other), and the frequency of occurrences of tonal vocalizations within each activity state. Videos from Pen 7 were excluded because it was difficult of identifying individual pups and heavy foliage prevented continuous observations of F7. Videos containing unusual events such as the introduction of a new animal or the presence of a caregiver in the compound were also excluded from analysis.

Continual sampling of individuals was done using video playback and Jwatcher™ 0.9 (Animal Behaviour Laboratory Macquarie University, 2000) behavioural coding software in 10 - 30 minute segments. The behaviour of each adult pair was coded simultaneously by two observers, with one observer viewing the male and one the female for each pen. Observers alternated wolves with each video to limit bias. J12 was coded separately by a single observer.

Coding reliability was increased through training, testing, and review. Observers chose videos at random and coded 10-minute clips totalling 2-3 hours of video until they felt confident in their coding reliability. They then coded the same 10-minute video clip independently and compared their codes. Coding was nearly identical and time was reliable within 2 seconds. During coding of adults, complex interactions were discussed and changes made in the coding output, when necessary.

Behaviours were scored in two ways: states and events. States were behaviours that had definite beginning and end points, thus a definite duration, and included all behaviours displayed during the observation time. A single occurrence of a state is referred to as a bout. The number and cumulative duration of all bouts in each state were used to construct activity budgets, or activity patterns, for each wolf and to calculate vocalization rates. Behaviours that occurred as part of these states were scored as events, occurrences with no defined durations. They were used to define more detailed description of each state. State codes were designed to be mutually exclusive and exhaustive, and to be consistent with the published literature whenever possible (see also Zimen, 1982; Wagener, 1998; Frézard and Le Pape, 2003). Behavioural states that were coded and associated events are as follows:

I. Unknown Activities

Not visible: wolf was out of sight behind vegetation, den, or hill or camera was off and behaviour not noted.

II. Individual Activities

Sleeping: wolf was lying down with head down and eyes closed, or camera was off or not on wolf and field notes indicate wolf was lying with head down or obviously sleeping.

Lying down: wolf was lying down, but head and ears were up and active.

Sitting: wolf was sitting with rear end on the ground and front legs straight

Standing: wolf was standing on all four feet without moving legs

Locomotion: wolf was walking, pacing, trotting, or running

Object play: play of one wolf directed at an object such as a stick, branch, bone, or dead crow; behaviour involved shaking, tossing, and pouncing on the object.

Self-directed maintenance: wolf behaviours that were natural body functions or maintenance activities including: *hygiene* (stretching, rubbing, or grooming), *nourishment*: (eating, drinking, gnawing on bones), and *elimination* (urinating, defecting, scrape marking).

Other (lumped with self-directed maintenance to simplify analysis): Digging, sniffing/investigating, and any other individual activity not included above.

III. Interactive Activities:

A. Affiliative Interactions: Affiliative interactions are directed at another wolf and serve to establish or reinforce the social bond between initiator and recipient (Poole, 1985).

Howling Session: included all wolf activity during a group howling session, which may involve vocalising (howls, barks, and squeaks), movement, socialising, etc. as long as the wolf was clearly engaged in the howling session.

Socialising: activities in which wolves initiated or received physical contact on any portion of their body that could not be classified as play or agonistic behaviour including: *A-G investigation* (wolf sniffed or licked another wolf's ano-genital region), *body contact* (wolf placed paw or muzzle on the main body of another wolf), and *muzzle contact* (wolf sniffed or licked another wolf's muzzle).

In this study play was classified into three sub-states based on the focus of the play. Object play has been addressed already. Before describing social play it is first necessary to discuss play as a general state. Play is a state of exuberance. It can utilise the same motor actions as other types of behaviour such as fighting or copulatory mounting, but can also be original and often unpredictable. It is often preceded and maintained by behaviours that indicate play intent (Bekoff, 1974). Play can be directed at other individuals, objects, or self. In captive situations play can also be directed at wolves in neighbouring pens, human observers, and other animals.

Neighbour play: play of one wolf directed at a wolf in an adjacent pen, or at the observer. The fence separating the animals constrained play behaviour by limiting physical contact. Behaviours included running up and down the fence line, digging at or scattering rocks along the fence, jumping against the fence, and biting or nibbling through the fence.

Pen-mate play: play directed at wolves within the same pen including: *initiation* (behaviour that initiated or attempted to initiate play with another individual, such play bow, leaping, exaggerated approach, tail wagging, head tossing, paw raising, etc.), *locomotor play* (play that involved minimal contact, but lots of movement such as stalking/ambush, chasing and circling with occasional body slamming or scruff or body bites), and *full-contact play* (play that involved extensive physical contact such as jaw wrestling, body slamming, scruff biting, tail pulling, pinning, wrestling, one up/one down, and prone play), and *social object play* (play that involved more than one wolf interacting with an object such as a stick, branch, bone, or dead crow).

B. Ambivalent Interactions: Ambivalent interactions are those between play and aggression that involve body postures intermediate to threat or play, and do not result in displacement or successful defence of physical space.

Ambiguous: included interactions that contained elements of both agonistic and play behaviour, such as aggressive vocalisations or relaxed body postures, with no clear distinction between categories.

C. Agonistic Interactions: Agonistic interactions include the behaviours of both the initiator and recipient in an aggressive encounter (Bekoff, 1981; Poole, 1985). It occurs when wolves display behaviour intended to either displace another individual by force (offensive) or use force or the threat of force to prevent displacement (defensive). It can end in a truce (both wolves displaced) or with a victor and loser (one wolf displacing the other or maintaining its place when challenged by another). Intense aggression can result in serious and even fatal injury. Aggression can occur over possession of physical space (resource use) or social space (social freedom) (Bekoff, 1981). Agonistic interactions were rare and the following were lumped for analysis.

Agonistic: includes all levels of aggression from *threat* (non-contact aggressive interactions that included gape, grin, bared teeth, tooth snapping, arched back, raised hackles, raising tail and/or growling) to *attack* (aggressive movement toward another individual such as a lunge or charge, resulting in either displacement of the individual (i.e. chasing) or physical contact such as biting, pawing, and hip or body slamming), and responds to an aggressor by reciprocating with any of these behaviours or rolling over on one's side or back, fleeing (i.e. being displaced), and/or tail tucking.

IV. Vocalisations were coded as events that could occur during any behavioural state.

(For more detailed definitions of vocalizations see McCarley, 1978; Schneider and Mace, 2005)

Tonal vocalisation: low amplitude, short duration, high-frequency sound (i.e. squeak, banded squeak, complex squeak, wuh, and squeak-wuh vocalizations) (Chapter 2)

Growl: moderate duration, noisy sound

Bark: short duration, noisy sound

Howl: long duration, high amplitude, varying frequency sound

Other: any other vocal sound produced by the wolf (e.g. yelp, huff)

Video Analysis-Relational States

Repeated replay of video was also used to determine the social contexts of the vocalising animal. For each vocalisation, the identity of the vocalising wolf, the potential recipients, the proximity to the nearest wolf, the activity state, and the relational state were coded whenever possible (see Appendix 8). The vocalising wolf's proximity to recipient wolves was estimated in wolf-lengths. Activity state was coded using the same scheme using for activity budgets. The vocalising wolf's relation to (movement/orientation toward or away from) the recipient(s) during or immediately following (< 1 sec) each vocalisation was also coded as follows:

Directed

Pen-mate: a decrease in the physical or social distance between individuals within a pen including orienting toward, orienting toward and approaching, or orienting toward and being approached by a pen-mate.

Other: a decrease in the physical or social distance between the vocaliser and wolf or unknown entity (e.g. caregiver) outside the pen including orienting toward and sometimes approaching a neighbouring pen or unknown object outside of the pen, or orienting toward a neighbour pen or unknown object outside of the pen and being approached by a pen-mate.

Termination: an increase in the physical or social distance between individuals including moving away from a recipient with or without orienting first or transitioning from one activity state to another—generally standing to locomotion.

Non-specific: movements with no obvious relation to other wolves including: vocalising while, while maintaining constant distance between itself and recipient, or while yawning..

Statistics

All statistical tests were performed using SPSS 12.0 unless otherwise noted. Correlation analysis was used to test for independence between activity, relational, and proximity contexts. Measures of proximity were correlated with relational state and dropped from analysis (Pearson $r = 0.44$, $p < 0.001$). In general, vocalisations produced in the directed pen-mate state were produced at closer proximity to the recipient than those produced in the directed-other state. Correlation analysis was also used to determine if acoustic measurements were independent. The five frequency variables were highly correlated and hence only start frequency was included in analyses (Appendix 6).

Preliminary exploratory analysis was used to determine if vocal structure differed by activity or relational states. Only states in which two or more vocalisations of a given type occurred were included in the analysis. For each vocalisation type, ANOVA was used to determine which acoustic variables potentially discriminated between states at the phrase and at the unit level. If only one variable differed and $p < 0.05$, this variable was graphed as a scatter plot with state as the independent variable and visually examined for degree of overlap of the dependent variable between states.

If two or more variables differed by $p < 0.15$, they were used in discriminate function analysis (DFA) to explore if and how these variables distinguish between states. An alpha of 0.15 was chosen to ensure that all variables that may contribute to discrimination when in combination with other variables were included. Discriminate functions were generated and used to categorise sounds by state, and the proportions of vocalisations correctly categorised were compared to chance. When group covariance matrixes were equal, cross-validation was used to categorise sounds (i.e. the sound being categorised was not included in the discriminate function). When group covariance matrixes were not equal, sounds were categorised using separate-group covariance matrixes and categorisation was validated using repeated random sampling and categorising. A state was considered distinguishable from others if vocalisations for that state were correctly categorised more often than chance and few vocalisations from other states were mis-categorised as belonging to that state.

Results

Distribution of Vocalisations across Relational States

The type of vocalisation, potential recipient(s), and contexts were coded for each of 111 occurrences of tonal vocalisations in which the identity of the vocalising wolf was known. The orientation and/or movement of the vocalising wolf in relation to other wolves was coded into three primary categories: directed, termination, and non-specific. Data from the five wolves that produced more than three vocalisations were included in the analysis. The first analysis treated all vocalisations with only high-frequency components (SV, CSV, BSV) as squeaks to parallel the analysis performed by Weir (1999). When uttering squeak-like vocalisation types, wolves were significantly more likely to be directed toward other wolves or entities than to be terminating an activity or engaging in a non-specific activity (GLM repeated measures $F_{2,8} = 5.00$, $p = 0.039$ (Fig. 3.1a).

Once non-linear vocal phenomena have been taken into account a more complicated pattern of results emerges. With the exception of squeak vocalisations, which occurred in similar proportions across the three relational states, all vocalisation types occurred most frequently in the directed state (GLM repeated measures $F_{2,32} = 4.89$, $p = 0.04$) (Fig. 3.1b). Fig. 3.1c shows the mean number of vocalisations directed toward others and pen-mates. Although not significant (GLM repeated measures $F_{4,16} = 1.12$, $p = 0.38$ interaction term), this figure reveals two noteworthy trends: wuh vocalisations were directed mostly toward pen-mates, and banded squeak vocalisations were directed most often outside the pen.

Activity Budget Profiles

Activity profiles for seven wolves (excluding wolves in Pen 7) were constructed using continual behavioural coding. All wolves spent the largest proportion of their time out of view, ranging from 25-60%, limiting the total time in view to 40.4 hours. When visible, wolves spent most of their time in individual activities such as sleeping, lying

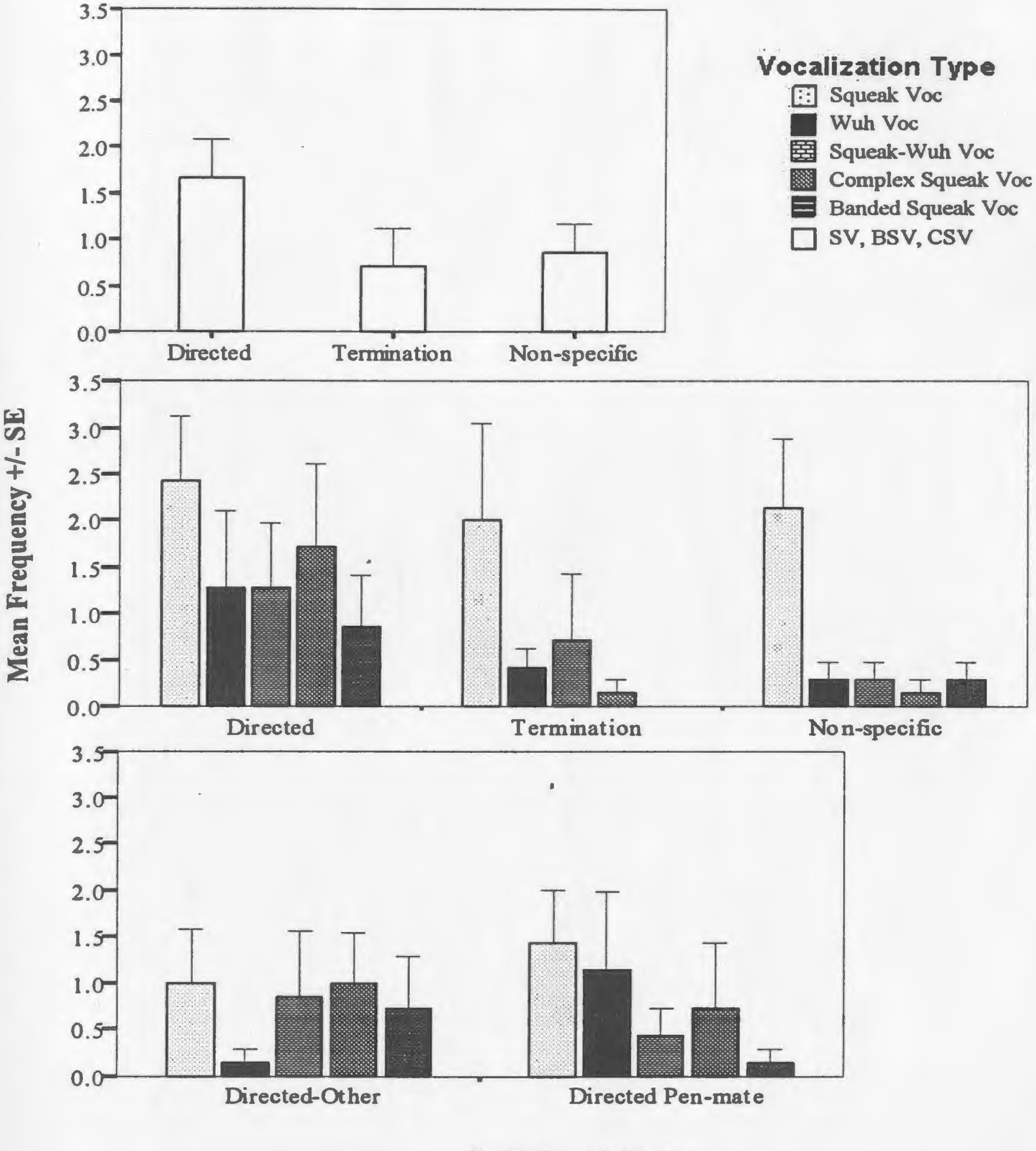

Figure 3.1 The mean number of tonal vocalisations produced by five captive red wolves, (*Canis rufus*) (a) differed significantly across relational states when all squeak-like vocalisations were pooled (n = 67), (b) differed significantly when comparing all five vocalisation types (n = 152), but (c) did not differ significantly when the target of directed states was specified (n = 53).

down, standing, or walking (54.3% duration, 73.4% bouts). Sitting was rare (Table 3.2). The seven wolves observed demonstrated individual activity patterns (Fig. 3.2). Wolves in Pen 8b showed similar activity patterns to each other, M8 spending more time in sight. F11 was more active than M11. Howling sessions were the most common affiliative interactions across pens. Wolves in Pen 12 participated in more affiliative interactions with each other than did wolves in Pen 8b or Pen 11a. M12 engaged in a higher proportion of neighbour play than other wolves, mostly directed at the observer. J12 engaged in the highest proportions of pen-mate and object play. Ambiguous and agonistic interactions were rare, with none occurring in Pen 8b.

Vocalisation Rates

The occurrence of growls, howls, barks, tonal vocalisations, and other vocalisations was coded along with activity patterns for the seven wolves. Howls and barks occurred exclusively during group howling sessions. Each howling session, including all howls, barks, and squeaks, was considered a single vocal event. Due to the difficulty of distinguishing an individual's vocalisations during a group howling session, precise numbers of vocalisations could not be determined. Howling sessions were composed of a minimum of 915 howls, 367 barks, and 44 squeaks.

Of the 293 vocal events recorded, 44% were tonal vocalisations, 32% growls, 19% howling sessions, and 6% other vocalisations. Table 3.3 shows the rate of vocal events for each wolf during in view observations (i.e. for each wolf: number of vocalisations of each type divided by the total number of hours the wolf was in view). Tonal vocalisations were the most common vocal events for three of the seven wolves

Table 3.2 Activity budgets of captive red wolves (*Canis rufus*) by duration (hr) and bout collapsed over three pens and seven red wolves.

	Duration		Bouts	
	Total (hr)	Percent	Total	Percent
Not visible	25.32	38.5	1865	21.3
Sleeping	11.40	17.3	364	4.2
Lying down	8.37	12.7	502	5.7
Sitting	0.16	0.2	48	0.5
Standing	5.11	7.8	1602	18.3
Locomotion	7.47	11.4	2941	33.5
Other solitary	2.79	4.2	804	9.2
Howling session	2.58	3.9	56	0.6
Socializing	0.50	0.8	168	1.9
Pen-mate play	0.67	1.0	162	1.8
Neighbour play	0.81	1.2	130	1.5
Object play	0.44	0.7	85	1.0
Ambiguous	0.04	0.1	10	0.1
Agonistic	0.10	0.2	31	0.4
Grand Total	65.76	100.0	8768	100.0

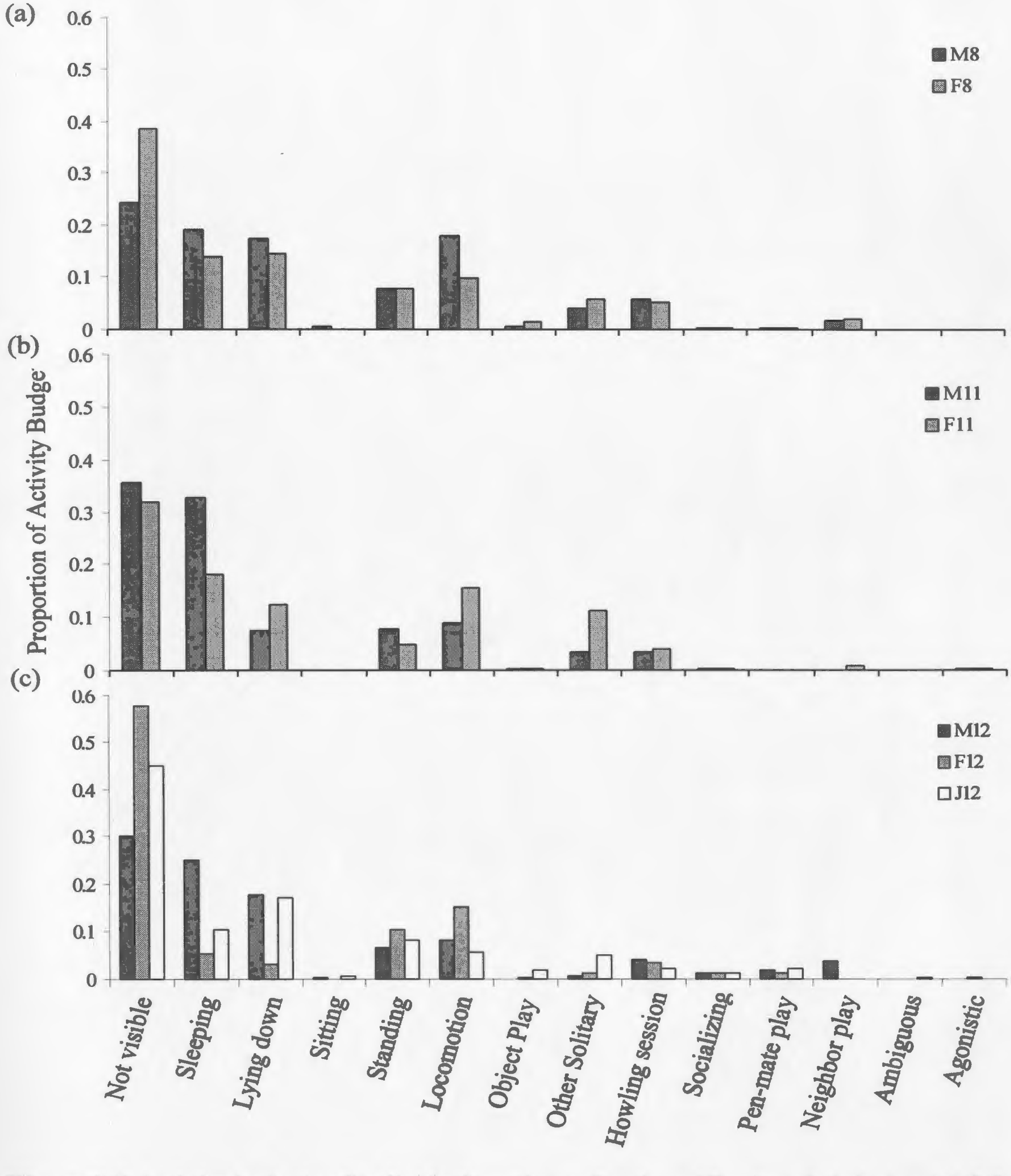

Figure 3.2 Activity budgets of individual captive red wolves (*Canis rufus*) during (a) 8.0 hours of observation per wolf in Pen 8b (b) 8.4 hours of observation per wolf in Pen 11a and (c) 11.0 hours of observation per wolf in Pen 12.

Table 3.3 Rates (hr) of vocalisation events produced by individual captive red wolves (*Canis rufus*) and percentage of total vocalisation events. Howling sessions were considered single vocalisation events and included howls, barks, and squeaks. (n = 293)

Vocalisation Event	M8	F8	M11	F11	M12	F12	J12	Total	Percentage
Tonal	1.3	0.6	4.6	0.7	5.8	4.7	4.3	3.2	44
Growls	1.0	1.2	0.6	0.0	6.6	1.7	5.1	2.2	31
Howling Sessions	1.3	1.6	1.8	1.6	1.3	1.0	1.2	1.4	19
Other	0.0	0.0	0.0	0.2	0.0	0.0	2.8	0.4	6

and the most common overall. The highest rate of vocal events for F8 and F11 involved howling, while M12 and J12 growled most often.

Activity budgets were used to calculate the production rates of tonal vocalisations in each activity and relational state. All 190 occurrences of tonal vocalisations were included, whether or not they could be identified by vocalisation type, or attributed to particular wolves. Tonal production rates were highest during pen-mate play and howling sessions, followed by sitting and standing, then socialising and agonistic interactions (Fig. 3.3). [Note: Rate is misleading here. Only one tonal vocalisation occurred during a single agonistic interaction.] Vocalisations that occurred during interactions were most often directed at pen-mates. During individual activities, wolves vocalised in all relational states, especially in directed-other and non-specific states. Vocalisations also occurred in the non-specific state during object play and other individual activities. Vocalisations during howling sessions occurred in the non-specific state by definition.

The next analysis was restricted to the 125 vocalisations, where the vocalisation type could be identified (SV, WV, SWV, CSV, BSV). The production rate of each vocalisation type was calculated for each of the seven wolves in each activity state where more than one wolf vocalised. Production rates varied significantly as a function of vocalisation type and activity state (GLM repeated measures $F_{16,96} = 2.346$, $p = 0.006$), as shown in Fig. 3.4. In general, production rates were highest for all vocalisation types during standing, socialising, and pen-mate play. Squeak vocalisations were the primary vocalisations used in individual activities (i.e. lying down, standing, locomotion), while wuh vocalisations were frequent in affiliative interactions (e.g. socialising, pen-mate

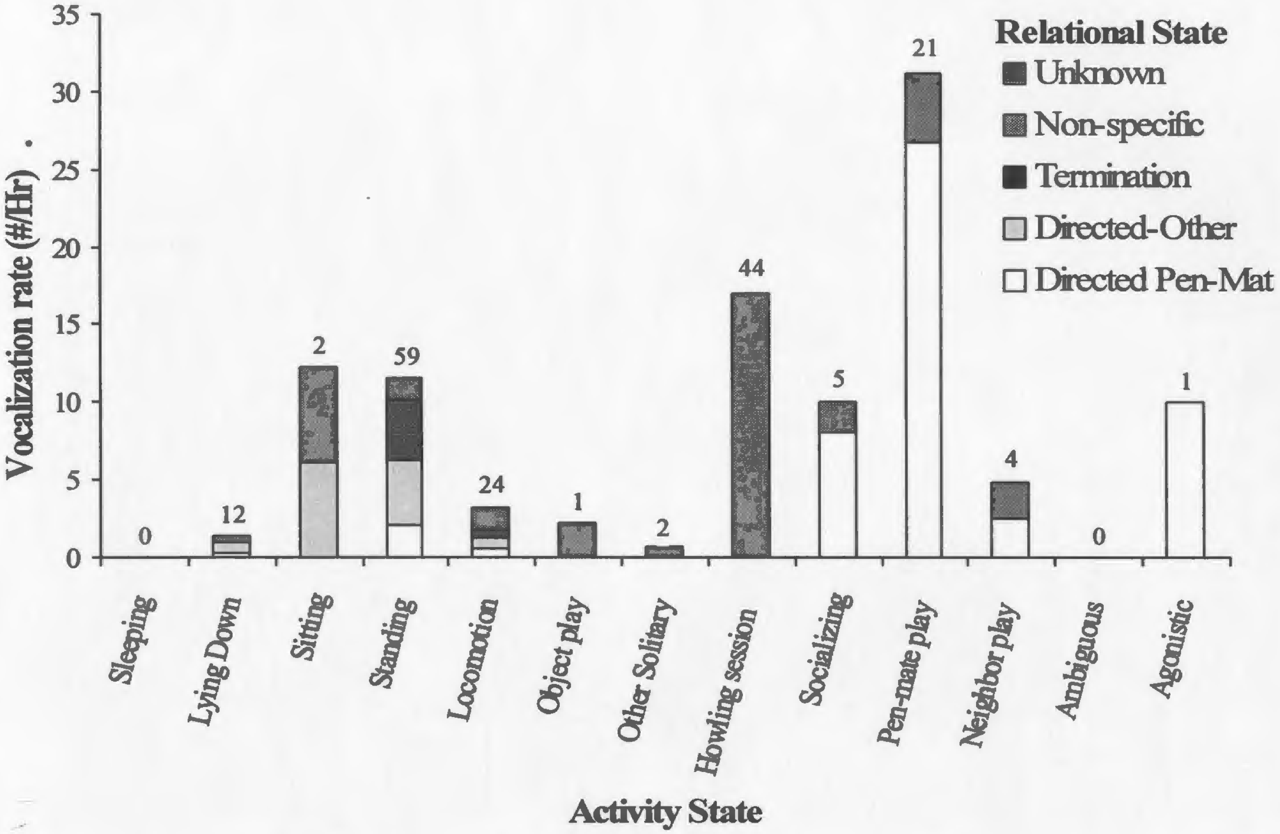

Figure 3.3 Production rates of tonal vocalisations produced by captive red wolves (*Canis rufus*) according to activity and relational states for 40.4 hours of in sight observations totalled over seven wolves. Numbers above the bars represent the number of vocalisations used to calculate each rate (n = 175 vocalisations).

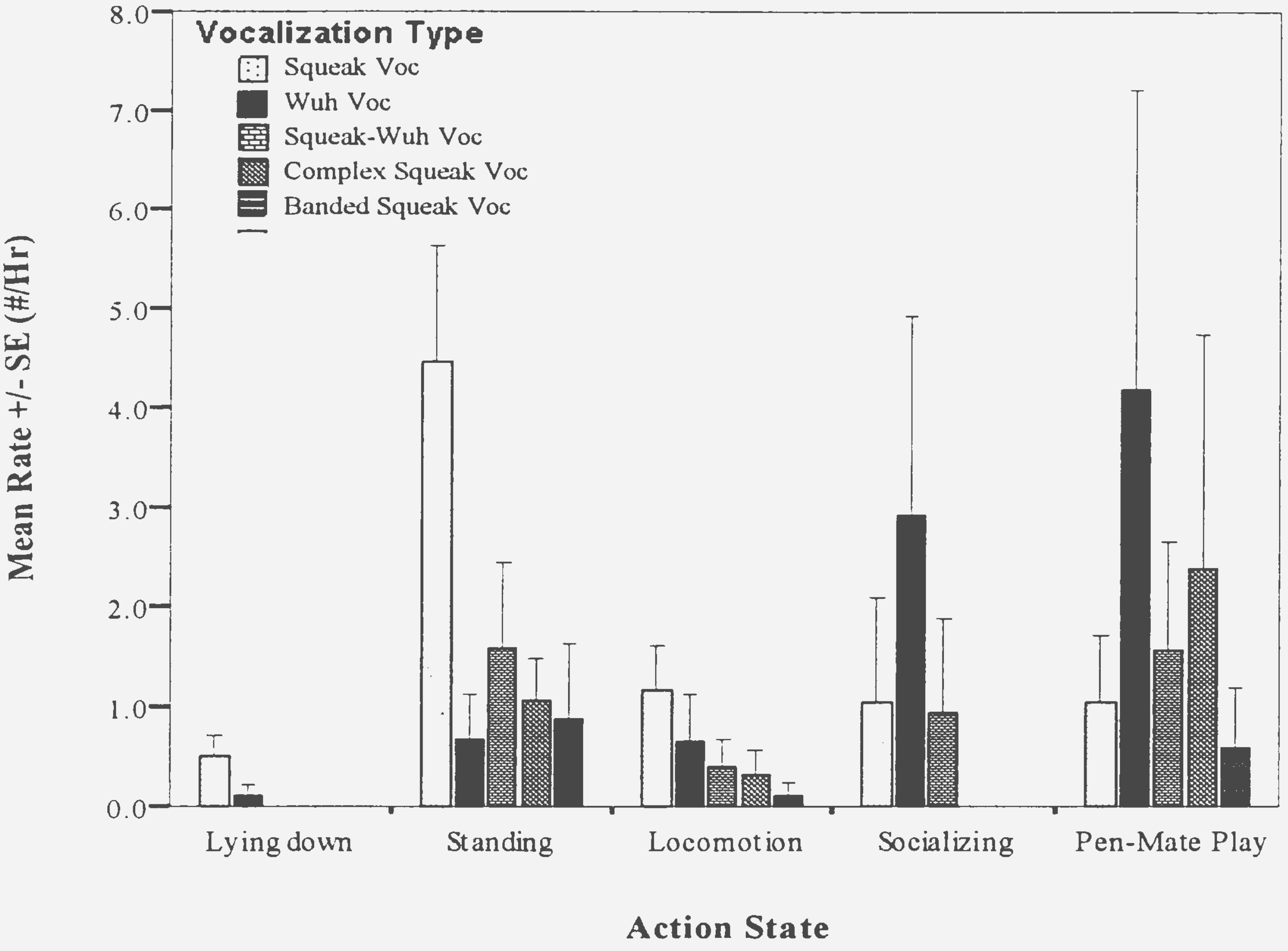

Figure 3.4 There was a significant interaction between the type of tonal vocalisations produced by captive red wolves (*Canis rufus*) and the activity states in which they occurred. Bars show mean hourly rates and SE for tonal vocalisations given in each activity state in which more than one wolf vocalised during 40.4 hours of in view observations of seven red wolves.

play). Complex squeak vocalisations were also frequent during pen-mate play.

Vocalisation Structure/Context Analysis

Exploratory analysis using discriminate function analysis and scatter plots was performed to determine if vocalisations differed structurally between relational and activity states. All vocalisations of significant quality to quantify from all videos for eight red wolves were used in this analysis. At the phrase level, vocalisation types did not differ significantly from each other and phrases did not differ between relational or activity states.

At the unit level, in the analysis of relational states, squeak vocalisations and complex squeak vocalisations directed toward others were distinguishable from other relational states (Table 3.4). Squeak vocalisations differed by start frequency, inter-unit interval, then duration (50% correct categorization), while complex squeak vocalizations differed by unit cycle, start frequency, then unit duration (96% correct categorization). Most SVs and CSVs produced during directed-other contained high-frequency components (6-10 kHz) that rarely occurred in other relational states. Activity states did not differ at the unit level.

Discussion

In this study I explored the contextual situations in which red wolves produce short-range tonal vocalisations. Activity budgets were determined for each of seven wolves, and vocalisation rates were calculated for each activity state. The orientation/movement of the vocalising wolf in relation to other wolves was coded for each vocalisation. Tonal vocalisations were associated with affiliative behaviours and

Table 3.4 Descriptive variables used by Discriminate Function Analysis to categorize vocalization by relational state. Vocalizations produced in the Directed-Other state could be differentiated from those produced in other relational states using functions produced from these variables. Means are weighted by wolf.

Relational State	Grand Mean (SD)			
	Start Frequency (Hz)	Inter-unit-interval (msec)	Unit Duration (msec)	Unit Cycle (msec)
Squeak Vocalization (n = 6)				
Directed Pen-mate	5114 (315)	216 (58)	145 (99)	-
Directed Other	6014 (1773)	178 (32)	76 (31)	-
Termination	4560 (434)	176 (18)	93 (61)	-
Non-specific	4988 (742)	142 (84)	131 (107)	-
Complex Squeak Vocalization (n = 4)				
Directed Pen-mate	4665 (-)	-	210 (-)	485 (-)
Directed Other	5916 (837)	-	98 (49)	309 (75)
Termination	-	-	-	-
Non-specific	-	-	-	-

were primarily directed toward other wolves. Production of tonal vocalisations was highest during howling sessions and pen-mate play, the most intense social interactions observed. Only one tonal vocalisation occurred during an agonistic interaction, which was also the only agonistic interaction displayed by M12. The wolves that had the highest rates of affiliative interactions, those in Pen 12, also produced the highest rates of tonal vocalisations (with the exception of M11 who will be discussed later). Pen 12 wolves also produced the highest rate of growls, which were often used during play. These results support the hypothesis that tonal vocalisations are affiliative and can be referred to as affiliative vocalisations.

Behavioural observations in the field support the conclusion that tonal vocalisations are affiliative in nature. At the beginning of the study period, wolves throughout the breeding facility were unfamiliar with me and would produce alarm barks as I walked to and from the blind. After a few days wolves acclimated to my presence. Their behaviour changed and many started directing play initiation behaviour accompanied by squeaks toward me.

Red Wolf –Gray Wolf Comparison

The affiliative nature of tonal red wolf vocalisations is consistent with what has been reported of gray wolf squeak vocalisations. Weir (1999) found that during seven 24-hour watches in 1997, 70% of squeaking vocalisations produced by the seven gray wolves at the Canadian Centre for Wolf Research (CCWR) when wolves were directed toward other wolves. When red wolf squeak-like vocalisations (SV, CSV, BSV) were combined a similar pattern was found. However, when non-linear sounds were considered, squeak vocalisations were fairly evenly distributed across relational states, while banded squeak and complex squeak vocalisations occurred primarily in directed states. Wuh and squeak-wuh vocalisations followed the same relational pattern as the banded squeak and complex squeak vocalisations. Thus far, wuh and squeak-wuh vocalisations have not been reported in gray wolves.

Red wolf tonal vocalisations also occur in similar activity states as gray wolf squeaks. Weir (1999) reported that gray wolves squeaked primarily in prosocial situations (64%) during the 24 hour watches, including when wolves were in close proximity to conspecifics, socialising, and playing. In addition, she noted that squeaks occurred frequently during howling sessions. Squeaks during howling sessions are difficult to find and quantify on a spectrogram due to the large number of simultaneous vocalizations involved in a howling sessions. To the human ear, these squeaks are greater amplitude and possibly contain more variation in frequency contour than squeaks given in other states for both species (J. Weir and R. Anderson, personal communication).

Red wolves differed from gray wolves in that red wolves also vocalised frequently during individual activities such as sitting and standing. Housing differences between study sites may have contributed to this difference. Red wolves were housed in adjoining pens. At CCWR in 1997, the seven pack-reared wolves were lived in a heavily forested 3.8 hectare enclosure with minimal human intervention (Weir, 1999). While the full extent of the impact of living conditions on vocal production cannot be determined, several factors can be considered.

Possible Housing Effects

While red wolves were able to communicate visually and acoustically across fences, only wolves in pens 8b and 11a were able to make physical contact with their neighbours in 8a and 11b, respectively. Wolves in other pens were not able to respond by engaging in affiliative behaviours with the vocalising wolf. This limitation to interactions led to a number of behaviours that would not occur in wild situations, and may have led to longer vocalisation bouts and increased chances of terminating an action immediately after the vocalisation. Wolves often stood vocalising while oriented toward a point outside of their pens, then walked away immediately after vocalising.

The complexity of the social situation created by the multi-pen design of the red-wolf breeding facility can be seen in the relational states in which affiliative vocalisations occurred. Wolves vocalising while participating in individual activities did so in a variety of relational states. They often produced affiliative vocalisations when they were clearly oriented toward someone or something outside their pen. Examination of the vocalisation structure indicated that squeak and complex squeak vocalisations occurring in the directed-other state might be higher in start frequency than when those types of vocalisations occurred in other relational states. However, sample sizes were small and further study is necessary before generalisations can be made.

The production rates of the various types of tonal vocalisations did not differ significantly between those directed toward pen-mates and those directed toward others. While wuh vocalisations were used primarily when wolves were directed toward pen-mates and banded squeak vocalisations were used primarily when wolves were directed toward others, those trends were driven by individual wolves (e.g. M11 produced 11/13 banded vocalisations). Larger sample sizes are necessary before definite conclusions can be made.

During the study period, the social situation was further compounded by the presence of pups in Pen 7. Wolves in neighbouring pens showed interest in the pups and frequently squeaked to them. When observing Pen 7, it was often impossible to identify from where squeaks were coming. M11 and F12 took particular interest in the pups. Both would stand or pace along the fence/corner adjacent to Pen 7 and vocalise. The majority of vocalisations attributed to M11 occurred while he was standing or walking along the fence adjacent to the pups. M11 demonstrated obvious desire to interact with the pups, again affirming the affiliative nature of tonal vocalisations. The use of tonal vocalisations in association with pups is consistent with what is known of gray wolf squeaks (Coscia *et al.*, 1991; Goldman *et al.*, 1995).

Between Pen Differences

Individuality and differences in social situation influenced the differences in social behaviour between pens. Each wolf was an individual, following its own behavioural patterns, which influenced the way the pair or group interacted. Each pen's social situation was unique. Wolves in Pen 8b had been housed together for some time. Their social interactions were brief, often only involving a quick orient when passing each other or touching of muzzles. Wolves in Pen 11a were introduced during the first day of observations. Initially these wolves displayed agonistic behaviour or avoided each other. By late summer they started to participate in affiliative interactions. Tonal vocalisation production for three of the four wolves in these pens was minimal.

The number of affiliative interactions and vocalisations increased with pack size. Most of the vocal interchanges observed in this study occurred in Pen 7 and 12. All but one of the interchanges in Pen 12 occurred during play, when the identity of the vocaliser was hard to determine. One clear interchange occurred between F12 and J12. The interaction began with F12 standing several wolf lengths behind J12 who was sitting. F12 vocalized, J12 oriented with his ears (Goldman *et al*., 1990) and gave a vocal response, F12 vocalized again, and J12 stood up, walked over to F12 and initiated greeting. This interaction shows the importance of ear orientation, which was not considered in this study.

Vocal interchanges also occurred between pups in Pen 7, however difficulty in identifying pups made these vocalization hard to quantify. Unfortunately, since many vocalizations were directed at Pen 7 and F7 often remained in parts of the pen with poor visibility, few vocalizations could be positively identified as hers. It is not surprising that the number and complexity of social interactions increased with the number and complexity of the group.

The presence of pups and the yearling served as a catalyst for social behaviour. The inclusion of the male yearling in Pen 12 probably increased the social behaviour of the group. J12 often initiated greeting ceremonies and pen-mate play. Wolves are known for their willingness to interact with pups. All pack members participate directly or indirectly in pup care, either through den digging, supplying mothers and pups with food, pup guarding, and playing (Packard, 2003). In particular, red wolves in captivity and the wild willingly foster pups that are not related to them (W. Waddell, personal communication).

Context of Tonal Vocalisation by Type

Different tonal vocalisation types were used in different activity and relational states. Squeaks were used more often than any other vocalisation in individual activities such as standing and walking and in all relational states, suggesting a possible solicitation function. Wuh vocalisations were common in affiliative interactions, especially play, and were primarily directed toward pen-mates. Wuh vocalisations may serve to reinforce the intent of the interaction, essentially saying, “This is still play” (Bekoff, 1974).

The wuh vocalisation provides an example of the difficulty in nomenclature associated with canid vocal sounds. Traditionally the term whimper has been used to refer to squeaks, whines, and a host of other tonal vocalisations. In fact, wuhs probably would be classified as a whimper (e.g. Schassburger, 1993). The term wuh was chosen to be a phonetic representation of the sound, without functional implications. The fact that wuhs occurred in play states in this study is inconsistent with the implied begging function of the term whimper. Discussion within the scientific community on how to analyse and name affiliative canid vocalizations, particularly non-linear vocalizations, is highly recommended.

With the exception of complex squeak vocalisations, mixed-unit vocalisations (i.e. BSVs and SWVs) occurred at moderate rates in both individual and affiliative states. All of the complex squeak vocalisations during play were produced by J12 and could be a product of age or individuality rather than state. The explanation cannot simply be age because adults also produced complex squeak vocalisations in other states. Vocalizations containing both squeak and wuh components may reflect the vocalising wolf's dual messages and/or subtle changes in the context (Wilden *et al*., 1998). Since vocalizations containing non-linear sounds occur across contexts, these sounds might be indicative of increased levels of excitement (Caldwell and Caldwell, 1979; Killebrew *et al*., 2001). These hypotheses can be tested with detailed analysis of the timing and structure of affiliative vocalisations in association with subtle changes in postural communication.

Summary

Tonal vocalisations were the most common vocalisation events produced by three of seven wolves for which activity budgets were available and comprised almost half of the vocalisation events observed. Of the tonal vocalisations that could be identified by type, half were squeaks. As reported in gray wolves (Weir, 1999), squeaking is an important aspect of wolf social interactions. Affiliative social interactions are a necessary part of pack life and tonal vocalisations are a necessary part of affiliative social interactions. As is the case with other social behaviour, one of the functions of tonal vocalisations is to reinforce the social bond between animals enabling the pack to work and live together effectively.

Further study of the association between acoustic structure and contextual usage of wolf tonal vocalizations, in a more ideal captive situation (i.e. less social complexity due to pen arrangement) is needed. Additional contexts such as pup rearing and sexual behaviour should be examined. Ultimately, short-range vocalizations must be studied in wild wolves.

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Chapter 4 Some conclusions and recommendations

Red wolves produce non-linear vocalisations similar to those identified in dholes and suggested for other social canids. These canid vocalisations are composed of a high-frequency whistle-like sound and a lower-frequency tonal sound, which occur separately or in combination as frequency jumps between the two sound types and as biphonations. As is true for dholes, non-linear sounds are common in red wolves. Although used in different contexts, this type of acoustic structure may be common among social canids. Further investigation into the production mechanism(s), variations in acoustical structure and contextual usage between species, and canid perception of these tonal vocalisations is warranted.

Red wolf squeaks, like many high-frequency canid vocalisations are higher in fundamental frequency than the upper range of most mammalian vocalisations produced by normal vocal-fold vibrations. High fundamental frequency, in combination with a general lack of visible harmonics, has lead to a proposal that these sounds are produced by a glottal whistle, rather than by normal vocal-fold vibration. Anatomical studies and computer modelling are needed to confirm this hypothesis. Red wolf wuhs, unlike tonal vocalisations of similar frequency range reported in other canids, frequently occur without visible harmonic bands, suggesting that the lack of harmonics in tonal vocalization might be a product of the resonant cavity. Study of the production mechanism of these vocalisations is also recommended. In addition, it remains to be determined if the production of non-linear phenomena is under the direct control of the vocalising animal or is a natural result of transitioning between linear sound types.

Studies of the production mechanism may help determine to what extent animals intend to make linear and non-linear sounds.

Of the tonal vocalisations produced by the red wolf, only the squeak has been reported in other North American canids. Lehner (1978) reported that coyotes produced wuh-like vocalisations with clear harmonic bands, but no pure-tone wuhs or squeak vocalisations. Gray wolves produce squeaks, and sonograms published by Harrington and Mech (1978) and Schassburger (1993) show evidence of a biphonatic structure between squeaks and harmonic wuh-like vocalisations. Further study of both coyote and gray wolf tonal vocalisations should investigate the occurrence of pure-tone wuhs, non-linear phenomena, and in the case of coyotes, squeaks. The study of affiliative vocalizations in coyotes has potential application to wolf conservation. Understanding the role of vocalizations in affiliative interactions will help biologists examine the events leading to hybridization, which is threatening the wild red wolf population. If possible, studies should be conducted on the interaction of red wolves and coyotes living in neighbouring pens.

Nothing is known about wolf perception of acoustic structure and non-linear phenomena. Studies are needed to determine the ability of wolves to detect and discriminate between sounds. Since wolves produce tonal sounds primarily when in visual range of each other, playback studies that mimic vocal behaviour in context are hard to create (Owings and Morton, 1998). The artificial situation created by the playback experiment makes it difficult to distinguish between a natural response and a response to an unusual stimulus (Bauers, 1993). More detailed functional analysis, which examines the response of recipients to the different types of vocalisations, is needed. To do this, one would need to observe either a single pack of red wolves or more than one pack at separate facilitates so that the study situation is not compounded by the presence of wolves in neighbouring pens. This study would need to be long term and to include all seasons: breeding, denning, and pup rearing. With additional information on the contexts of each type of vocalisation, it may be possible to design controlled studies to examine vocalisations in specific social situations.

The red wolves in this study demonstrated individual patterns of behaviour and vocal output. Individual wolves had a variety of reactions to my presence, from hiding until I had been in my blind for several minutes, to jumping on the fence as I entered the facility. Some showed active interest in the pups for weeks after their arrival, others for only a few days. Wolves in one pen never demonstrated aggression, while the interactions between wolves in another pen slowly changed from agonistic to affiliative. Some vocalised often, while others did not. Some wolves produced high proportions of non-linearities; others didn't produce any. While it is often desirable to understand the behaviour of a species at the population level, it is pertinent, when making generalisations, to remember that populations are composed of individuals and that there are genuine individual differences.

Although wolves within pens demonstrated individual behaviour patterns, there were also differences between pens. These differences may reflect something about the pair bonds between these animals. To increase our knowledge of the use of affiliative vocalisations and our understanding of pair bonds I recommend an in-depth study of the social interactions of wolf pairs. This study would entail a comparison of several wolf pairs, looking at the number and length of social interactions, the type of behaviours displayed during the social interaction (e.g. vocalisations, physical contact, postures, etc.), and the reactions of the partners to each other. From a management persepective, the goal of such as study would be to develop behavioural assessment criteria that could be used to judge the willingness of pairs of wolves to interact and perhaps allow managers to predict which pairs will be able to successfully breed and rear young.

In addition to studying wolf pairs, an in-depth study of adult-pup interactions is needed. Many of the wolves in this study demonstrated a desire to interact with pups by squeaking. Past study has highlighted the role of squeaking in pup-rearing in gray wolves (Coscia *et al*., 1991). The possibility of setting up remote recorders in the dens of expectant mothers should be explored. A transmitter would allow data gathering with minimal disruption to the wolves.

Limitations of this study should be considered in designing future research on captive wolves. The complexity of the pen situation was far from natural and made interpretation of behavioural context difficult. While digital video recorders are ideal for detailed analysis of acoustic and visual signals, they are limited in their ability for recording low amplitude sounds such as non-linear sounds. While squeaks were emitted during howling sessions, the level of noise during the howling session made acoustical analysis of these squeaks impossible. The set-up and equipment used in future studies should be appropriate to the research questions being addressed.

A true understanding of affiliative vocalisations requires study of wild wolf packs.

Although much can be learned from captive wolves, captivity is ultimately an artificial situation. Captive studies have provided an opportunity to create a knowledge base about affiliative vocalisations. It is now possible to determine if what is known also exists in wild wolves. Situations exist today in which filming wild wolves may be possible. Gray wolves in Yellowstone National Park are more tolerant of human activities then wolves elsewhere. Red wolves living on island propagation sites also provide an ideal situation for studying wild wolves. It is possible that motion triggered remote cameras set up near denning sites will provide us with valuable records of the social interactions of wild wolves.

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Appendix 1: Technical Note:

Are digital video recordings adequate for audio analysis of low—amplitude animal vocalisations?: Red wolf (*Canis rufus*) affiliative vocalisations as a test case.

Introduction

Adequate audio and visual records are essential to the study of animal communication. Relationships between vocal and visual behaviour are especially important when the vocalising animal is in visual range of the recipient. In such situations vocal behaviour will always be augmented by visual behaviour. Simultaneous observations of vocal and visual behaviours, such as can be produced using video recordings, are required. The purpose of this note is to assess the adequacy of audio recording made by a digital video recorder through comparison with audio recordings made simultaneously with a DAT recorder.

Affiliative vocalisations of red wolves are brief (10 – 1900 msec), low amplitude sounds that occur in phrases of 1 - 30 units. These vocalisations occur in several contexts such as greeting, play, and adult-pup interactions. Determining the function of these vocalisations requires identifying the vocalising animal, potential recipients, and subtle changes in the visual behaviour of the interacting wolves, necessitating video playback. Since the vocalisations are brief and occur frequently, the link between audio and video records must be accurate. This level of accuracy is only achievable with a single recording device.

Three types of affiliative sounds have been identified in the red wolf: squeaks, wuhs, and non-linear sounds. Squeaks are high (2600 – 9500 Hz) and wuhs relatively low

(110 – 1600 Hz) in frequency. Four non-linear sound unit types have been identified: squeak with sidebands (squeaks with non-harmonic bands that are parallel to the fundamental frequency); squeak-wuh biphonations (two overlapping fundamental frequencies); within-type frequency jumps (abrupt, discontinuous changes in frequency), and squeak-wuh frequency jumps (abrupt, discontinuous changes from squeak to wuh) (for further definition of non-linear sounds in canids see Wilden *et al.*, 1998 and Volodin and Volodina, 2002). For detailed information on acoustic structure, individuality, and related contexts see Chapters 2 and 3.

Weir (1999) found that audio recordings from analog video recordings were of equal or better quality than analog audio recordings. Temporal and frequency variables were consistently higher in video recordings with minimal deviation, and the shapes of frequency contours were unaltered. However, with the advent of digital computer technology and related analysis software, it is desirable to compare digital camcorders with DAT audio recorders.

While many digital video cameras offer superior optical quality, commercial camcorders do not offer the same control over audio recordings as DAT audio recorders. Digital video and DAT audio recorders use identical sampling rates, however, only professional digital video cameras allow for manual audio gain control. The built-in automatic gain control in non-professional cameras could potentially distort the sound recording, especially when using an external microphone. This distortion is most likely to be a concern when dealing with high amplitude vocalisations, when measuring relative amplitude (dB), or when examining the acoustic structure of low amplitude, complex sounds. This paper is concerned with the differences in recording quality between digital video and DAT audio recordings of low-amplitude red wolf vocalizations.

Methods

A pair of wolves and their male yearling were observed at a breeding facility associated with Point Defiance Zoo and Aquarium in Graham, WA for one hour on the evening of 17 July, 2003. Wolves were housed in a 929 m^2 enclosure with soil and river rock substrate with scattered conifers, low shrubs, and patches of tall grasses. The enclosure had an artificial den that was approximately 1.2 m wide, 2.4 m long, and 1.5 m high. The slope, vegetation, and the den occasionally blocked some observations. Wolves were distinguishable by individual markings.

Simultaneous video and audio recordings were made from inside a nylon tent placed on the path adjacent to the pen. Recordings began 5 - 10 minutes after the observer entered the blind. Video recordings were made with a Sony Digital-8 DCR-TRV240 Handycam (16 bit - 48 kHz PCM digital stereo audio) and an external Audio-Technica AT835b shotgun microphone. Audio recordings were made with a Tascam DA-P1 Digital Audio Recorder (16 bit – 48 kHz sampling rate, single channel), Audio-Technica AT835b shotgun microphone, and Maxell Dat Cassette. Video camera and microphones were placed side-by-side on a stand attached to a tripod, allowing for simultaneous directional adjustments of the equipment. Both microphones were 5 - 20 cm from the fence.

Seventy sound units from 21 vocalisations were analyzed, including squeaks, wuhs, and non-linear units (composed of two or more frequency components) for a total of 85 frequency components. Acoustic structure was quantified by spectrogram analysis using Wavesurfer 1.6.0 (Sjölander and Beskow, 1993, http://www.speech.kth.se/wavesurfer/index.html) for sounds above 2 kHz and Raven 1.0 (Cornell Lab of Ornithology) for sounds below 2 kHz, each software package providing the best graphics at the given frequency range. AVI video clips containing affiliative vocalisations were copied from raw videos and analyzed in Wavesurfer using wideband (FFT window length 256, Hamming analysis, bandwidth 375) and narrowband (FFT window length 1024, Hamming analysis, bandwidth 58) settings. For low-frequency vocalisations, AVI clips were converted to WAV files and analyzed in Raven using wideband (FFT window length 512, Hamming analysis, bandwidth 122) and narrowband (FFT window length 2048, Hamming analysis, bandwidth 30.5) settings. Audio recordings were converted to WAV files and analyzed under the same wideband and narrowband settings.

Measurements were taken from spectrographs on the following variables: fundamental frequency at the start and end of each unit (Hz), maximum frequency (Hz), minimum frequency (Hz), and average frequency (greater than 2 kHz only). Duration of each unit (msec) and number of units per vocalisation were also recorded. The presence or absence of sidebands, biphonations, and frequency jumps was noted.

Results

Temporal and frequency variables measured on spectrograms produced from the audio track of digital video recordings (referred to as video analysis here after) and DAT audio recordings were significantly correlated (Pearson's correlation = 0.950 to 0.989, $p < 0.001$). Although frequency measurements did not differ significantly between recording methods ($t_{84} = -1.12$ to -0.99, $p > 0.05$), durations in the audio recording were significantly longer ($t_{84} = 2.49$, $p = 0.015$) (Table 1). DAT recordings revealed the addition of three squeaks, the presence of sidebands on three squeaks, and the presence of within-type frequency jumps in one squeaks that were not visible in the digital video recordings. In addition, two of the units that were classified as squeak-wuh frequency jumps (abrupt jumps from squeak to wuh) in the video analysis were classified as biphonations (overlapping squeak and wuh portions) in the audio analysis (Fig. 1). One wuh component was not visible in the audio analysis that was coded as a biphonation during video analysis. Background noise was more prevalent in DAT recordings. Except for one vocalization which changed classifications from a squeak vocalization to a banded squeak vocalization, the numberr and classification of whole vocalizations was consistent between recording methods.

Table 1. Comparison of time and frequency measurements of spectrograms of red wolf sound units from digital video and DAT audio recordings (n = 85)

	Mean ± SE Video	Mean ± SE Audio	y	t
Duration (msec)	69±8	76±9	0.950**	2.49*
Average Frequency (Hz)	4857±328	4807±324	0.988**	-0.99
Start Frequency (Hz)	4850±324	4802±321	0.989**	-1.02
End Frequency (Hz)	4828±328	4770±330	0.988**	-1.12
Maximum Frequency (Hz)	4915±329	4860±325	0.988**	-1.10
Minimum Frequency (Hz)	4788±327	4734±326	0.989**	-1.12

*$p < 0.05$ **$p < 0.001$

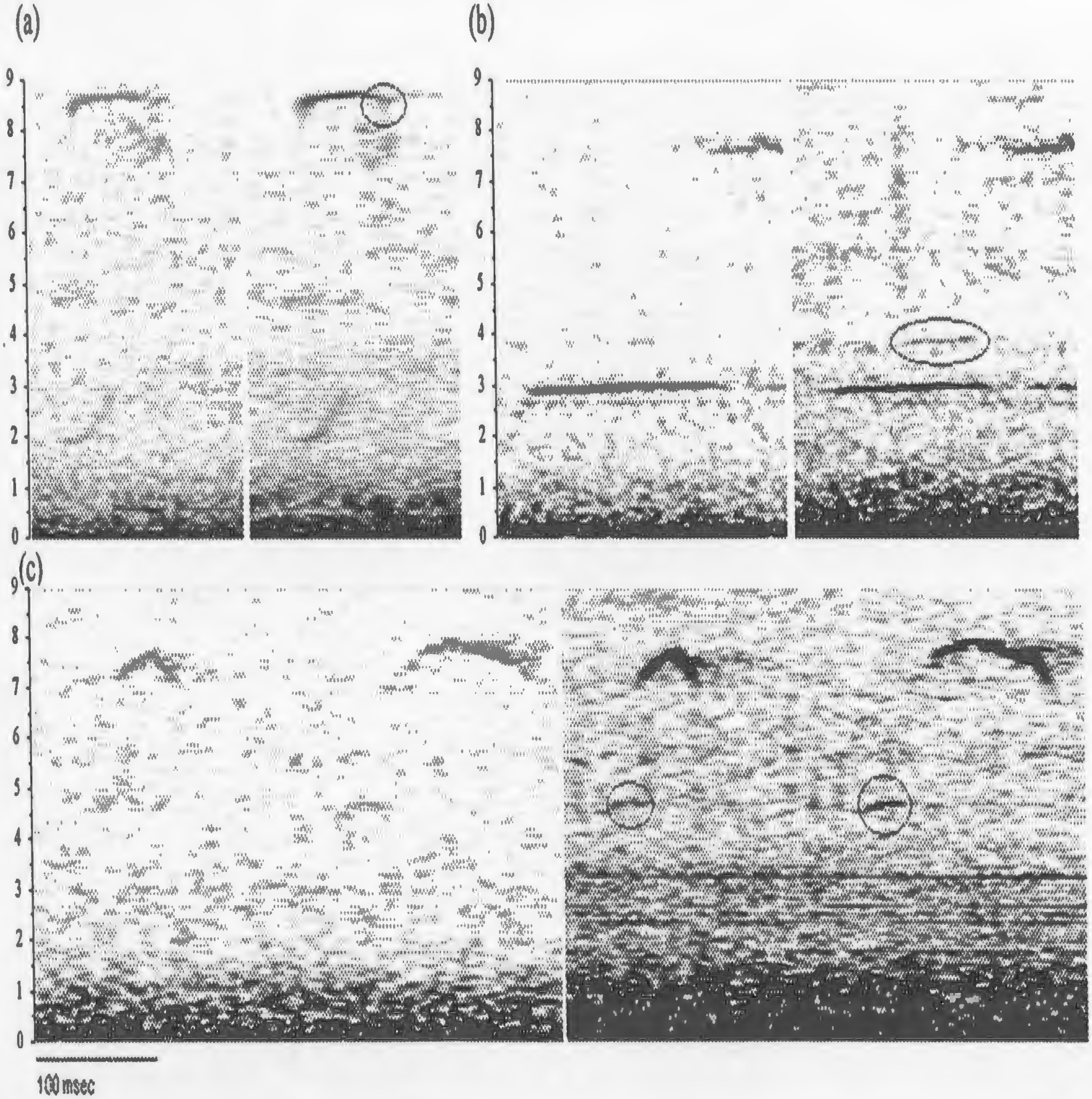

Fig 1.1 Spectrograms of red wolf sound units produced from digital video and DAT audio recordings demonstrating (a) the longer duration of the high frequency component leading to the reclassification of a squeak-wuh frequency jump to a squeak-wuh biphonation (Note: the low frequency component is hard to identify at these spectrum settings) (b) the appearance of a sideband and (c) the appearance of a second high frequency element and new squeak in DAT audio recordings. Changed and new components are circled on the DAT spectrograms.

Discussion

Comparisons of digital video and DAT audio recordings revealed some differences in quality of the recordings. DAT recordings revealed greater unit durations and more non-linear detail (e.g. presence of frequency jumps and sidebands) than video recordings. Red wolf affiliative vocalisations often fade in amplitude toward the end of the vocalisations and non-linear detail is often low in amplitude, indicating that the DAT recorder was more sensitive to low-amplitude sounds than the audio recorder on the video camera. However, background noise was more prevalent in DAT recordings, which caused greater difficulty in measuring low-frequency wuhs. The DAT recorder allows the researcher to adjust the gain control manually for optimal recording of the desired sounds. Since the type of microphone used and the sampling rate of the recorders were the same (Russell, 2003), our results suggest that the automatic gain control on the digital video camera was probably responsible for dampening sounds. The impact of the dampening effect on the analysis of non-linear sounds is noteworthy.

Post-analysis viewing of the video revealed that the differences in unit classification between video and audio produced spectrograms occurred primarily in situations where the vocalising wolf could not be identified or the vocalisation was being produced by an animal in a different pen. Therefore these differences in coding are related to the orientation of the shotgun microphone and would have little impact on studies of simultaneous vocal and visual behaviours, which occur in front of the camera/microphone. However, the possibility remains that complex detail of very low amplitude sounds produced in front of the microphone may not be detected by the video recorder. Although measured duration tended to be shorter in video recordings than in audio recordings, this measurement difference was relatively consistent and would have little impact on comparative studies. In this study, the benefits of detailed audio-video correlation far out-weigh the loss in quality associated with using a digital video camera.

The automatic gain control on the digital video camera could potentially alter measurements of relative amplitude. Since amplitude is relative to distance from the observer and distance was not kept constant in this study, we were not concerned by changes in amplitude produced by the video camera. We do not recommend the use of a commercial video camera when amplitude measurements are being used. Wolf affiliative vocalisations are low amplitude sounds. We can not determine from this study the effects of the digital video camera on high amplitude sounds. Professional digital video recorders with manual gain control may overcome these shortcomings.

We conclude that digital video analysis is acceptable for audio analysis of low amplitude animal vocalisations and recommend its use in simultaneous video/audio analysis during detailed observations of social behaviour. Current digital video recording technology has limited application in detailed acoustical comparisons and relative frequency measures of non-linear sounds. Further technological developments are necessary for accurate acoustic contextual analysis.

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Wilden, I., H. Herzel, G. Peters, & G. Tembrock. (1998). Subharmonics, biphonation, and deterministic chaos in mammal vocalizations. *Bioacoustics, 9,* 1-26.

Appendix 2: Observation Area

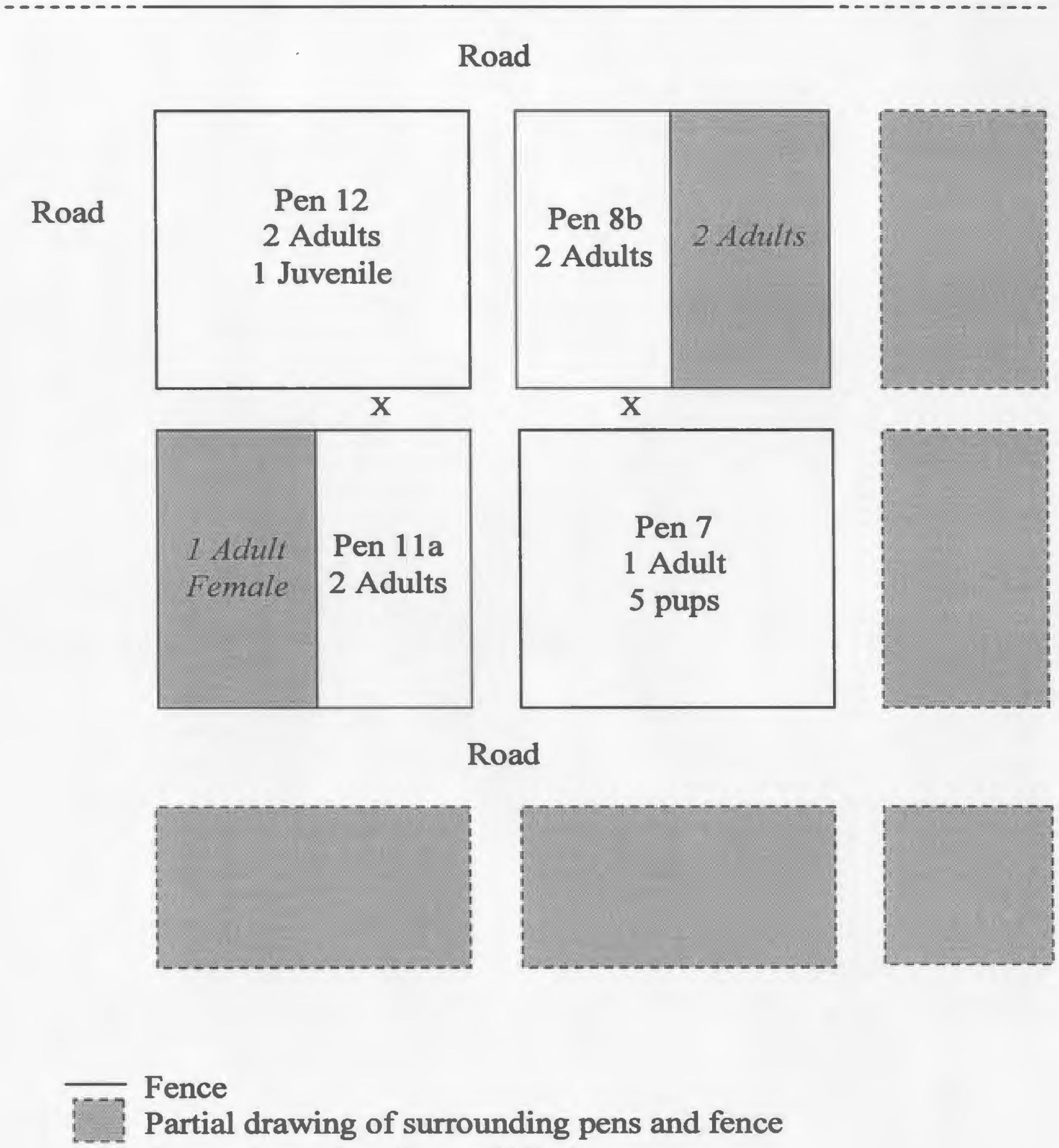

Figure 1. Diagram of observation area showing pens of interest, locations of blind, and immediate surroundings. Not drawn to scale.

Appendix 3: Distinguishing Phrases

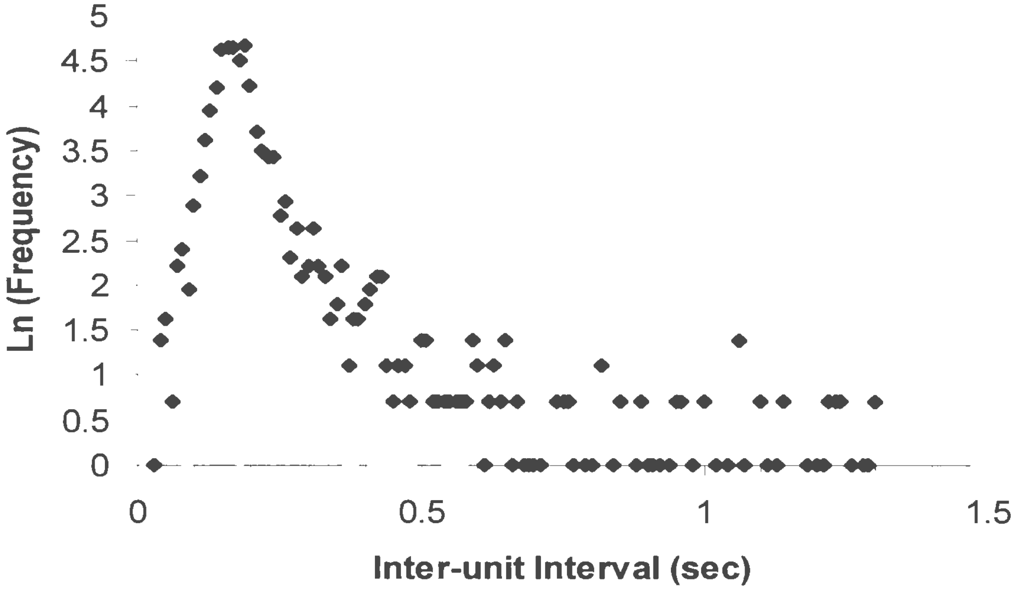

Figure 1. The ln-frequency plot of inter-unit intervals of tonal vocalisation of red wolves demonstrates a change in slope at 0.5 seconds indicating that intervals longer that 0.5 seconds constitute a new phrase.

Appendix 4: Descriptive Tables

Table 1. Descriptive statistics of temporal and frequency variables of squeak vocalisations, phrases, and units including grand central tendencies weighted by wolf* and extreme boundaries of all observations**.

	Phrases	*Units*	*Dur (msec)*	*IPI (msec)*		
Squeak Vocalisation						
Grand Mean (SD)	1.1 (0.1)	2.4 (0.7)	570 (234)	942 (497)		
Range of Means	1.0-1.2	1.8-3.4	299-905	591-1294		
Grand Median (SD)	1.0 (0.0)	1.5 (0.9)	256 (167)	942 (497)		
Range of Medians	1.0-1.0	0.0-3.0	101-527	591-1294		
Absolute Minimum	1.0	1.0	21	502		
Absolute Maximum	4.0	19.0	7350	2788		
	Units	*Dur (msec)*	*Duty Cycle*	*Rate (#/sec)*	*ISI (msec)*	*Cycle (msec)*
Squeak Phrase						
Grand Mean (SD)	2.2 (0.5)	482 (155)	0.705 (0.151)	7.7 (6.0)	220 (60)	287 (74)
Range of Means	1.8-3.0	299-647	0.482-0.873	3.0-18.1	160-305	194-395
Grand Median (SD)	1.3 (0.4)	244 (158)	0.841 (0.265)	4.3 (0.7)	209 (56)	279 (80)
Range of Medians	1.0-2.0	101-490	0.388-1.000	3.0-18.1	159-302	229-421
Absolute Minimum	1.0	20	0.119	2.9	23	66
Absolute Maximum	19.0	4541	1.000	30.0	499	906
	Dur (msec)	*Ave Fq (Hz)*	*Start Fq (Hz)*	*End Fq (Hz)*	*Max Fq (Hz)*	*Min Fq. (Hz)*
Squeak Unit						
Grand Mean (SD)	106 (53)	5089 (315)	5066 (313)	5053 (330)	5140 (301)	5001 (336)
Range of Means	43-191	4759-5534	4755-5502	4632-5491	4810-5573	4618-5447
Grand Median (SD)	70 (40)	5073 (211)	5037 (251)	4951 (337)	5124 (190)	4921(333)
Range of Medians	34-135	4784-5316	4708-5367	4379-5215	4886-5367	4379-5215
Absolute Minimum	11	2632	2632	2632	2632	2632
Absolute Maximum	1404	8683	8683	8734	8734	8632

*n voc =5, n phrase =5, n units = 5 wolves

** n = 144 vocalisation, 169 phrases, 515 units

Table 2 Descriptive statistics of temporal and frequency variables of wuh vocalisations, phrases, and units including grand central tendencies weighted by wolf* and extreme boundaries of all observations**.

	Phrases	*Units*	*Dur (msec)*	*IPI (msec)*
Wuh Vocalisation				
Grand Mean (SD)	2.4 (0.4)	7.7 (2.4)	4737 (236)	1806 (406)
Range of Means	2.1-2.7	6.0-9.4	4571-4904	1519-2094
Grand Median (SD)	1.0 (0.0)	6.3 (3.9)	2924 (2666)	1081 (225)
Range of Medians	1.0-1.0	3.5-9.0	1037-4810	922-1240
Absolute Minimum	1.0	1.0	22	519
Absolute Maximum	7.0	19.0	14808	6174

	Units	*Dur (msec)*	*Duty Cycle*	*Rate (#/sec)*	*ISI (msec)*	*Cycle (msec)*
Wuh Phrase						
Grand Mean (SD)	3.3 (1.5)	482 (155)	0.719 (0.060)	4.5 (1.0)	230 (1)	327 (22)
Range of Means	2.3-4.4	299-647	0.677-0.762	3.7-5.2	229-230	312-343
Grand Median (SD)	1.5 (0.7)	366 (281)	0.813 (0.265)	4.3 (0.7)	222 (12)	286 (22)
Range of Medians	1.0-2.0	167-564	0.625-1.000	3.3-5.4	213-230	270-301
Absolute Minimum	1.0	22	0.155	2.3	71	127
Absolute Maximum	17.0	4810	1.000	15.7	443	763

	Dur (msec)	*Ave Fq (Hz)*	*Start Fq (Hz)*	*End Fq (Hz)*	*Max Fq (Hz)*	*Min Fq. (Hz)*
Wuh Unit ***						
Grand Mean (SD)	122 (17)	333 (33)	350 (42)	293 (18)	366 (27)	280 (4)
Range of Means	99-124	324-343	321-380	281-305	347-385	278-283
Grand Median (SD)	78 (22)	314 (12)	340 (18)	251 (44)	349 (5)	238 (25)
Range of Medians	63-94	305-323	328-353	220-283	345-353	220-255
Absolute Minimum	10	165	160	115	170	115
Absolute Maximum	1975	1505	1525	1505	1525	1505

*n voc = 2, n phrase = 5, n units =5 wolves

** n = 57 vocalisations, 83 phrases, and 237 units

***Wuh Vocalisation containing frequency jump (within) excluded from analysis

Table 3 Descriptive statistics of temporal and frequency variables of banded squeak vocalisations, phrases, and units including grand central tendencies weighted by wolf* and extreme boundaries of all observations**.

	Phrases	*Units*	*Dur (msec)*	*IPI (msec)*
Banded squeak vocalisation				
Grand Mean (SD)	2.3 (-)	7.7 (-)	3827 (-)	1817 (-)
Range of Means	-	-	-	-
Grand Median (SD)	1.0 (-)	5.0 (-)	1049 (-)	1158 (-)
Range of Medians	-	-	-	-
Absolute Minimum	1.0	1.0	116	500
Absolute Maximum	7.0	30.0	19370	4441

	Units	*Dur (msec)*	*Duty Cycle*	*Rate (#/sec)*	*ISI (msec)*	*Cycle (msec)*
Non-linear Squeak Phrase						
Grand Mean (SD)	3.4 (-)	667 (-)	0.526 (-)	7.2 (-)	162 (4)	242 (4)
Range of Means	-	-	-	-	159-165	240-245
Grand Median (SD)	2.0 (-)	473 (-)	0.429 (-)	4.3 (0.7)	161 (1)	223 (16)
Range of Medians	-	-	-	-	160-162	217-240
Absolute Minimum	1.0	19.0	0.172	2.3	43	151
Absolute Maximum	14.0	3338.0	1.000	13.2	477	635

	Dur (msec)	*Ave Fq (Hz)*	*Start Fq (Hz)*	*End Fq (Hz)*	*Max Fq (Hz)*	*Min Fq. (Hz)*
Squeak Unit						
Grand Mean (SD)	57 (-)	4842 (-)	4832 (-)	4845 (-)	4888 (-)	4808 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	46 (-)	4835 (-)	4835 (-)	4835 (-)	4886 (-)	4784 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	12	3949	3949	3949	4101	3949
Absolute Maximum	642	8683	8556	8556	8683	8556
Sideband Unit						
Grand Mean (SD)	115 (47)	6840 (2534)	6756 (2517)	6838 (2508)	6875 (2515)	6752 (2522)
Range of Means	82-148	5049-8632	4976-8535	5064-8611	5097-8654	4968-8535
Grand Median (SD)	95 (16)	6835 (2542)	6765 (2551)	6822 (2524)	6860 (2542)	6765 (2551)
Range of Medians	84-106	5037-8632	4962-8569	5037-8607	5063-8658	4962-8569
Absolute Minimum	23	4379	4227	3848	4455	3848
Absolute Maximum	872	9189	9189	9063	9341	9063

*n voc = 1, n phrase = 5, n unit = 5, 2 wolves

** n = 36 vocalisations, 70 phrases, and 277 units

Table 4 Descriptive statistics of temporal and frequency variables of complex squeak vocalisations, phrases, and units including grand central tendencies weighted by wolf* and extreme boundaries of all observations**.

	Phrases	*Units*	*Dur (msec)*	*IPI (sec)*
Complex squeak vocalisation				
Grand Mean (SD)	1.8 (0.0)	7.9 (4.4)	2846 (735)	951 (50)
Range of Means	1.8-1.8	4.8-11.0	2326-3365	915-986
Grand Median (SD)	1.3 (0.4)	5.3 (3.2)	2122 (1078)	795 (227)
Range of Medians	1.0-1.5	3.0-7.5	1359-2884	635-956
Absolute Minimum	1.0	2.0	301	507
Absolute Maximum	5.0	26.0	10064	4067

	Units	*Dur (msec)*	*Duty Cycle*	*Rate (#/sec)*	*ISI (msec)*	*Cycle (msec)*
Squeak Jump Phrase					n=4	n=4
Grand Mean (SD)	4.4 (2.4)	1219 (398)	0.536 (0.120)	5.0 (0.9)	208(40)	312 (93)
Range of Means	2.7-6.1	937-1500	0.451-0.621	4.3-5.6	160-249	257-452
Grand Median (SD)	3.0 (1.4)	712 (95)	0.480 (0.128)	4.3 (0.7)	196 (36)	298 (95)
Range of Medians	2.0-4.0	644-779	0.390-0.571	3.7-4.8	156-228	247-441
Absolute Minimum	1.0	53	0.099	2.2	73	174
Absolute Maximum	21.0	5709	1.000	11.5	491	757

	Dur (msec)	*Ave Fq (Hz)*	*Start Fq (Hz)*	*End Fq (Hz)*	*Max Fq (Hz)*	*Min Fq. (Hz)*
Squeak Unit (n=5)						
Grand Mean (SD)	101 (76)	5967 (1358)	5916 (1378)	5876 (1350)	6031 (1331)	5809 (1396)
Range of Means	17-200	4833-7932	4694-7888	4696-7811	4747-7969	4516-7784
Grand Median (SD)	69 (43)	5825 (1702)	5765 (1757)	5794 (1680)	5927 (1651)	5996 (1736)
Range of Medians	15-119	4493-8303	4329-8303	4405-8215	4977-8354	4202-8177
Absolute Minimum	8	3797	3721	3670	3848	3670
Absolute Maximum	613	8734	8734	8734	8734	8683
Sideband Unit (n=2)						
Grand Mean (SD)	136 (26)	6474 (2945)	6389 (2860)	6443 (2820)	6566 (2860)	6340 (2817)
Range of Means	118-155	4392-8556	4367-8412	4449-8437	4544-8589	4348-8332
Grand Median (SD)	108 (1)	6544 (2883)	6525 (2873)	6430 (2793)	6594 (2882)	6379 (2721)
Range of Medians	107-109	4506-8582	4493-8556	4456-8405	4557-8632	4456-8303
Absolute Minimum	34	3797	3797	3898	3898	3797
Absolute Maximum	359	8734	8683	8683	8734	8556
Squeak Jump Unit (n=2)						
Grand Mean (SD)	166 (22)					
Range of Means	131-234					
Grand Median (SD)	136 (33)					
Range of Medians	127-213					
Absolute Minimum	34					
Absolute Maximum	965					

Portion A (n=2w)						
Grand Mean (SD)	32 (26)	4798 (39)	4778 (7)	4853 (48)	4886 (85)	4761 (6)
Range of Means	14-50	4770-4826	4773-4783	4819-4887	4826-4945	4757-4766
Grand Median (SD)	26 (17)	4633 (179)	4563 (278)	4664 (170)	4664 (170)	4563 (278)
Range of Medians	14-38	4506-4759	4367-4759	4544-4784	4544-4784	4367-4759
Absolute Minimum	10	4101	4101	4101	4101	4101
Absolute Maximum	121	9367	9291	8936	9392	8936
Portion B (n=2w)						
Grand Mean (SD)	110 (26)	6732 (2249)	6545 (1803)	6551 (1905)	6871 (2140)	6385 (1768)
Range of Means	92-129	5142-8322	5271-7820	5204-7898	5358-8384	5135-7636
Grand Median (SD)	79 (14)	6873 (2094)	6595 (1701)	6645 (1700)	6924 (2094)	6531 (1611)
Range of Medians	70-89	5392-8354	5392-7757	5443-7847	5443-8405	5392-7670
Absolute Minimum	11	3012	3341	2962	3341	2936
Absolute Maximum	898	8683	8455	8405	8708	8405
Portion C (n=1w, 5u)						
Grand Mean (SD)	43 (-)	5320 (-)	5225 (-)	5159 (-)	5250 (-)	5134 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	27 (-)	4929 (-)	4784 (-)	4506 (-)	4784 (-)	4430 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	14	4430	4329	4278	4329	4278
Absolute Maximum	119	6987	6987	7037	7037	6987
Portion D (n=1w, 1u)						
Grand Mean (SD)	11 (-)	5245 (-)	5645 (-)	5697 (-)	5697 (-)	5645 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	-	-	-	-	-	-
Range of Medians	-	-	-	-	-	-
Absolute Minimum	-	-	-	-	-	-
Absolute Maximum	-	-	-	-	-	-

*n voc = 2, n phrase = 5 wolves

** n = 19 vocalisations, 34 phrases, and 147 units

Table 5 Descriptive statistics of temporal and frequency variables of squeak-wuh vocalisations, phrases, and units including grand central tendencies weighted by wolf* and extreme boundaries of all observations**.

	Phrases	*Units*	*Dur (msec)*	*IPI (sec)*
Squeak-wuh vocalisation				
Grand Mean (SD)	2.2 (0.1)	11.0 (2.0)	5269 (1097)	1528 (336)
Range of Means	2.2-2.3	9.7-12.4	4493-6044	1290-1766
Grand Median (SD)	1.8 (0.4)	9.8 (0.4)	4304 (386)	1163 (46)
Range of Medians	1.5-2.0	9.5-10.0	4031-4577	1130-1195
Absolute Minimum	1.0	1.0	82	504
Absolute Maximum	5.0	30.0	18175	6191

	Units	*Dur (msec)*	*Duty Cycle*	*Rate (#/sec)*	*ISI (msec)*	*Cycle (msec)*
Squeak-wuh Phrase					n=5	n=5
Grand Mean (SD)	4.9 (0.7)	1227 (18)	0.528 (0.043)	5.2 (0.3)	188 (25)	322 (177)
Range of Means	4.5-5.4	1214-1240	0.497-0.559	4.9-5.4	153-215	209-634
Grand Median (SD)	4.0 (0.0)	938 (161)	0.440 (0.038)	4.3 (0.7)	181 (25)	272 (104)
Range of Medians	4.0-4.0	824-1052	0.413-0.467	4.9-5.0	157-222	205-455
Absolute Minimum	1.0	23.0	0.117	1.9	30	73
Absolute Maximum	19.0	4938.0	1.000	19.1	466	1089

Unit Type	*Dur (msec)*	*Ave Fq (Hz)*	*Start Fq (Hz)*	*End Fq (Hz)*	*Max Fq (Hz)*	*Min Fq. (Hz)*
Wuh Unit (n=5)						
Grand Mean (SD)	122 (81)	397 (87)	425 (113)	352 (81)	446 (109)	331 (54)
Range of Means	43-357	297-501	255-540	292-488	317-544	275-410
Grand Median (SD)	57 (44)	381 (110)	404 (130)	341 (98)	429 (138)	312 (56)
Range of Medians	26-357	258-540	300-544	250-493	258-540	243-380
Absolute Minimum	12	225	205	210	235	205
Absolute Maximum	1242	685	765	750	765	665
Squeak Unit (n=2)						
Grand Mean (SD)	65 (21)	6186 (1466)	6173 (1431)	6171 (1398)	6233 (1440)	6127 (1405)
Range of Means	50-80	5149-7222	5162-7185	5183-7159	5215-7251	5133-7120
Grand Median (SD)	55 (21)	6671 (2131)	6645 (2094)	6620 (1987)	6696 (2094)	6595 (2023)
Range of Medians	40-70	5164-8177	5164-8126	5215-8025	5215-8177	5164-8025
Absolute Minimum	8	2977	2962	2977	3017	2962
Absolute Maximum	334	8784	8734	8784	8835	8734
Sideband Unit (n=3)						
Grand Mean (SD)	117 (86)	7370 (1827)	7331 (1811)	7298 (1750)	7402 (1813)	7256 (1766)
Range of Means	63-216	5261-8455	5240-8417	5277-8316	5309-8476	5217-8308
Grand Median (SD)	82 (26)	7384 (1835)	7337 (1796)	7333 (1791)	7426 (1827)	7270 (1780)
Range of Medians	63-111	5265-8481	5265-8456	5265-8405	5316-8481	5215-8329
Absolute Minimum	30	4936	4936	4987	4987	4936
Absolute Maximum	800	8734	8734	8734	8734	8658
Squeak Jump Unit (n=1w,3u)						
Grand Mean (SD)	79 (-)					
Range of Means	-				4826-4945	

Grand Median (SD)	90 (-)					
Range of Medians	-					
Absolute Minimum	56					
Absolute Maximum	331					
Portion A (n=1w,3u)						
Grand Mean (SD)	19 (-)	5856 (-)	5856 (-)	5881 (-)	5886 (-)	5856 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	15 (-)	4658 (-)	4658 (-)	4708 (-)	4708 (-)	4658 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	9	4658	4658	4607	4658	4607
Absolute Maximum	29	8253	8253	8253	8253	8253
Portion B (n=1w,3u)						
Grand Mean (SD)	30 (-)	7063 (-)	7063 (-)	6996 (-)	7063 (-)	6996 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	30 (-)	7898 (-)	7898 (-)	7848 (-)	7898 (-)	7848 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	24	4455	4506	3645	4506	3645
Absolute Maximum	286	8734	8734	8683	8734	8683
Frequency Jump Unit (n=1w,10u)						
Grand Mean (SD)	183 (-)					
Range of Means	-					
Grand Median (SD)	119 (-)					
Range of Medians	-					
Absolute Minimum	36					
Absolute Maximum	697					
Squeak Portion (n=1w,11u)						
Grand Mean (SD)	75 (-)	8022 (-)	7949 (-)	8105 (-)	8131 (-)	7923 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	45 (-)	8455 (-)	8354 (-)	8455 (-)	8455 (-)	8253 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	15	3898	3898	3848	3949	3848
Absolute Maximum	294	9493	9493	9620	9620	9493
Wuh Portion (n=1w,9u)						
Grand Mean (SD)	93 (-)	566 (-)	599 (-)	532 (-)	607 (-)	517 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	49 (-)	565 (-)	585 (-)	515 (-)	585 (-)	500 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	16	350	425	260	425	260
Absolute Maximum	661	980	980	970	980	970
Biophonation (Pure) (n=1w,7u)						
Grand Mean (SD)	94 (-)					
Range of Means	-					

Grand Median (SD)	84 (-)					
Range of Medians	-					
Absolute Minimum	52					
Absolute Maximum	1267					
Squeak Portion (n=1w,6u)						
Grand Mean (SD)	57 (-)	8430 (-)	8413 (-)	8447 (-)	8489 (-)	8375 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	64 (-)	8531 (-)	8481 (-)	8531 (-)	8556 (-)	8405 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	14	3848	3848	3848	3898	3848
Absolute Maximum	1267	9392	9392	9518	9518	9392
Wuh Portion (n=1w,5u)						
Grand Mean (SD)	29 (-)	621 (-)	644 (-)	559 (-)	644 (-)	559 (-)
Range of Means	-	-	-	-	-	-
Grand Median (SD)	25 (-)	610 (-)	630 (-)	585 (-)	630 (-)	585 (-)
Range of Medians	-	-	-	-	-	-
Absolute Minimum	12	355	360	235	360	235
Absolute Maximum	258	1570	1570	1410	1570	1410
Biphonation (Harmonic) (n=2)						
Grand Mean (SD)	156 (76)					
Range of Means	102-210					
Grand Median (SD)	88 (9)					
Range of Medians	82-95					
Absolute Minimum	40					
Absolute Maximum	1016					
Squeak Portion (n=2)						
Grand Mean (SD)	117 (24)	6624 (2001)	6603 (2014)	6656 (2011)	6699 (2046)	6567 (1987)
Range of Means	100-134	5208-8039	5179-8028	5234-8078	5253-8146	5168-7966
Grand Median (SD)	73 (20)	6911 (2327)	6886 (2399)	6911 (2327)	6961 (2363)	6848 (2345)
Range of Medians	59-87	5265-8556	5190-8582	5265-8556	5291-8632	5190-8506
Absolute Minimum	18	3848	3848	3848	3848	3848
Absolute Maximum	618	9164	9164	9341	9341	9164
Wuh Portion (n=2)						
Grand Mean (SD)	57 (14)	562 (67)	633 (36)	487 (89)	639 (28)	481 (97)
Range of Means	48-67	515-609	607-659	424-550	619-659	413-550
Grand Median (SD)	42 (6)	588 (103)	625 (49)	513 (67)	630 (42)	513 (67)
Range of Medians	38-46	515-660	590-660	465-560	600-660	465-560
Absolute Minimum	10	265	335	235	335	235
Absolute Maximum	536	1440	1440	1290	1440	1290

*n voc = 2, n phrase = 5, n unit = see table wolves

** n = 99 vocalisations, 74 phrases, and 394 units

Appendix 5: Histogram of Frequency (Hz) Components of Tonal Units

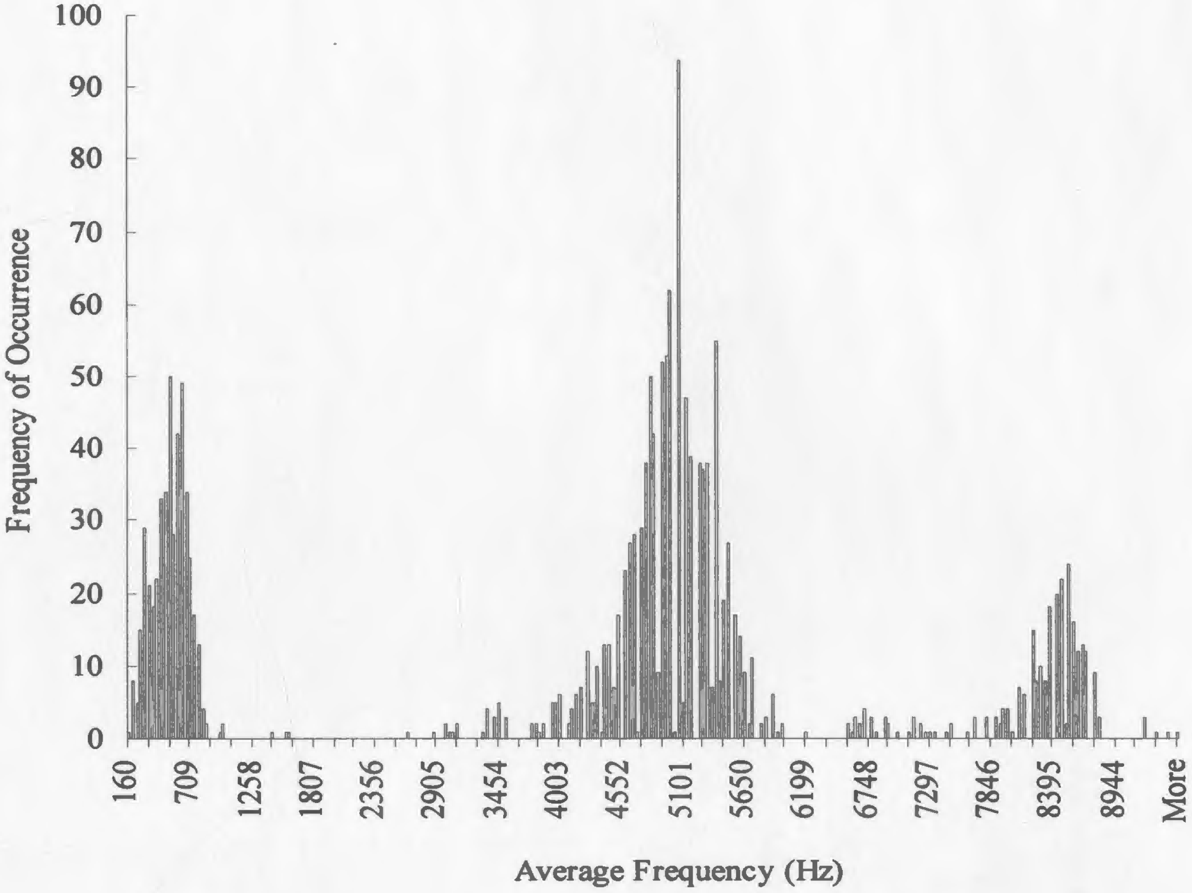

Figure 1. Histogram demonstrating the distribution of the average frequencies of components of tonal units. Wuhs and low-frequency components peak around 550 Hz, and squeaks and high-frequency components peak at 5000 and 8500 Hz.

Appendix 6: Variable Correlation Analysis Tables

Table 1 Correlation analysis table of all acoustic variables of squeak vocalizations

		Dur	Duty	Phrase	P/V	Pdur	Rate	U/V	U/P	Udur	ISI	ISPI	Cycle	AveF	StartF	EndF	MaxF	MinF
Dur	r	1.00	-0.31	a	0.65	0.56	-0.27	0.77	0.54	-0.03	a	a	a	0.14	0.15	0.14	0.13	0.15
	p		0.00		0.00	0.00	0.01	0.00	0.00	0.69				0.10	0.08	0.09	0.12	0.07
	N	144	144	144	144	144	92	144	144	144	0	0	0	144	144	144	144	144
Duty	r	-0.31	1.00	0.05	0.01	-0.50	0.22	-0.45	-0.53	0.30	a	0.13	a	-0.13	-0.13	-0.13	-0.11	-0.15
	p	0.00		0.56	0.88	0.00	0.02	0.00	0.00	0.00		0.54		0.10	0.08	0.09	0.16	0.05
	N	144	169	169	144	169	105	144	169	169	0	25	0	169	169	169	169	169
Phrase	r	a	0.05	1.00	a	-0.03	-0.09	a	-0.05	-0.04	a	0.52	a	0.14	0.14	0.15	0.13	0.15
	p		0.56			0.65	0.39		0.52	0.62		0.01		0.07	0.08	0.05	0.08	0.06
	N	144	169	169	144	169	105	144	169	169	0	25	0	169	169	169	169	169
P/V	r	0.65	0.01	a	1.00	-0.02	-0.08	0.40	0.00	-0.08	a	a	a	0.10	0.11	0.11	0.10	0.12
	p	0.00	0.88			0.80	0.47	0.00	0.97	0.33				0.22	0.19	0.19	0.25	0.16
	N	144	144	144	144	144	92	144	144	144	0	0	0	144	144	144	144	144
Pdur	r	0.56	-0.50	-0.03	-0.02	1.00	-0.47	0.83	0.95	0.05	a	-0.17	a	0.10	0.10	0.10	0.09	0.10
	p	0.00	0.00	0.65	0.80		0.00	0.00	0.00	0.50		0.41		0.21	0.18	0.19	0.22	0.18
	N	144	169	169	144	169	105	144	169	169	0	25	0	169	169	169	169	169
Rate	r	-0.27	0.22	-0.09	-0.08	-0.47	1.00	-0.35	-0.37	-0.28	a	0.05	a	-0.17	-0.17	-0.17	-0.18	-0.16
	p	0.01	0.02	0.39	0.47	0.00		0.00	0.00	0.00		0.87		0.08	0.08	0.09	0.07	0.10
	N	92	105	105	92	105	105	92	105	105	0	13	0	105	105	105	105	105
U/V	r	0.77	-0.45	a	0.40	0.83	-0.35	1.00	0.88	-0.18	a	a	a	0.18	0.19	0.20	0.17	0.20
	p	0.00	0.00		0.00	0.00	0.00		0.00	0.04				0.03	0.03	0.02	0.04	0.02
	N	144	144	144	144	144	92	144	144	144	0	0	0	144	144	144	144	144
U/P	r	0.54	-0.53	-0.05	0.00	0.95	-0.37	0.88	1.00	-0.14	a	-0.13	a	0.13	0.13	0.13	0.12	0.14
	p	0.00	0.00	0.52	0.97	0.00	0.00	0.00		0.06		0.55		0.10	0.10	0.08	0.13	0.07
	N	144	169	169	144	169	105	144	169	169	0	25	0	169	169	169	169	169
Udur	r	-0.03	0.30	-0.04	-0.08	0.05	-0.28	-0.18	-0.14	1.00	-0.05	-0.05	0.06	-0.05	-0.05	-0.04	-0.02	-0.07
	p	0.69	0.00	0.62	0.33	0.50	0.00	0.04	0.06		0.38	0.81	0.27	0.28	0.31	0.42	0.73	0.13
	N	144	169	169	144	169	105	144	169	513	344	25	344	513	513	513	513	513
ISI	r	a	a	a	a	a	a	a	a	-0.05	1.00	a	0.83	0.06	0.06	0.04	0.06	0.05
	p									0.38			0.00	0.29	0.29	0.42	0.31	0.39
	N	0	0	0	0	0	0	0	0	344	344	0	344	344	344	344	344	344
ISPI	r	a	0.13	0.52	a	-0.17	0.05	a	-0.13	-0.05	a	1.00	a	-0.04	-0.03	-0.05	-0.04	-0.04
	p		0.54	0.01		0.41	0.87		0.55	0.81				0.85	0.89	0.82	0.84	0.86
	N	0	25	25	0	25	13	0	25	25	0	25	0	25	25	25	25	25
Cycle	r	a	a	a	a	a	a	a	a	0.06	0.83	a	1.00	0.09	0.09	0.08	0.09	0.08
	p									0.27	0.00			0.10	0.11	0.14	0.11	0.14
	N	0	0	0	0	0	0	0	0	344	344	0	344	344	344	344	344	344
AveF	r	0.14	-0.13	0.14	0.10	0.10	-0.17	0.18	0.13	-0.05	0.06	-0.04	0.09	1.00	1.00	0.99	1.00	0.99
	p	0.10	0.10	0.07	0.22	0.21	0.08	0.03	0.10	0.28	0.29	0.85	0.10		0.00	0.00	0.00	0.00
	N	144	169	169	144	169	105	144	169	513	344	25	344	513	513	513	513	513
StartF	r	0.15	-0.13	0.14	0.11	0.10	-0.17	0.19	0.13	-0.05	0.06	-0.03	0.09	1.00	1.00	0.99	1.00	1.00
	p	0.08	0.08	0.08	0.19	0.18	0.08	0.03	0.10	0.31	0.29	0.89	0.11	0.00		0.00	0.00	0.00
	N	144	169	169	144	169	105	144	169	513	344	25	344	513	513	513	513	513
EndF	r	0.14	-0.13	0.15	0.11	0.10	-0.17	0.20	0.13	-0.04	0.04	-0.05	0.08	0.99	0.99	1.00	0.99	1.00
	p	0.09	0.09	0.05	0.19	0.19	0.09	0.02	0.08	0.42	0.42	0.82	0.14	0.00	0.00		0.00	0.00
	N	144	169	169	144	169	105	144	169	513	344	25	344	513	513	513	513	513
MaxF	r	0.13	-0.11	0.13	0.10	0.09	-0.18	0.17	0.12	-0.02	0.06	-0.04	0.09	1.00	1.00	0.99	1.00	0.99
	p	0.12	0.16	0.08	0.25	0.22	0.07	0.04	0.13	0.73	0.31	0.84	0.11	0.00	0.00	0.00		0.00
	N	144	169	169	144	169	105	144	169	513	344	25	344	513	513	513	513	513
MinF	r	0.15	-0.15	0.15	0.12	0.10	-0.16	0.20	0.14	-0.07	0.05	-0.04	0.08	0.99	1.00	1.00	0.99	1.00
	p	0.07	0.05	0.06	0.16	0.18	0.10	0.02	0.07	0.13	0.39	0.86	0.14	0.00	0.00	0.00	0.00	
	N	144	169	169	144	169	105	144	169	513	344	25	344	513	513	513	513	513

r Pearson's r

a Cannot be computed because at least one of the variables is constant.

Table 2 Correlation analysis table of all acoustic variables of wuh vocalizations

		Dur	Duty	Phrase	P/V	Pdur	Rate	U/V	U/P	Udur	ISI	IWI	Cycle	AveF	StartF	EndF	MaxF	MinF
Dur	r	1.00	-0.06	a	0.90	0.19	-0.23	0.80	0.18	-0.06	a	a	a	-0.05	-0.09	0.01	-0.04	-0.05
	p		0.64		0.00	0.16	0.24	0.00	0.19	0.66				0.70	0.52	0.91	0.79	0.70
	N	57	57	57	57	57	28	57	57	57	0	0	0	57	57	57	57	57
Duty	r	-0.06	1.00	0.09	0.14	-0.61	-0.02	-0.43	-0.71	0.27	a	-0.18	a	-0.13	-0.17	-0.09	-0.13	-0.12
	p	0.64		0.42	0.30	0.00	0.89	0.00	0.00	0.01		0.40		0.23	0.12	0.43	0.25	0.27
	N	57	83	83	57	83	39	57	83	83	0	25	0	83	83	83	83	83
Phrase	r	a	0.09	1.00	a	-0.06	-0.18	a	-0.10	0.02	a	0.06	a	-0.40	-0.40	-0.38	-0.41	-0.39
	p		0.42			0.60	0.27		0.35	0.88		0.77		0.00	0.00	0.00	0.00	0.00
	N	57	83	83	57	83	39	57	83	83	0	25	0	83	83	83	83	83
P/V	r	0.90	0.14	a	1.00	-0.16	0.03	0.56	-0.15	-0.09	a	a	a	0.02	-0.05	0.15	0.02	0.05
	p	0.00	0.30			0.23	0.87	0.00	0.26	0.48				0.89	0.72	0.28	0.89	0.69
	N	57	57	57	57	57	28	57	57	57	0	0	0	57	57	57	57	57
Pdur	r	0.19	-0.61	-0.06	-0.16	1.00	-0.54	0.60	0.92	0.09	a	0.17	a	-0.02	0.02	-0.12	0.04	-0.11
	p	0.16	0.00	0.60	0.23		0.00	0.00	0.00	0.44		0.43		0.86	0.85	0.27	0.75	0.32
	N	57	83	83	57	83	39	57	83	83	0	25	0	83	83	83	83	83
Rate	r	-0.23	-0.02	-0.18	0.03	-0.54	1.00	-0.33	-0.34	-0.48	a	0.19	a	0.06	0.07	0.08	0.00	0.15
	p	0.24	0.89	0.27	0.87	0.00		0.08	0.03	0.00		0.57		0.72	0.68	0.64	0.98	0.36
	N	28	39	39	28	39	39	28	39	39	0	11	0	39	39	39	39	39
U/V	r	0.80	-0.43	a	0.56	0.60	-0.33	1.00	0.68	-0.23	a	a	a	-0.08	-0.07	-0.11	-0.07	-0.13
	p	0.00	0.00		0.00	0.00	0.08		0.00	0.09				0.55	0.63	0.43	0.58	0.34
	N	57	57	57	57	57	28	57	57	57	0	0	0	57	57	57	57	57
U/P	r	0.18	-0.71	-0.10	-0.15	0.92	-0.34	0.68	1.00	-0.22	a	0.22	a	-0.01	0.05	-0.12	0.01	-0.09
	p	0.19	0.00	0.35	0.26	0.00	0.03	0.00		0.04		0.30		0.92	0.69	0.27	0.95	0.41
	N	57	83	83	57	83	39	57	83	83	0	25	0	83	83	83	83	83
Udur	r	-0.06	0.27	0.02	-0.09	0.09	-0.48	-0.23	-0.22	1.00	0.08	-0.11	0.21	0.03	0.01	0.03	0.04	0.02
	p	0.66	0.01	0.88	0.48	0.44	0.00	0.09	0.04		0.29	0.61	0.01	0.66	0.84	0.68	0.50	0.78
	N	57	83	83	57	83	39	57	83	243	155	25	155	241	241	241	241	241
IWI	r	a	a	a	a	a	a	a	a	0.08	1.00	a	0.79	0.00	-0.02	0.03	-0.02	0.03
	p									0.29			0.00	0.97	0.82	0.68	0.80	0.68
	N	0	0	0	0	0	0	0	0	155	155	0	155	153	153	153	153	153
IPI	r	a	-0.18	0.06	a	0.17	0.19	a	0.22	-0.11	a	1.00	a	-0.16	-0.13	-0.09	-0.13	-0.10
	p		0.40	0.77		0.43	0.57		0.30	0.61				0.45	0.54	0.66	0.55	0.62
	N	0	25	25	0	25	11	0	25	25	0	25	0	25	25	25	25	25
Cycle	r	a	a	a	a	a	a	a	a	0.21	0.79	a	1.00	-0.06	-0.08	-0.02	-0.08	-0.02
	p									0.01	0.00			0.48	0.32	0.81	0.33	0.80
	N	0	0	0	0	0	0	0	0	155	155	0	155	153	153	153	153	153
AveF	r	-0.05	-0.13	-0.40	0.02	-0.02	0.06	-0.08	-0.01	0.03	0.00	-0.16	-0.06	1.00	0.99	0.99	0.99	0.99
	p	0.70	0.23	0.00	0.89	0.86	0.72	0.55	0.92	0.66	0.97	0.45	0.48		0.00	0.00	0.00	0.00
	N	57	83	83	57	83	39	57	83	241	153	25	153	241	241	241	241	241
StartF	r	-0.09	-0.17	-0.40	-0.05	0.02	0.07	-0.07	0.05	0.01	-0.02	-0.13	-0.08	0.99	1.00	0.97	1.00	0.98
	p	0.52	0.12	0.00	0.72	0.85	0.68	0.63	0.69	0.84	0.82	0.54	0.32	0.00		0.00	0.00	0.00
	N	57	83	83	57	83	39	57	83	241	153	25	153	241	241	241	241	241
EndF	r	0.01	-0.09	-0.38	0.15	-0.12	0.08	-0.11	-0.12	0.03	0.03	-0.09	-0.02	0.99	0.97	1.00	0.98	1.00
	p	0.91	0.43	0.00	0.28	0.27	0.64	0.43	0.27	0.68	0.68	0.66	0.81	0.00	0.00		0.00	0.00
	N	57	83	83	57	83	39	57	83	241	153	25	153	241	241	241	241	241
MaxF	r	-0.04	-0.13	-0.41	0.02	0.04	0.00	-0.07	0.01	0.04	-0.02	-0.13	-0.08	0.99	1.00	0.98	1.00	0.98
	p	0.79	0.25	0.00	0.89	0.75	0.98	0.58	0.95	0.50	0.80	0.55	0.33	0.00	0.00	0.00		0.00
	N	57	83	83	57	83	39	57	83	241	153	25	153	241	241	241	241	241
MinF	r	-0.05	-0.12	-0.39	0.05	-0.11	0.15	-0.13	-0.09	0.02	0.03	-0.10	-0.02	0.99	0.98	1.00	0.98	1.00
	p	0.70	0.27	0.00	0.69	0.32	0.36	0.34	0.41	0.78	0.68	0.62	0.80	0.00	0.00	0.00	0.00	
	N	57	83	83	57	83	39	57	83	241	153	25	153	241	241	241	241	241

r Pearson's r

a Cannot be computed because at least one of the variables is constant.

Appendix 7: Autocorrelation

Table 1. Autocorrelation function analysis (ACF) of 10% of squeak and wuh vocalizations for unit level variables demonstrating minimal correlation between successive units. Presence of + indicates a significant correlation ($p < 0.05$) between units.

	Lag 1								Lag 2							
	Dur	IUI	Cycle	AveF	StartF	EndF	MaxF	MinF	Dur	IUI	Cycle	AveF	StartF	EndF	MaxF	MinF
Squeak 6																
Squeak 8			+								+					
Squeak 13																
Squeak 43																
Squeak 76																
Squeak 108				+	+	+	+	+				+	+	+	+	+
Squeak 119																
Squeak 157		+								N/A						
Squeak 163																
Squeak 165				+	+	+	+	+				+	+	+	+	+
Squeak 209																
Squeak 224																
Squeak 240				+								+				
Squeak 267																
Wuh 18																
Wuh 36																
Wuh 64				+	+		+					+	+		+	
Wuh 66																
Wuh 68																
Wuh 187																

Appendix 8: Contextual Distribution of Vocalisations

Table 1. The production of each vocalisation type produced by individual red wolves in activity states.

		Wolf								
Vocalisation	**Activity State**	**F7**	**M8**	**F8**	**M11**	**F11**	**M12**	**F12**	**J12**	**Grand Total**
SV	Resting			1	1		1		1	4
	Sitting								1	1
	Standing	1	2		6	1	4	11	6	31
	Locomotion		2		4	1		3	1	11
	Object Play								1	1
	Socializing							1		1
	Pen-mate play								1	1
	Total	1	4	1	11	2	5	15	11	50
WV	Resting		1							1
	Standing		3				1			4
	Locomotion			1			3			4
	Socializing						2	1		3
	Pen-mate play								1	1
	Neighbour play		2							2
	Threat						1			1
	Total		6	1			7	1	1	16
SWV	Standing				5		1	5		11
	Locomotion				1			2		3
	Socializing	1					1			2
	Pen-mate play								1	1
	Total	1			6		2	7	1	17
CSV	Sitting								1	1
	Standing				1		1	3	3	8
	Locomotion							1		1
	Pen-mate play								4	4
	Total				1		1	4	8	14
SNV	Standing				7			1		8
	Locomotion				4	1				5
	Pen-mate play								1	1
	Total				11	1		1	1	14
Grand Total		2	10	2	29	3	15	28	22	111

Table 2. The production of each vocalisation type produced by individual red wolves in relational states.

		Wolf								
Vocalisation	**Relational State**	**F7**	**M8**	**F8**	**M11**	**F11**	**M12**	**F12**	**J12**	**Grand Total**
S	Directed Pen-Mate					1	3	3	3	10
	Directed Other		1		5			1		7
	Termination	1			4	1	1	8	2	17
	Non-specific		3	1	2		1	3	6	16
	Total	1	4	1	11	2	5	15	11	50
W	Directed Pen-Mate						5	1	1	7
	Directed Other		2							2
	Termination		1	1			1			3
	Non-specific		2				1			3
	Unknown		1							1
	Total		6	1			7	1	1	16
SWV	Directed Pen-Mate	1					2		1	4
	Directed Other				4			1		5
	Termination				1			5		6
	Non-specific				1			1		2
	Total	1			6		2	7	1	17
SJ	Directed Pen-Mate								6	6
	Directed Other				1		1	4	1	7
	Termination								1	1
	Total				1		1	4	8	14
NS	Directed Pen-Mate				1				1	2
	Directed Other				7			1		8
	Non-specific				3	1				4
	Total				11	1		1	1	14
Grand Total		2	10	2	29	3	15	28	22	111

Table 3. The production of each vocalisation type produced by individual red wolves in proximity states.

		Wolf								
Vocalisation	**Proximity (wolf lengths)**	**F7**	**M8**	**F8**	**M11**	**F11**	**M12**	**F12**	**J11**	**Grand Total**
SV	0-1				1			3	4	8
	2-5				1		5	8	3	17
	6-9				1				2	3
	10+	1	4	1	8	2		4	2	22
	Total	1	4	1	11	2	5	15	11	50
WV	0-1		1				6	1	1	9
	2-5		1							1
	6-9						1			1
	10+		3	1						4
	Unknown		1							1
	Total		6	1			7	1	1	16
SWV	0-1	1			1		1		1	4
	2-5							3		3
	6-9							2		2
	10+				5		1	2		8
	Total	1			6		2	7	1	17
CSV	0-1						1	1	4	6
	2-5							2	3	5
	6-9				1			1		2
	10+								1	1
	Total				1		1	4	8	14
BSV	0-1				3				1	4
	2-5				1					1
	6-9				2			1		3
	10+				5	1				6
	Total				11	1		1	1	14
Grand Total		2	10	2	29	3	15	28	22	111

Table 4. The production of each vocalisation type produced by red wolves in activity and relational states.

		Relational State					
Vocalisation	**Activity State**	**Directed Pen-mate**	**Directed-Other**	**Termination**	**Non-specific**	**Unknown**	**Grand Total**
SV	Lying Down		1		3		4
	Sitting				1		1
	Standing	8	5	13	5		31
	Locomotion	1	1	4	5		11
	Object play				1		1
	Socializing				1		1
	Pen-mate play	1				1	2
	Neighbour play	1					1
	Total	11	7	17	16	1	52
WV	Lying Down				1		1
	Standing		1	1	2		4
	Locomotion	2		2			4
	Socializing	3					3
	Pen-mate play	7				1	8
	Neighbour play		1			2	3
	Threat	1					1
	Total	13	2	3	3	3	24
SWV	Standing	1	3	6	2		12
	Locomotion		2		1		3
	Socializing	2					2
	Pen-mate play	3					3
	Total	6	5	6	3		20
CSV	Sitting		1				1
	Standing	2	5	1			8
	Locomotion		1				1
	Pen-mate play	4					4
	Total	6	7	1			14
BSV	Standing		8				8
	Locomotion	1			4		5
	Pen-mate play	1					1
	Total	2	8		4		14
Grand Total		38	29	27	26	4	124

Table 5. The production of each vocalisation type produced by red wolves in activity and proximity states.

		Proximity (wolf lengths)				
Vocalisation	Activity State	0-1	2-5	6-9	10+	Grand Total
SV	Lying down		1	1	2	4
	Sitting		1			1
	Standing	4	14	1	12	31
	Locomotion	2	1	1	7	11
	Socializing	1				1
	Object Play				1	1
	Pen-mate Play	2				2
	Neighbour play	1				1
	Total	10	17	3	22	52
WV	Lying down				1	1
	Standing	1	1		2	4
	Locomotion	2		1	1	4
	Socializing	3				3
	Pen-mate Play	8				8
	Neighbour play	1				1
	Threat	1				1
	Total	16	1	1	4	22
SWV	Standing	1	3	1	7	12
	Locomotion		1	1	1	3
	Socializing	2				2
	Pen-mate Play	3				3
	Total	6	4	2	8	20
CSV	Sitting		1			1
	Standing	2	4	1	1	8
	Locomotion			1		1
	Pen-mate Play	4				4
	Total	6	5	2	1	14
BSV	Standing	2	1	1	4	8
	Locomotion	1		2	2	5
	Pen-mate Play	1				1
	Total	4	1	3	6	14
Grand Total		42	28	11	41	122

Table 6. The production of each vocalisation type produced by red wolves in relational and proximity states.

		Proximity (wolf lengths)					
Vocalisation	**Relational State**	**0-1**	**2-5**	**6-9**	**10+**	**Unknown**	**Grand Total**
SW	Directed Pen-mate	4	5		2		11
	Directed-Other	2	1		4		7
	Termination		8	1	8		17
	Non-specific	3	3	2	8		16
	Unknown	1				1	2
	Total	10	17	3	22	1	53
WV	Directed Pen-mate	13					13
	Directed-Other	1			1		2
	Termination			1	2		3
	Non-specific	1	1		1		3
	Unknown	1				2	3
	Total	16	1	1	4	2	24
SWV	Directed Pen-mate	5			1		6
	Directed-Other			1	4		5
	Termination	1	2	1	2		6
	Non-specific		2		1		3
	Total	6	4	2	8		20
CSV	Directed Pen-mate	4	2				6
	Directed-Other	2	3	2			7
	Termination				1		1
	Total	6	5	2	1		14
BSV	Directed Pen-mate	1		1			2
	Directed-Other	2	1	1	4		8
	Non-specific	1		1	2		4
	Total	4	1	3	6		14
Grand Total		42	28	11	41	3	125