The Ecology and Conservation of Cave Roosting Bats in the Yorkshire Dales

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others

Hibernating *Myotis* bats in Buckden Gavel Mine. Photo: Anita Glover

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And for the amusement of those who watched me write up, especially John………

Bill Watterson (1995) The Calvin and Hobbes Tenth Anniversary Book. Time Warner Books, London.

Abstract

The caves of the Yorkshire Dales National Park are potentially an important assemblage of underground bat sites, but prior to this study nothing was known about the ecology of cave use. Between 2002 and 2005, 53 cave entrances were logged for swarming activity using automated echolocation call loggers. A subset was chosen for continuous monitoring outside the hibernation period and another for winter logging. Logging was followed up by trapping at caves with significant autumn bat activity. All bats caught were ringed, identified to species and sexed. Biopsies (3 mm diameter) were taken from each wing for genetic analysis.

A large proportion of the caves surveyed were used by bats, but activity was concentrated in a small number of hotspots. Peak activity occurred during August and September, but significant activity was recorded in all months between March and October. All five swarming species were caught at most sites: *Myotis brandtii, M. daubentonii, M. mystacinus, M. nattereri* and *Plecotus auritus*. *M. nattereri* was the most abundant species late in September, but earlier other species made up a significant proportion of those caught. The sex ratio of swarming bats was heavily skewed towards males.

Swarming activity was positively correlated with the degree of cave chamber development, cave length and shelter at the entrance, and negatively correlated with the amount of water the cave carried. Entrance size, altitude and the degree of connectivity between cave and summer habitat were not correlated with activity. In a multiple regression model, chamber development and hydrology were the best predictors of activity. Entrance orientation and shelter contributed towards the model but were less important. The regression model could explain 55% of the variance in bat activity.

Genetic diversity of *Myotis nattereri*, based on an analysis of six microsatellite loci, was high in swarming populations suggesting high gene flow and large effective populations. Most of the genetic variation was explained by variation among individuals within populations, but some was due to differences between swarming areas and by differences between populations within swarming areas. Isolation by distance was found between sites in six swarming areas between Cumbria and Sussex, but not between sites in the Yorkshire Dales. The differentiation observed was more logically explained on the basis of local geography and probable catchment areas.

Preliminary experiments suggest that Time Domain Signal Coding has potential as a sound analysis system for real time bat call identification.

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Chapter 1: General Introduction

1.1 Scope

This chapter aims to review the important background literature to the research reported in this thesis. The research concerns the role of caves in the annual cycle of temperate bats in one of Europe's most important karst landscapes. The scope is therefore broad, covering many aspects of bat ecology, but each will necessarily be brief. Life history strategies of bats will be discussed, with emphasis on the temperate cycle. The roosting ecology of bats will be discussed with reference to seasonal changes in requirements and sites used. The social structure of bats will be described, looking at colony composition and stability and the reasons for sexual segregation explored, before discussing mating systems. The role of dispersal and migration in bats will then be discussed, before describing swarming behaviour in some detail, since this is the central theme of the thesis. The rationale behind the study and a brief description of the study area and species will be presented before finishing with the principle aims of the research.

1.2 Bat ecology

1.2.1 Life history strategies and annual cycle

1.2.1.1 Life history traits

Considering the ecological diversity of the Chiroptera, it is perhaps surprising that there is relatively little variation in their life history strategies (see Barclay & Harder 2003 for a review). One interesting feature they have in common is that they exhibit many life history traits that are typical of much larger mammals:

- They are long lived - 30 years or more (e.g. Tuttle & Stevenson 1982).
- They exhibit low fecundity - typically one litter containing one offspring per year (although see Wilson 1971 and Isaac *et al.* 1994 for examples of multiple litters and twins in tropical species).
- They show high levels of parental investment (Kunz and Hood 2000).

- They are relatively slow to reach sexual maturity (Racey and Entwistle 2000), although this is less pronounced in the tropics than in temperate regions.

Bats are the only mammals capable of powered flight and this ability has influenced bat life history strategies in two ways (Barclay & Harder 2003). Firstly, flight places constraints on body mass, development and reproduction, and these underlie many of the similarities in bat life histories. Secondly, flight reduces predation risk and increases access to resources, presumably placing bat life histories under different selection pressures to those of other mammals.

1.2.1.2 Torpor and hibernation in temperate bats

Daily torpor and the ability to enter seasonal hibernation are important physiological traits in temperate bats, influencing both their distribution and their ecology. For this reason, the concepts and physiology of torpor and hibernation are introduced here, with further discussion of their ecological relevance in the following sections.

Bats, like all mammals, are endothermic. Endotherms maintain a high body temperature by metabolising their food to generate heat internally. However, the process of endothermy is reliant on a high intake of food, and temperate insectivorous bats face several major energetic problems (Altringham 1996). Like other small mammals their low body mass/surface area ratio means that they loose heat more quickly. They therefore require a high metabolic rate in order to maintain a constant core temperature. Their diet of insects is low in energy and unlike other insectivores, such as shrews, they are restricted to nocturnal foraging. In addition, insect populations may be ephemeral and fluctuate with prevailing weather conditions - most aerial insects cease to fly at temperatures below 7 °C (Taylor 1963; Peng *et al.* 1992). There is also a prolonged seasonal shortage of food during the winter coupled with increased thermoregulatory costs.

Bats in temperate regions are heterothermic i.e. they regulate their body temperature to minimise energy expenditure. It is thought that heterothermy evolved in the tropics in response to fluctuating food resources and that this capability enabled bats to expand into temperate regions (McNab 1969). Temperate bats use daily torpor to conserve energy throughout the year and undergo seasonal hibernation during the winter.

Daily torpor and hibernation are distinguished as distinctive heterothermic states on the basis of torpor bout duration (Geiser and Ruf 1995). However in temperate bats, the progression from one state to another is a continuum as torpor bouts increase in length at the end of autumn and decrease in spring. This also makes the precise period of hibernation difficult to define.

Torpor typically involves a controlled reduction in body temperature to within 1-2 °C of ambient temperature. Breathing rate and heart rate fall, thus reducing the supply of oxygen to the tissues, metabolic rate declines and peripheral vasoconstriction occurs (Altringham 1996; Speakman and Thomas 2003).

Hibernation is not a continuous state and bats arouse periodically throughout the winter. Arousals are energetically expensive because in order to generate the heat required to arouse, bats metabolise some of their fat reserves (Davis 1970). Arousal (in the absence of disturbance) must therefore have functional significance. Possible explanations as to why bats arouse include: (i) foraging on warmer nights: this is probably dependent on species and the type of prey taken (see Ransome 1971; Avery 1985; Brigham 1987; Whitaker and Rissler 1993); (ii) drinking to counteract evaporative water loss (Speakman and Racey 1989; Hays *et al.* 1992; Thomas and Cloutier 1992; Thomas and Geiser 1997); (iii) metabolic imbalance and immunological function (see Willis 1982; Schmidt 1967); (iv) sleep deprivation (Daan *et al.* 1991; Trachsel *et al.* 1991; Deboer 2005); (v) moving roosting location in response to changes in microclimate within a hibernaculum (Twente 1955; Ransome 1968; Daan 1968; 1973); (vi) mating (Guildhay 1948; Thomas *et al.* 1979; Avery 1985; Gebhard 1995).

1.2.1.3 The temperate cycle

The annual cycle in temperate bats consists of three main phases (1) parturition and lactation, (2) mating and (3) hibernation (Altringham 1996; Racey and Entwistle 2000). The exact timing of these phases is species dependent and may vary from year to year in accordance with environmental fluctuations and physiological status. There is also significant overlap between certain phases (see Figure 1.1).

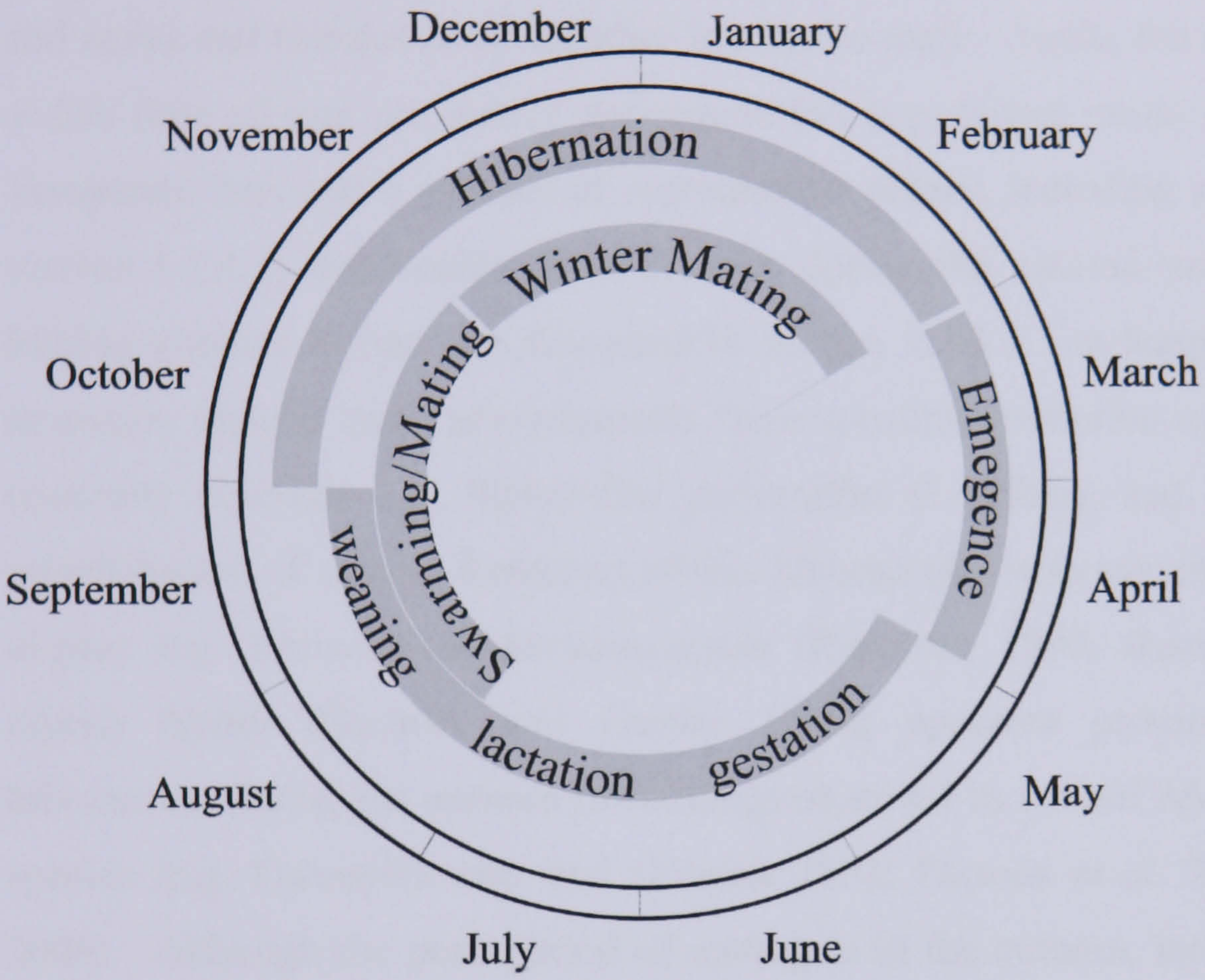

Figure 1.1 The annual cycle in north temperate bats (from Altringham 2003)

The timing of emergence from hibernation depends on weather conditions in the spring and hence food availability. Bats may not move directly to summer roosts, instead remaining for some time at hibernacula but using progressively shorter bouts of torpor (Altringham 2003). They may also occupy transitional roosts, although this aspect in the roosting ecology of temperate bats is poorly understood. In general, males are solitary or roost in small bachelor groups during the summer, whereas females form maternity colonies of tens to hundreds of individuals. The energetic demands of pregnancy and lactation place constraints on females leading to the selection of warmer roost sites (Lausen and Barclay 2002; 2003). Communal roosting among females can reduce the cost of thermoregulation (Trune and Slobodchikoff

1976; Roverud and Chappell 1991) and confer benefits through information transfer between conspecifics (Wilkinson 1992). Males are not under the same constraints as females and can utilise cold roosts (including hibernation sites) throughout the summer, using torpor to conserve energy (Hamilton and Barclay 1994; Grinevitch *et al.* 1995). The degree of sexual segregation during the summer varies with species, geographical location and altitude (see section 1.2.3.2).

At the end of the summer as the young are weaned, summer colonies begin to disperse and males and females come together in order to mate. Again, the movements of bats at this time of year are poorly understood but transitional roosts may be important. Temperate bats use a number of reproductive delays, including sperm storage (see section 1.2.4.2) that enable them to mate during the autumn prior to hibernation. Mating systems in bats are discussed in section 1.2.4.1. In temperate bats, mating strategies include male advertisement from roosting territories established close to maternity colonies e.g. *Pipistrellus pipistrellus* (Lundberg and Gerell 1986); the establishment of mating territories within hibernacula, with varying degrees of male display e.g. *Rhinolophus ferrumequinum* (Ransome 1990; Rossiter *et al.* 2000), *Myotis blythii* (Horácek and Gaisler 1986); apparent promiscuous mating at hibernacula during the autumn (swarming) observed in several *Myotis* and *Plecotus* species (e.g. Furmankiewicz and Górniak 2002; Parsons *et al.* 2003; Rivers *et al.* 2006). Although the peak period of mating is in the autumn, mating may continue through the winter and even into spring (Strelkov 1962; Furmankiewicz and Górniak 2002).

The onset of hibernation is a transitional process in response to decreasing temperatures and a reduction in food availability (see section 1.2.1.2). Over the course of the autumn, the resident populations of hibernacula increase and bats use progressively longer bouts of torpor. Hibernating populations contain both sexes and many are mixed-species assemblages.

1.2.2 Roosting ecology

Bats spend a great deal of their lives in their roosts and the selection of suitable roosting sites is crucial to the success of an individual or colony. However, bats are adaptable and opportunistic roosters and this has allowed them to exploit a wide range of roost types, both natural and man-made (Kunz 1982; Altringham 1996). The availability of suitable roosts influences the distribution and density of bats at both a local and global scale, and generalist roosters tend to be more widespread than specialists. The following sections describe the roosting requirements of temperate species during the active and hibernation season. Aspects of roosting ecology related to social structure are discussed further in section 1.2.3.

1.2.2.1 Active season

The roosting requirements of temperate bats during the active season are to some degree sex-dependent due to the energetic demands of reproduction (Hamilton and Barclay 1994). Females must select sites where the energetic costs of pregnancy and lactation can be met. Warm roosts, and the formation of large maternity colonies, reduce thermoregulation costs and promote rapid growth and development of young (Tuttle 1976a). The selection of roosts close to areas of high and reliable food resources decreases the cost of commuting to foraging sites and increases reproductive success (Tuttle 1976a). Roost sites must also offer protection from predators and the weather.

Worldwide, caves support the largest aggregations of bats, but in temperate regions they are less likely to offer suitable roosting conditions for females, particularly at higher latitudes. Caves are often a limited resource and may be located considerable distances from profitable foraging areas. In addition, they may be too cold to promote efficient thermoregulation. Many tropical bat species use foliage roosts (reviewed in Kunz and Lumsden 2003) but this tendency decreases away from the equator and temperate bats rarely use exposed roosts.

Most temperate species roost in trees and man-made structures. Solitary bats can roost beneath flaking bark (e.g. Cervený and Bürger 1989) but these roosts offer limited scope for larger aggregations. Several species of temperate vespertilionid bats use tree cavities as summer roosts including *Myotis nattereri*, *M. daubentonii*, *M. bechsteinii* and *Nyctalus noctula* (Gaisler *et al.* 1979; Cervený and Bürger 1989; Senior *et al.* 2005). Bat boxes erected in woodland areas may be seen as substitutes for tree cavity roosts, and have been adopted by *M. nattereri*, *M. bechsteinii*, *Pipistrellus pipistrellus* and *P. pygmaeus* (Park *et al.* 1996; 1998; Kerth *et al.* 2001; Rivers *et al.* 2006). Roosts in man-made structures include mines, tunnels, buildings (roof cavities, cellars and crevices) and bridges.

1.2.2.2 Hibernation season

Bats require hibernation sites that offer a stable, cool and humid microclimate and are free from disturbance. Some temperate species hibernate in trees e.g. *Nyctalus noctula* and *Barbastellus barbastellus*, but these sites are less stable in terms of their microclimate and tree-hibernators must arouse and move roost site more frequently than those bats that roost in caves (Sluiter *et al.* 1973). Most temperate species hibernate in caves and man-made underground sites (mines, tunnels, cellars, ice houses). Microclimate inside underground sites is buffered against fluctuations in ambient temperature although this relationship is affected by distance from the entrance, number and orientation of entrances and complexity of the system (Tuttle and Stevenson 1977). The precise microclimatic conditions required vary from species to species (Webb *et al.* 1996), but most species prefer temperatures between 2 and 10 °C.

1.2.3 Social structure

1.2.3.1 Why do bats aggregate?

The distribution, density and permanency of roosts all influence colony size and social structure (Altringham and Senior 2005). Bats that form the largest colonies typically roost in caves, although colonies of tree-roosting Old World fruit bats (Pteropodidae) may also form large colonies that number over 100,000 (Kunz and Lumsden 2003). The cave-roosting Brazilian free-tailed bat (*Tadarida brasiliensis*) forms the largest aggregations, numbering several million in its nursery colonies (Davis *et al.* 1962). Caves are effectively permanent roost sites but are often limited in number and patchy in their distribution. Bats that roost in potentially abundant but ephemeral roosts, such as foliage and beneath exfoliating bark, form smaller groups (for a review, see Kunz and Lumsden 2003).

Roost availability may go some way to explaining the degree to which bats aggregate but what are the main benefits that encourage/necessitate colonial behaviour? They can be divided into two groups: (i) factors that benefit both transient and stable groupings (e.g. thermoregulation and reduced predation risk), (ii) social benefits that may rely on long-term associations between conspecifics.

1.2.3.1.1 Benefits to both transient and stable groups

Colonial roosting can confer thermoregulatory benefits to individuals through clustering (Trune and Slobodchikoff 1976). Clustering reduces an individual's surface area and hence heat loss to its surroundings (Speakman and Thomas 2003). Large colonies are even capable of raising ambient temperature inside the roost (Tuttle 1976a). Within a species, maternity roosts contain more individuals than non breeding roosts as reproductive females have the most to gain from reduced thermoregulatory costs (Roverud and Chappell 1991). Clustering also allows bats that hibernate in less thermally stable sites, such as tree cavities, to tolerate periods of extreme cold weather rather than arouse (Sluiter *et al.* 1973). Bats may also cluster to reduce evaporative water loss, over 99% of which occurs across the body surface (Thomas and Cloutier 1992). This is particularly important in hibernating bats because limiting water loss reduces both the risk of desiccation and the frequency of energetically expensive arousals in order to drink.

Roosts themselves offer protection from predators but individuals can reduce predation risk during emergence by roosting in large numbers, through the dilution effect. Small colonies emerge later than large ones, presumably due to the greater risk from diurnal predators earlier in the evening (Fenton *et al.* 1994). Bats can further reduce predation risk through temporal clustering during emergence (Speakman *et al.* 1999).

Roost members can gain useful information from each other (reviewed by Wilkinson 1995). In the evening bat, *Nycticeus humeralis* (Wilkinson 1992) females returning to the roost after an unsuccessful foraging trip followed other departing females and were more successful than females that did not adopt this behaviour. The bats followed appeared to be chosen at random so this behaviour could benefit even transient associations of bats.

1.2.3.1.2 Benefits to stable social groups

Coloniality in stable groups can lead to the evolution of co-operative behaviour such as group foraging. This would, for example, benefit species that feed on prey that are patchy in both time and space, such as aerial hawkers. This is a little a studied area with only a few well-documented examples, mainly in tropical species (see Wilkinson 1995 for a review).

1.2.3.2 Sexual segregation

Males and females typically roost apart during the nursery season, but few studies have attempted to determine why and most of the evidence supporting possible explanations is indirect or anecdotal. Tuttle (1979) has shown that foraging is often more efficient and infant mortality lower if the females have to commute only short distances for food. Therefore, females may exclude males from the roost to reduce competition for food during the maternity season (Kunz 1974). This assumes that males and females sharing the same roost share foraging ranges, but this is not always the case. Male and female *Myotis daubentonii* did so in one study (Senior *et al.* 2005), but in a study by Entwistle *et al.* (1996), male *Plecotus auritus* foraged on the periphery of the maternity colony's home range.

It is likely that different thermal requirements in temperate bats demand that males and females roost apart for part of the year. As discussed above, males often choose cooler roosts during the summer to take advantage of the energy savings that come from torpor, an option not available to pregnant and lactating females (e.g. Hamilton and Barclay 1994).

Segregation may have evolved for many other reasons, none of which have been explored. These include the avoidance of aggression, either between males or between males and the females and their offspring; the avoidance by males of the high parasite numbers found in many maternity roosts; and limitations in the size and number of suitable roosts. Sexual segregation in bats is discussed in some detail by Altringham and Senior (2005).

1.2.4 Mating systems

1.2.4.1 Factors which affect mating systems

The mating strategies that exist among bats are incredibly diverse, perhaps reflecting the ecological diversity of the order. Furthermore, single species may employ more than one mating strategy (e.g. Senior *et al.* 2005). The diversity and flexibility of mating strategies among bats, coupled with a lack of knowledge in the case of many species, mean that attempts to classify bat mating systems (see Bradbury and Vehrencamp 1976; 1977; Altringham 1996; McCracken and Wilkinson 2000) often simplify complex situations. As McCracken and Wilkinson (2000) point out 'any attempt to categorise bat mating systems is a compartmentalisation of a continuum'.

Mating systems in mammals are influenced by population density and the distribution of individuals, which in turn are determined by the abundance and distribution of resources (Clutton-Brock and Harvey 1978).

Emlen and Oring (1977) classify mating systems on the basis of the ability of one sex to monopolise access to one or several mates and the means by which this is achieved. The potential to monopolise access to several mates (polygamy) will depend on whether multiple mates, or the resources necessary to attract multiple mates, are economically defendable. This will be dependent on the spatial and temporal distribution of both resources and mates. Also important is the emancipation of one sex from parental care, allowing them to pursue multiple matings.

Monogamy will develop when (i) monopolisation of one mate is viable, but not more than one and/or (ii) juvenile survival depends upon, or is increased by, parental care afforded by both sexes. Monogamous pairs form in the neotropical emballonurid *Saccopteryx leptura*, and males defend small feeding territories used by one to a few females (Bradbury and Vehrencamp 1976; 1977). The only species observed in which the males provide directly for the young are *Vampyrum spectrum* (Vehrencamp *et al.* 1977) and *Lavia frons* (Vaughan and Vaughan 1987).

Polygyny arises when individual males gain access to multiple females. Males may control access to females indirectly, by monopolising resources (resource defence polygyny) or directly, by defending aggregations of females (female/harem defence polygyny). Limited resources favour resource defence, and gregariousness in females will favour the formation of harems. However the two are inextricably linked, especially in bats where the limited resource may be roost sites, and distinguishing between resource defence and female defence is not always possible (Ostfeld 1987). Perhaps for this reason, McCracken and Wilkinson (2000) classify polygynous mating systems according to seasonality and female group stability. Although individual males may actively defend groups of females, or the resources they require, genetic paternity studies reveal the extent of extra-harem copulations. Only a third of juveniles were fathered by the harem holder in *Saccopteryx bilineata* (Heckel *et al.* 1999) and nearly a quarter of juveniles were fathered by satellite males in *Artibeus jamaicensis* (Ortega *et al.* 2003). These studies demonstrate how social structure cannot provide a complete picture of the complex mating systems that may be operating.

Examples of both resource and female defence polygyny have been described within a single species. *Artibeus jamaicensis* roosting in forest habitats in Panama and Mexico use two different roost types. Solitary males and small groups of males roost in ephemeral foliage roosts, whereas females show a marked preference for tree hollows. The tree hollows, which offer better protection from the elements and predation, are a limited resource enabling dominant males to defend them and gain access to multiple mates (Morrison 1979). Males use patrolling movements and only forage over short distances in order to defend tree hollows (Morrison and Morrison 1981). On the other hand, in caves in Puerto Rico (Kunz *et al.* 1983) and Mexico (Ortega and Arita 1999), *A. jamaicensis* roost in solution cavities. These cavities are numerous and dominant males directly defend harem groups through agonistic interactions with male visitors (Ortega and Arita 2000).

Where mates and resources cannot be monopolised by males, male dominance polygyny may arise. Males aggregate during the breeding season and females select mates from these aggregations. Emlen and Oring (1977) describe two forms of male dominance polygyny: (i) explosive breeding assemblages and (ii) leks. Explosive breeding assemblages involve the convergence of both sexes during a short and highly synchronised mating period. The operational sex ratio is close to unity and sexual selection is minimal. It could be argued that many temperate species fall into this category, mating in the autumn before hibernation. In three European species: *Nyctalus noctula* (Sluiter and van Heerdt 1966), *Pipistrellus pipistrellus* (Gerell and Lundberg 1985; Lundberg and Gerell 1986; Park *et al.* 1998) and *Pipistrellus nathusii* (Gerell Lundberg and Gerell 1994), males establish seasonal mating territories in roosting boxes where they roost and mate with multiple females. At first, this appears to be a case of resource defence polygyny, but because females are transient and may move between male territories mating with more than one male, a case of male dominance polygyny could be argued. To my knowledge, there have been no paternity studies to assess the reproductive success of territorial males in these species, but multiple paternity has been reported in *Nyctalus noctula* (Mayer 1995).

Large aggregations of bats, of several temperate species, gather at hibernacula during the autumn and the behaviour has been termed 'swarming'. Swarming behaviour has been linked to both information transfer regarding hibernacula and to mating, and could be an example of male dominance polgyny. Swarming is discussed further in section 1.2.6.

Leks are characterised by (i) no male parental care; (ii) male display at mating arenas smaller than both sexes' home range; (iii) male territories containing no resources; and (iv) female mate choice (Bradbury 1981). The only bat species known to exhibit true lekking behaviour is *Hypsignathus monstrosus* (Bradbury 1977). This species exhibits extreme sexual dimorphism due to the enlarged larynx of the male, which enables emission of loud, repetitive vocalisations during display. Females show active mate choice, mating success is highly skewed towards a small number of males and other than the males themselves, mating territories contain no resources.

Despite the examples of multiple mating by females (Mayer 1995; Vonhof *et al.* 2006), polyandry has not been described in any bat species.

1.2.4.2 Sperm storage

Sperm storage (reviewed by Racey 1979, and more recently by Crichton. 2000) is the prolonged retention of viable spermatozoa in the reproductive tract. Sperm storage is usually associated with heterothermy, and is typical in bat species where the male and female gametogenic cycles are asynchronous. In temperate species the hibernation period separates the start of the female oestrus and the onset of gestation. Many bats mate in the autumn and sperm is stored in the female reproductive tract until the spring when ovulation occurs. Delayed ovulation and fertilisation is not the only mechanism that is employed by hibernating species, although it is the most common. *Miniopterus schreibersii* is a widespread species complex (Appleton *et al.* 2004) that inhabits both tropical and temperate regions. In the tropics normal foetal development occurs, but at higher latitudes implantation of the embryo can be delayed by as much as five months (Richardson 1977).

The principal site of sperm storage in females varies between species, for example: the oviduct e.g. *Rhinolophus ferrumequinum* (Mori *et al.* 1982) and *R. hipposideros* (Racey 1975); the utero-tubal junction e.g. *Myotis daubentonii* (Racey 1975) and *M. lucifugus* (Krutzsch *et al.* 1982); and the uterus e.g. *Pipistrellus pipistrellus* (Racey and Potts 1970). Uchida and Mori (1987) suggest that spermatozoa receive nutritive substances from epithelial cells in the female reproductive tract. The retention of sperm may be aided in some species by the formation of a vaginal plug. The plug may originate from the male or female gland - this is dependent upon species (Crichton 2000) and may also serve to reduce sperm competition (see section 1.2.4.3).

Because spermatogenesis ceases in late summer/early autumn, male bats also store sperm. The principal storage organs are the caudal epididymes although Krutzsch (1975) and Krutzsch & Crichton (1986) report sperm storage in the testes of several *Pipistrellus* species. This enables males to mate with females through the hibernation period and upon emergence in the spring. Males have even been observed attempting to copulate with torpid females although the reproductive efficacy of such events is unknown.

1.2.4.3 Sperm competition

In any mating system where females mate with more than one male, the opportunity for sperm competition will arise (Parker 1970). In a large number of Chiropteran species (most notably temperate vespertilionids and rhinolophids), the chance of sperm competition is increased due to prolonged sperm storage in females (Fenton 1984). Stored sperm can remain fertile for the duration of female receptivity (Racey 1973; Hosken 1998a). Testes mass in both the Megachiroptera (Hosken 1998b) and the Microchiroptera (Hosken 1997) varies in accordance with sperm competition theory, which states that male investment in spermatogenesis will increase in relation to sperm competition risk (Parker 1982; 1990a; b). Social group size influences the risk of sperm competition through increased risk of multi-male copulations (Møller and Birkhead 1989). Testes mass is positively correlated with group size in both sub orders (Hosken 1997; 1998b). In addition, there is a trend towards greater testis size in species with a male-biased sex ratio, consistent with results obtained from birds (Møller 1991). The influence of mating order and cryptic female choice in sperm competition are yet to be investigated in bats.

1.2.5 Dispersal and migration

1.2.5.1 Dispersal

The definition of dispersal most widely adopted for vertebrates is that of Howard (1960): 'the movement the animal makes from its point of origin to the place where it reproduces or would have reproduced if it had survived and found a mate'. This definition refers to natal dispersal - the permanent dispersal of juveniles from their place of birth to where they intend to breed. Not included in this definition is breeding dispersal which is the movement of reproductive adults between breeding sites. Both natal and breeding dispersal are often sex-biased and the occurrence and possible causes of this bias have been reviewed in Greenwood (1980). Traditionally, mark-recapture methods have been used to measure dispersal patterns (e.g. Kurta and Murray 2002; Rivers *et al.* 2006), but unless accompanied by behavioural observations that enable breeding success to be ascertained, this only provides a measure of gross dispersal. More recently, molecular techniques have facilitated the study of effective dispersal (movements followed by reproductive success) and the consequences for population structure (e.g. Kerth *et al.* 2002; Winters and Waser 2003; Rivers *et al.* 2005).

The mechanisms that influence the evolution of dispersal strategies are summarised in Dieckmann *et al.* (1999). Dispersers often suffer high mortality rates (Gaines and McClenaghan 1980) due to increased risk of exposure, predation and disease. A lack of familiarity with a new habitat may impair foraging success and increase the chance of attack from established individuals (Moore and Ali 1984). Individuals may also incur costs through investment in dispersal morphology (Harrison 1980). So why do animals disperse given the costs? Permanent natal dispersal (either forced or voluntary) reduces competition among conspecifics (Moore and Ali 1984) and prevents inbreeding (Pusey 1987). Breeding dispersal may promote gene flow in species that benefit from philopatry. No single study addresses this directly, but in combination, several papers show its importance (e.g. Kerth and Morf 2004; Burland *et al.* 1999; 2001; Veith *et al.* 2004; Furmankiewicz and Altringham 2006).

1.2.5.2 Seasonal movements and migration

Seasonal dispersal may be driven by factors other than the need for outbreeding. Temporal variability in habitat quality is the major driving force behind the migration of many bat species, particularly but not exclusively, those in temperate zones (Fleming and Eby 2003). Many insectivorous species migrate to hibernate, for example to a less harsh winter climate (e.g. *Nyctalus noctula*, Strelkov 1969); Petit and Mayer 2000), or to localities with suitable underground hibernacula (e.g. *Myotis grisescens*, Tuttle 1976b). In North America, *Tadarida brasiliensis* migrates from the south central United States to Mexico so as to be able to feed throughout the year (Cockrum 1969). Interestingly, populations in southern California, where the winter climate is mild, are sedentary. Fruit and nectar feeding bats also migrate to follow food, for example *Pteropus poliocephalus* in Australia (Eby 1996) and *Leptonycteris curasoae* in Mexico (Ceballos *et al.* 1997).

1.2.5.3 Dispersal ability

Bats typically have lower wing loadings and aspect ratios than birds and are not therefore suited to long distance, fast and efficient flight (Norberg and Rayner 1987). Even those species with wing forms best suited to rapid and cheap migration typically migrate only up to 1,600 km, for example *Nyctalus noctula* (Strelkov 1969; Petit and Mayer 2000), *Tadarida brasiliensis* (Cockrum 1969) and the *Lasiurus* species of the USA (Fleming and Eby 2003). Given the low wing loadings and aspects ratios, and small size of most bats it is perhaps surprising that many of them undertake the journeys they do. *Plecotus auritus* has a wing form for slow, manoeuvrable flight that facilitates gleaning and hovering (Norberg and Rayner 1987). During the summer it forages within 1 km of its roost and frequently less (Entwistle *et al.* 1997), but will make a return journey of over 70 km in a single night to visit a swarming site (Furmankiewicz 2004). *Myotis nattereri*, with a similar wing form can undertake similar flights (Parsons and Jones 2003).

To be able to make long distance flights, bats must be able to navigate. Echolocation operates over relatively short ranges in most species and is not therefore suited to long-range navigation (Altringham and Fenton 2003). The roles of other senses in migration are very poorly understood and our knowledge has hardly advanced since Altringham's brief review (1996). Vision does seem to be important and some bats appear to have the visual acuity necessary for navigation by landmarks or even by stars (Altringham and Fenton 2003).

1.2.6 Swarming behaviour in bats

Swarming was defined by Fenton (1969) as 'the flights of bats through hibernacula in the late summer and early fall', although the exact timing of swarming and subsequent hibernation varies with altitude and latitude (e.g. Humphrey *et al.* 1977; Schowalter 1980).

Early in the swarming season, bats may visit underground sites for just a few hours, arriving several hours after sunset and leaving again before sunrise. The end of the swarming season sees a net influx as progressively more bats remain within the sites during the day (Degn 1995; Parsons *et al.* 2003; Rivers *et al.* 2006). Swarming is characterised by chasing behaviour in and around the entrance and a strongly male-biased sex ratio (e.g. Thomas *et al.* 1979; Parsons *et al.* 2003; Rivers *et al.* 2006).

Swarming probably allows individuals to assess the current suitability of traditional hibernacula (Davis and Hitchcock 1965; Rehak *et al.* 1994) and enables information transfer between adults and juveniles regarding the location of hibernation sites (Fenton 1969; Humphrey and Cope 1976). However, neither of these functions accounts for the male-biased sex ratio or the high levels of chasing that are observed, a behaviour that is most likely linked to mating.

Because many swarming species spend the summer in isolated colonies exhibiting high degrees of philopatry (Burland *et al.* 1999; 2001; Kerth *et al.* 2000), a likely function of swarming is that it brings individuals from these colonies together during the main mating period. This not only aids the location of mates that might otherwise be dispersed in the environment (see Clutton-Brock and Harvey 1978), but also promotes gene flow between colonies and in the population at large. Ringing, radio tracking and genetic studies provide evidence that swarming populations are indeed comprised of bats from many summer colonies (Parsons and Jones 2003; Kerth *et al.* 2003; Veith *et al.* 2004; Furmankiewicz 2004; Rivers *et al.* 2005; 2006). Additional genetic studies suggest that mating occurs predominantly outside the breeding habitat (Kerth and Morf 2004) and that mating at hibernacula can explain genetic population structure (Rivers *et al.* 2005; Furmankiewicz and Altringham 2006).

Copulations have been observed during swarming but males exhibit no obvious signs of territorial behaviour and there is no evidence of male display other than short looping flights from cavities within the sites (Thomas *et al.* 1979). The mating system has therefore been described as promiscuous (Thomas *et al.* 1979; Wai-Ping and Fenton 1988; McCracken and Wilkinson 2000), although the opportunity for direct female choice through mate selection, or indirect female choice through sperm competition, means that it may not be indiscriminate (see Watt and Fenton 1995).

The high turnover of bats and the lower numbers later found in hibernation led Fenton (1969) to suggest that swarming sites might serve as meeting points along migration routes. However there are no ringing data to back this, high turnover can be explained by low rates of visitation prior to entry into hibernation, and many crevice-dwelling bats go uncounted in hibernacula.

Swarming has been described in approximately 15 temperate bat species (mainly from the genus *Myotis*) in Europe and North America. In Europe, swarming has been observed in *Myotis nattereri*, *M. daubentonii*, *M. brandtii*, *M. mystacinus*, *M. bechsteinii*, *M. emarginatus*, *Plecotus auritus*, *P. austriacus* and *Barbastellus barbastellus* (Horácek and Zima 1978; Bauerová and Zima 1988; Rehák *et al.* 1994; Hanzal and Prucha 1996; Furmankiewicz and Górniak 2002; Kerth *et al.* 2003; Parsons *et al.* 2003; Veith *et al* 2004; Rivers *et al.* 2006). North American swarming species include *M. lucifugus*, *M. grisescens*, *M. sodalis*, *M. keenii*, *M. volans* and *Pipistrellus subflavus* (Davis 1964; Hall and Brenner 1968; Fenton 1969; Mumford and Whitaker 1975; Tuttle 1976b; Cope and Humphrey 1977; Thomas *et al.* 1979; LaVal and LaVal (1980); Schowalter 1980; Navo *et al.* 2002).

1.3 Background to study, study area and species

As will be apparent from the rest of this chapter, caves are a critical resource in the lives of many temperate bat species. The Yorkshire Dales contains the UK's largest area of karst landscape. In addition to almost 2,000 cave entrances and the longest cave system in the UK, the area has a huge number of old and abandoned lead mines. These underground sites could hold nationally important populations of all five species of cave-hibernating bat resident in the north of England, and a systematic and comprehensive survey was long overdue. A very small proportion of the more accessible caves and mines in Wharfedale and Swaledale were briefly surveyed in the early 1990s by local bat conservationists (J.D. Altringham, pers. comm.). The surveys took the form of winter hibernation counts and very few bats were found, leading to the general impression that the caves of the Dales were relatively unimportant to bats. However, the size and complexity of the caves and the habit of *Myotis* species of hibernating in concealed locations suggested that this conclusion was probably unreliable. Further research was clearly necessary. Given the difficulty of finding bats underground, further survey and research demanded the identification of a suitable survey method. A probable method was identified during ongoing studies in the North York Moors National Park, as part of a project that had already rekindled interest in the Yorkshire Dales.

In the North York Moors National Park it has been shown that all five of the cave-hibernating species resident in the north of England gather at a small cluster of caves in the late summer and autumn to swarm and mate (Rivers *et al.* 2005; 2006). These caves attract as many as 4,000 *M. nattereri* alone from a catchment area over 60 km in radius. Swarming bats are potentially easy to monitor using automated loggers that count the echolocation calls of passing bats. Swarming bats are also relatively easy to catch and identify as they pass in and out of caves. By monitoring swarming we may also be identifying hibernation sites: there is a net movement into caves throughout the autumn and a net movement out in the spring (Rivers *et al.* 2006). This suggests that towards the end of the swarming season, swarming bats are staying to hibernate in their swarming caves. Monitoring swarming can therefore identify sites critical to bats for mating, and potentially for hibernation, since in many cases the same sites are probably used for both purposes.

Further impetus for work in the Dales came when the Yorkshire Dales National Park Authority (YDNPA) launched its Biodiversity Action Plan, *Nature in the Dales* (2000). Bats feature in several of the habitat action plans and a grouped species action plan (SAP) for bats was later developed. One major aim of this SAP is to identify those underground sites in the Park that are used by bats and determine their relative importance.

The project thus evolved to address two issues: (i) the development of an effective survey and monitoring technique for bats using underground sites and (ii) the furthering of our knowledge of the ecology of cave-roosting bats and the role caves play in their population ecology.

1.3.1 The Yorkshire Dales

Figure 1.2 Ingleborough National Nature Reserve with Whernside in the background. The limestone pavement in the foreground is part of the Great Scar Limestone in which most of the larger caves have formed.

The landscape of the south western part of the Yorkshire Dales National Park is largely defined by the 200 m horizontal beds of Carboniferous limestone, dominated by the Great Scar Limestone. It is in these beds that the most extensive cave systems have developed. Several of the largest caves are just outside the boundary of the park. Many of the highest peaks are capped by the Yoredale Series of rocks, a complex layering of sandstones and limestones, with small beds of other rocks, including coal seams and mineral rich veins. Many cave sinks have formed along the junction between these often impervious rocks and the underlying limestone. In some localities, such as Grassington and lower Wharfedale, there are extensive disused lead mines. The surface rocks over much of the north and east of the park are those of the Yoredale Series. Small caves have formed in many of these dales, but old lead mines make up the majority of the underground sites (Waltham 1987). The caves and mines of the Yorkshire Dales are described in some detail in a number of publications (Brook *et al*. 1988; 1991; 1994; Gill 1993; 1994).

1.3.2 Cave-roosting species in the study area

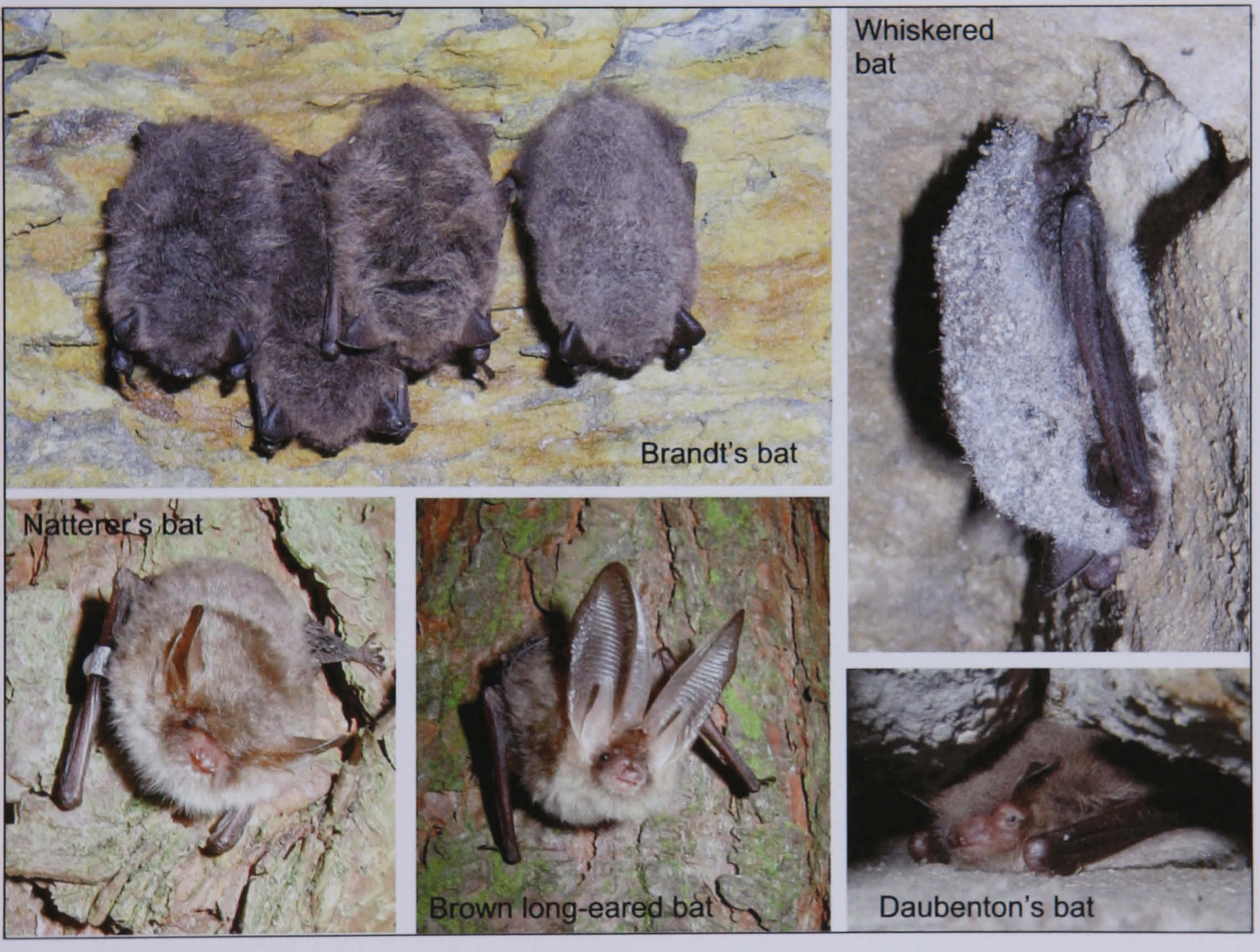

Figure 1.3 The five species of cave-roosting bat present in the Yorkshire Dales.

M. nattereri (Natterer's bat) and *M. daubentonii* (Daubenton's bat) are relatively widespread and common in the Yorkshire Dales. *M. nattereri* forage in woodland and on the woodland edge. Nursery colonies are primarily in buildings and in tree holes and the species hibernates in caves and tunnels, where it is typically found in crevices, singly or in small groups. *M. daubentonii* feed predominantly over water. Large nursery colonies are found in bridges and similar structures close to water, while smaller groups of males may be found in riverside tree roosts. They also hibernate in underground sites, again singly or in small groups. Little is known about the ecology of *M. brandtii* (Brandt's bat) and *M. mystacinus* (whiskered bat), which were only separated as two species 35 years ago. In Yorkshire, they are close to the northern limits of their range and populations may be more fragmented than those of *M. nattereri* and *M. daubentonii*. Both species forage and roost in or close to woodland. Both are found hibernating in caves, mines and tunnels. *P. auritus* (brown long-eared bat) is also a woodland bat, but may be less dependent than *Myotis* species on underground sites for hibernation, sometimes making use of buildings and trees. Differences in the distribution and ecology of these species could have important implications for population structure and the role of underground sites. More detailed information on these bats can be found in Altringham (2003).

1.4 Project aims and thesis outline

1.4.1 Project aims

The broad aims of this PhD project were to investigate the ecology of those bat species using underground sites in the Yorkshire Dales, to develop methods for surveying and monitoring bat activity at caves and to identify those sites of particular importance to bats. At the end of the project the results will be prepared for addition to the appropriate national park databases and recommendations made for safeguarding important bat sites.

1.4.1.1 Methodological aims

To develop methods for surveying and monitoring underground sites based on the automated detection and logging of the echolocation calls of passing bats.

To investigate the potential of an alternative sound analysis method (Time Domain Signal Coding, TDSC), in conjunction with artificial neural networks (ANNs), in automated logging systems. TDSC analysis may allow us to identify bats to species without the need for intensive and intrusive capture.

1.4.1.2 Primary conservation aims

To identify underground sites in the Yorkshire Dales important to bats and answer the questions:

- At what time of year are they used?
- For what purpose?
- By which species?
- What factors influence the use of caves and mines by bats?

At the end of the project to prepare the data collected for addition to the park's Geographical Information System (GIS) database and to produce guidelines for the conservation of bats at underground sites and draw up a code of practise for cavers.

1.4.1.3 Scientific and long term conservation aims

An understanding of the ecology of cave use by bats and the consequences to population structure are interesting in their own right and essential for informed conservation management.

The aims were therefore to look at genetic diversity of the most abundant species using the caves of the Yorkshire Dales and answer the questions:

- Does it comprise a single, homogeneous genetic population or is there geographic differentiation?
- Is there any evidence to suggest that physical features, such as areas of high altitude, present a barrier to gene flow?
- What role do swarming sites play in determining population structure?

1.4.2 Thesis outline

Chapter 2 describes the methodologies used in surveying caves for bats: automated logging and bat capture. It then gives the core descriptive findings such as species composition, sex ratios, temporal activity patterns (seasonal and diurnal) and the movement patterns of ringed bats. Chapter 3 investigates the relationships between swarming activity and the physical characteristics of caves and describes a multiple regression model for predicting activity based on these characteristics. Chapter 4 describes experiments to evaluate the potential of TDSC and ANNs for bat species identification. In Chapter 5 the population ecology and genetics of *M. nattereri* are explored on regional (Yorkshire Dales) and national scales using microsatellite markers, and DNA obtained by wing biopsy. Measures of genetic diversity and differentiation are used to explore the influence swarming has on population structure. Finally, Chapter 6 explores the implications to conservation of swarming and its effects on population structure and examines the threats to bats. It then considers some of the practical issues surrounding two areas of activity that offer achievable short term benefits in the Yorkshire Dales: survey and monitoring and the replacement of unsuitable cave barriers.

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Chapter 2: Seasonal Changes in the Use of Underground Sites and Patterns in Swarming Bats

2.1 Abstract

Between 2002 and 2005, 53 cave entrances in the Yorkshire Dales were logged for autumn swarming activity using automated echolocation call loggers. A small number of selected caves were chosen for continuous monitoring outside the hibernation period. Another subset was selected for winter logging and loggers were placed inside the caves. Logging was followed up by trapping (using mist nets and harp traps) at most of the caves shown to have significant autumn bat activity. All bats caught were ringed, identified to species and sexed. Biopsies (3 mm diameter) were taken from both wings of each bat for genetic analysis.

A large proportion (>60%) of the caves surveyed were used by bats, but activity was concentrated in a relatively small proportion of hotspots (9 cave systems). Continuous logging confirmed that peak activity occurred during autumn swarming in August and September, but significant activity was recorded in all months between March and October. All five swarming species were caught at most of the sites trapped: *Myotis brandtii, M. daubentonii, M mystacinus, M. nattereri* and *Plecotus auritus*. *M. nattereri* was by far the most abundant species late in the swarming season (September), but earlier other species made up a significant proportion of those caught. The sex ratio of swarming bats was heavily skewed towards males in all species (77 - 91%).

2.2 Introduction

The resource requirements of an animal do not remain constant throughout the year. The function and/or importance of a particular resource may change according to changes in the environment as well as the physiological/reproductive status of the animal. This is particularly true in a fluctuating environment such as that experienced by temperate bats. Temperate bats change their roosting locations and social structure throughout the course of their annual cycle.

The importance of underground sites as roosts has been best documented in the tropics and sub tropics where large aggregations may use the same site all year round. Their functions include maternity colonies (e.g. *Tadarida brasiliensis mexicana* - McCracken 1994) and mating sites (e.g. *Artibeus jamaicensis* - Ortega and Arita 2000, 2002; Ortega *et al.* 2003). Caves are also used as maternity roosts in warm temperate regions (e.g. *Miniopterus schreibersii* - Dwyer 1966; Palmeirim and Rodrigues 1995). Generally speaking, underground sites are too cold to be used as maternity roosts at higher latitudes (Altringham 1996). However, underground sites may be important transitional or summer roosts for males under less prohibitive energetic constraints (Degn *et al.* 1995).

In temperate regions, it is the use of caves as hibernacula that has received most attention (e.g. Hooper and Hooper 1956; Tinkle and Patterson 1965; Daan 1968; Kuipers and Daan 1970; Daan 1973; Richter *et al.* 1978; Jones 1991; Nagel and Nagel 1991). Underground sites provide the stable, cold and humid conditions bats require to undergo prolonged deep torpor and survive a seasonal shortage of food. In the UK, survey and research has been biased towards hibernating horseshoe bats (e.g. Ransome 1990; Park *et al.* 1999; 2000) as they hang out in the open and are more easily identified. Other species that hibernate in caves (e.g. *Myotis* species) are crevice dwellers and as such, are more difficult to locate. This has lead to a situation in the north of England, beyond the distribution of horseshoe bats, where potentially important hibernacula have not been identified, studied or protected.

More recently, attention has turned to the importance of underground sites as places of visitation during the autumn. Swarming - defined by Fenton (1969) as 'the flights of bats through hibernacula in the late summer and early fall' - is most common in, but not restricted to, the genus *Myotis*. Autumn is the main period of mating in temperate bats which then undergo reproductive delay during hibernation. Several behavioural studies and an increasing number of genetic studies (e.g. Barclay and Thomas 1979; Thomas *et al.* 1979; Kerth *et al.* 2003; Kerth and Morf 2004; Veith *et al.* 2004; Rivers *et al.* 2005) have reinforced the idea that swarming has a mating function (Fenton 1969). The consensus is that, in many cases, swarming sites are important hibernacula and it seems likely that swarming also involves the assessment of suitable sites prior to hibernation (Davis and Hitchcock 1965) and information transfer between adults and juveniles (Fenton 1969; Humphrey and Cope 1976). Most of the research on swarming has been carried out in North America (e.g. Davis and Hitchcock 1965; Fenton 1969; Humphrey and Cope 1976; Cope and Humphrey 1977) and continental Europe (e.g. Horácek and Zima 1978; Furmankiewicz and Górniak 2002; Kerth *et al.* 2003). To date, only two series of studies have addressed swarming in the UK (Parsons and Jones 2003; Parsons *et al.* 2003a; b; Rivers *et al.* 2005; 2006). These are all detailed studies of a small number of sites in a restricted geographical location.

This is the first study to attempt to identify and determine the importance of underground sites in an extensive, cave-rich area. The Yorkshire Dales is the UK's largest karst area with over 1800 cave entrances and hundreds of kilometres of passage. Despite this, information on the use of its caves and mines by bats is limited to a handful of winter surveys at a small number of sites. The winter surveys revealed few bats and the caves were given low priority conservation status with regards to bats. In 2000, the Yorkshire Dales National Park Authority launched its Biodiversity Action Plan, *Nature in the Dales*. Bats feature in several of the habitat action plans and a grouped species action plan (SAP) has recently been developed. One major aim of this SAP is to identify and determine the importance of underground sites within the park.

The aim of this chapter is to describe seasonal patterns in the use of underground sites by bats, within the Yorkshire Dales. This not only provides essential baseline data for the YDNPA Biodiversity Action Plan, but also goes some way to furthering our understanding of the ecology of those bat species that rely on underground sites as part of their annual cycle.

The primary questions to be addressed in this chapter are:

- At what times of the year are caves important to bats? This informs us about the ecological function of caves and facilitates conservation management plans.
- What patterns are observed during swarming (i.e. timing of activity during the autumn and through the night; species composition; sex ratio) and how do these relate to those seen in other swarming studies? Do the northern, upland location and the abundance of caves lead to unique behavioural patterns?
- Are the key swarming sites also important hibernation sites? This could give some sites very high conservation status.
- Is there a reliable method of identifying and subsequently monitoring hibernacula? No suitable method is currently available and this is a major barrier to conservation monitoring and research.
- Are bats faithful to a single underground site? This has important implications to conservation and population biology.

In order to answer these questions, keys sites first had to be identified and this was done using several field techniques including automated logging, capture at cave entrances and winter surveys. Figure 2.1 shows the sites that were studied, highlighting those shown to be of major importance. One area of key importance is the Easegill system. This is the largest cave system in the UK and although designated a SSSI, it currently lies outside the boundary of the National Park. Sites selected for further study were those that were identified early on in the project and where safe and effective catching was possible. Full details of this survey work are presented in a final project report to the YDNPA (A.M. Glover and J.D. Altringham, in prep.).

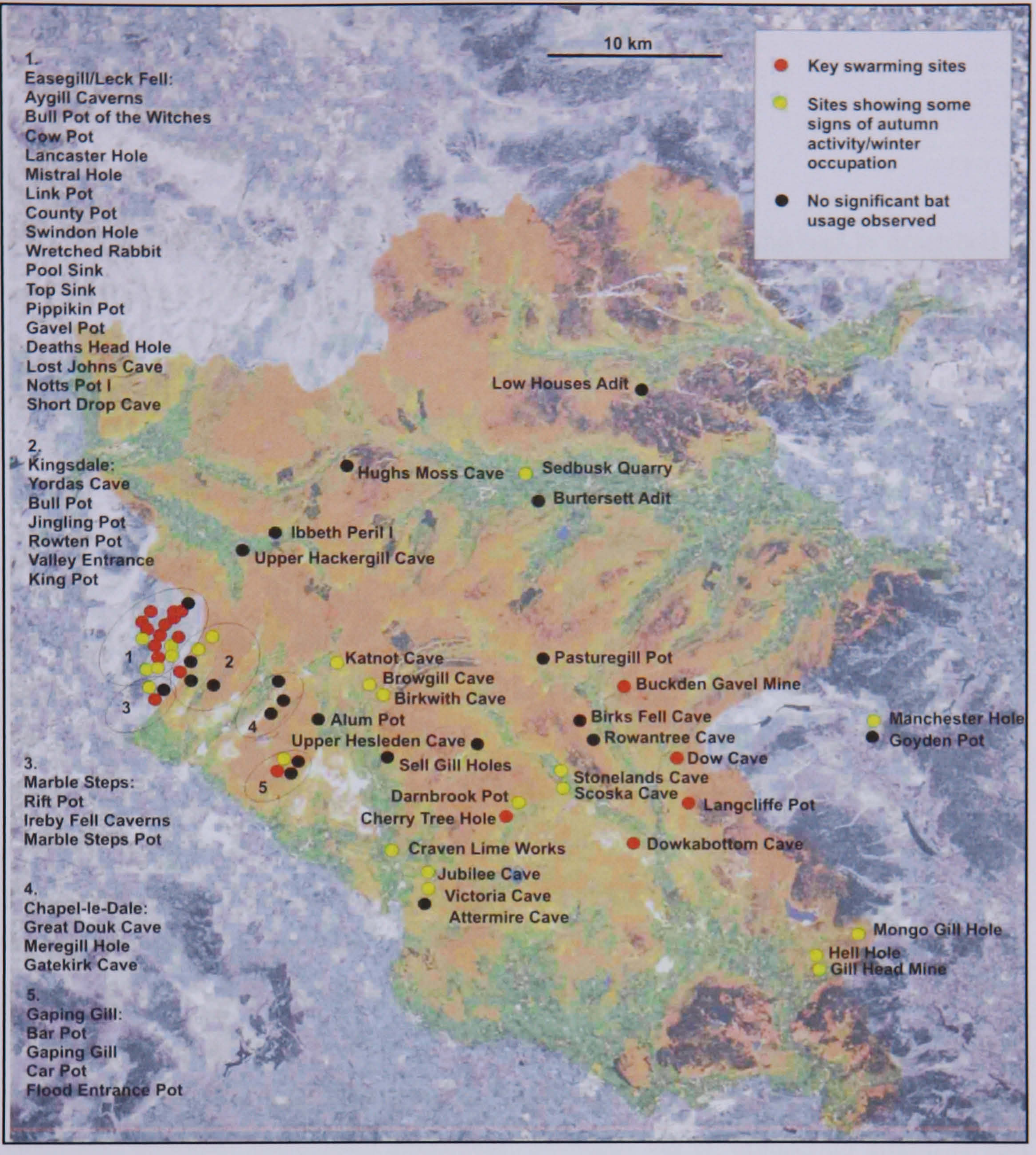

Figure 2.1 Identification of key underground sites in the Yorkshire Dales. The Yorkshire Dales National Park (1762 km^2) is highlighted in false natural colours in this satellite image, which will be used throughout this thesis.

2.3 Methods

2.3.1 Study area

The study area was the Yorkshire Dales in the north of England and is described in more detail in Chapter 1.

2.3.2 Field techniques

2.3.2.1 Automated loggers for measuring bat activity

Automated loggers based on the detection of echolocation calls were used to measure bat activity at caves. Bats echolocate using short (<50 ms), high intensity (up to 120 dB at 10cm), high frequency (typically 15 - 150 kHz) sound pulses generated in the larynx. These calls are used for orientation, target detection and target discrimination. All sounds decrease in intensity as they travel away from a point source due to spherical spreading and attenuation. Bat echolocation calls are detectable only over short distances as attenuation increases with frequency. The sensitivity of the loggers was tested in the field to ensure that all bats entering, exiting or circling the cave entrances would be detected. Several Natterers' bat passes of approximately 10 chirps, recorded via a Pettersson D980 bat detector to a Sony Walkman Pro, were used in testing. These passes were replayed from a laptop containing a data acquisition card (National Instruments DAQ6062E, sampling rate 500 kHz), using BatSound software (Pettersson Elektronic, Sweden) via an ultrasound amplifier and loudspeaker (UltraSound Advice, London) directly towards the logger at an estimated maximum amplitude of 100 dB at 10 cm (based on amplifier specifications provided by UltraSound Advice, London). All calls played within a 180° arc directly in front of the logger (in vertical and horizontal planes) were picked up at 5 m (figure 2.2). Sensitivity began to decline at 10 m outside the central 90° arc, where a maximum of 10% of passes went unrecorded. Loggers were placed so that all bats entering or exiting the cave flew well within the detection range.

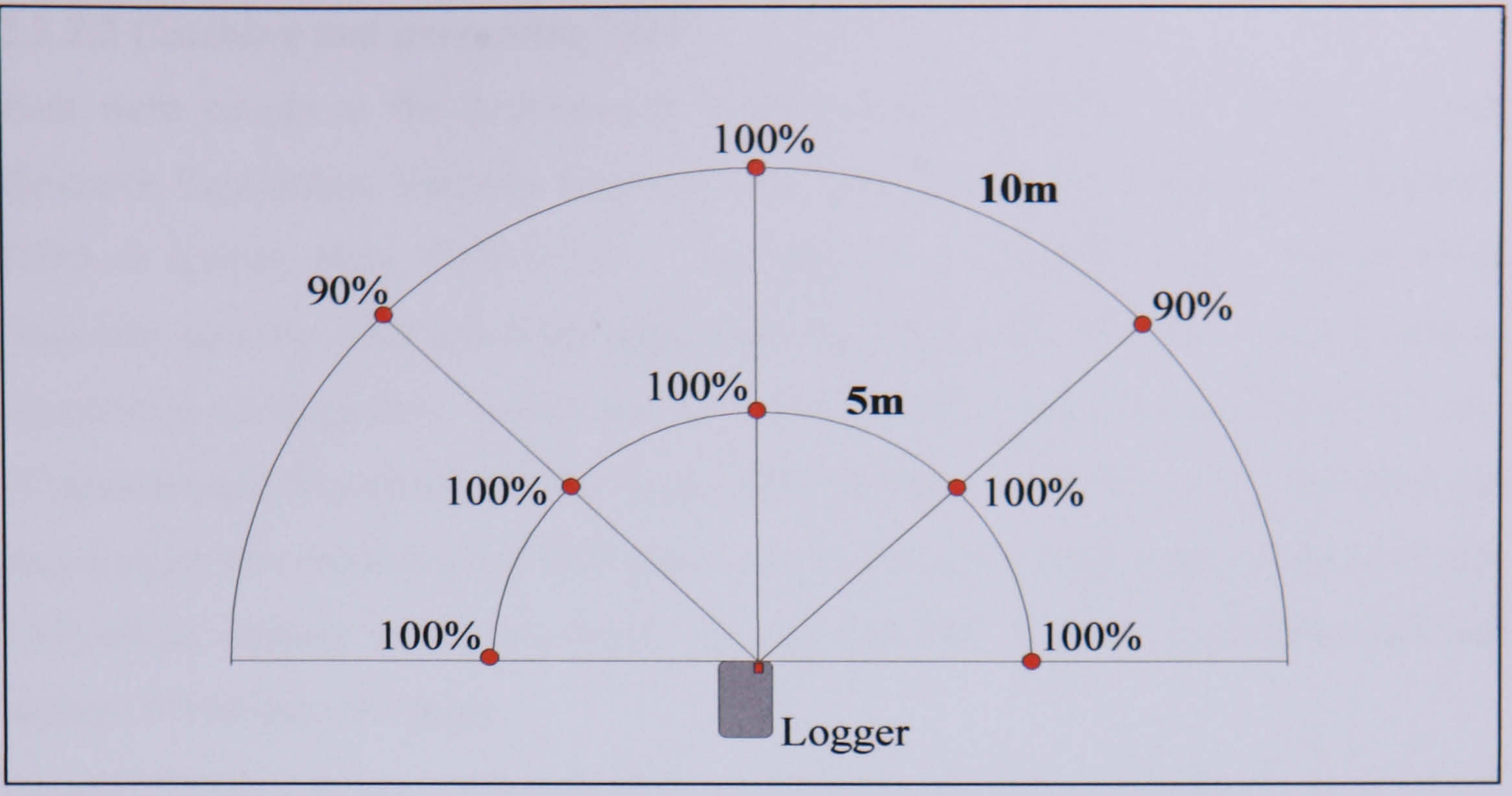

Figure 2.2 Detection range (in any plane) of the automated logger when *Myotis nattereri* call sequences (~100 dB at 10 cm) were played directly towards it.

The automated loggers measure bat activity (number of bat passes) against time. In the case of logging systems based on ultrasound, a bat pass is the discrete train of echolocation pulses heard as a bat enters and leaves the range of the bat detector. The logging system therefore gives an index of activity in the vicinity of the device, not a count of the number of individual bats.

Automated logger components: a heterodyne bat detector (Batbox III, Stag Electronics from Batbox Ltd., UK) tuned to 45kHz is connected to a counter (Skye Instruments Ltd., Powys, UK) which counts bat passes by triggering on the first chirp of a pass. The counter then de-activates for 0.5 seconds - this is a compromise between ensuring all passes are logged and minimising the number of passes logged twice. Each pass is output as a single square wave pulse to a data logger (Tinytag Plus Re-ed TGPR1201, Gemini Dataloggers (UK) Ltd.) and recorded as number of passes min^{-1}. A 12 V (4 Ah) lead-acid rechargeable battery (Yuasa NP4-12) with a 120 Ω resistor connected to the positive terminal replaces the 9 V battery in the bat detector, increasing the running time of the system from ~36 hours to ~7 days. The entire system is contained within a waterproof case (Peli model 1200, Peli Products (UK) Ltd.), which has a hole (11 mm diameter) drilled in front of the detector's microphone. Data are downloaded to TinyTag Explorer (Gemini dataloggers, UK) software either directly or via a Palm Pilot palmtop computer.

2.3.2.2 Catching and processing bats

Bats were caught at the entrances to underground sites using harp traps (Austbat Research Equipment, Victoria, Australia) and mist nets (North Ronaldsay superfines, BTO or Avinet, New York, USA). The trap/net combinations and configurations were site specific, although harp traps were used in preference where appropriate to speed up processing time, reduce stress to the bats and to improve catching efficiency (Tidemann and Woodside 1978; Francis 1989; Berry *et al.* 2004). The time each bat was caught was recorded and bats were kept in cotton holding bags (Alana Ecology, UK) while waiting to be processed. It was standard practise to process each bat within 30 minutes of capture.

Bats were identified to species level according to Altringham (2003). The only species that presented a problem for identification were *Myotis brandtii* and *Myotis mystacinus*. The relative size of the protocone on P_3 to P_4 on the upper jaw was used to separate these species. The shape of the outer edge of the tragus and penis shape (in males) were also used as a guide, although both features are somewhat subjective. Where teeth were worn or missing, and other features were ambiguous, the bat was classified as Mb/m. Of the 188 bats identified as either *M. brandtii* or *M. mystacinus*, 5 (<3%) were classified as Mb/m.

Sex, age and reproductive status were recorded. Age (adult/juvenile) was determined according to the degree of ossification of the epiphyseal joints in the finger bones (Racey 1974; Anthony 1988). The reproductive status of male bats was determined according to testes size and the degree of epididymal distension (Racey 1974; 1988).

Bats were ringed on the right forearm with an individually numbered aluminium bat ring (Mammal Society, UK). For genetic analysis (see Chapter 5) a biopsy sample (3 mm diameter) was taken from each wing membrane according to Worthington Wilmer and Barratt (1996). Biopsy samples were stored at room temperature in 70 - 100% ethanol.

All catching and processing were carried out under licences from English Nature and the Home Office.

2.3.3 Data collection protocols

2.3.3.1 Seasonal changes in bat activity at caves

From those caves originally identified as significant swarming sites, Dow Cave (SD 984743), Bar Pot (SD 752724), Cherry Tree Hole (SD 882743) and Link Pot (SD 668803) were chosen for longer term study (Figure 2.3). Automated loggers were placed at the entrances to these caves from April to September 2004, and March to October 2005. The loggers ran almost continuously throughout these periods and nightly counts of bat passes were used to monitor changes in bat activity. The hibernation period was studied separately.

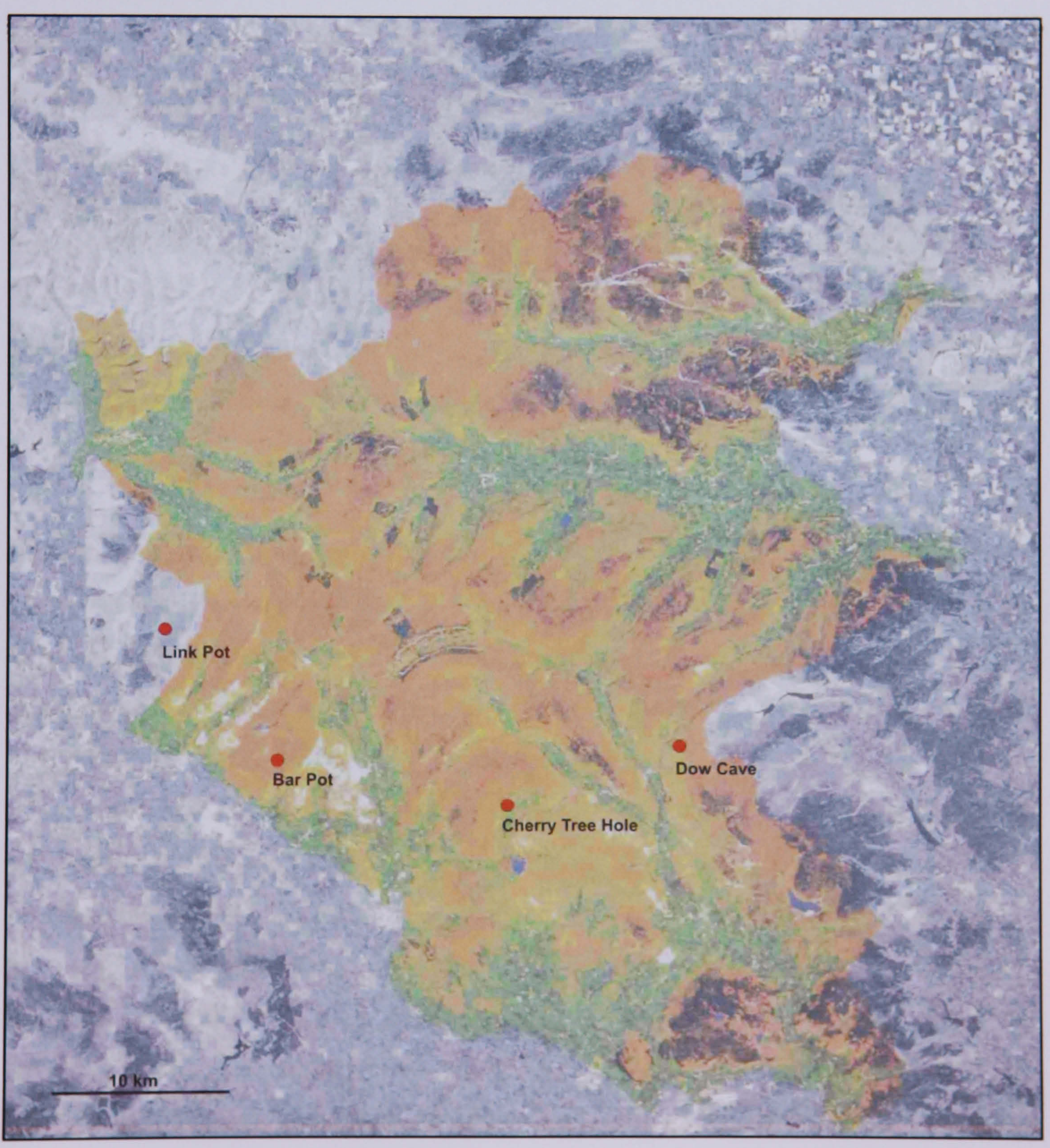

Figure 2.3 Locations of sites where logging throughout the active season was conducted.

2.3.3.2 Temporal patterns in swarming activity

Data from logging (section 2.3.3.1) and from captures (section 2.3.3.3) were used to investigate changes in activity both through the swarming season and through the night.

2.3.3.3 Patterns in swarming bats (species composition, sex ratio and age)

Bats were caught and ringed throughout the project (August 2002 - October 2005). Intervals between visits to any one site were never less than two weeks, to minimise disturbance. Bats were also caught at a small number of caves/mines in the south east of the study site in 1996 (two nights), 2000 (three nights) and 2001 (11 nights). However, only *M. nattereri* and *M. daubentonii* were ringed on these occasions.

2.3.3.4 Assessing methods of identifying and monitoring hibernation sites

2.3.3.4.1 Visual surveys

Many of the caves and mines investigated as part of this project were visited (in part or whole) at least once during the study. Most of these sites were visited more than once and during the hibernation period. During all underground visits, torpid bats and droppings were actively sought and recorded without disturbance. Winter surveys were carried out under licence from English Nature.

2.3.3.4.2 Automated logging of arousal activity

From February to April 2003, automated loggers were placed inside six caves and mines: Dow Cave (SD 984743), Buckden Gavel Mine (SD 955782), Cherry Tree Hole (SD 882704), Browgill Cave (SD 670789), Great Douk Cave (SD 747770) and Short Drop Cave (SD 670789). Loggers were positioned in locations that were likely to be major thoroughfares or hibernation sites (large chambers, major passages or junctions). Since bats arouse at irregular but frequent intervals during hibernation, it was hoped that these loggers would detect any bat arousing and flying in that part of the cave. Throughout November 2003 and again throughout March 2004, this approach was used in some of the larger systems: Bar Pot (SD 752724), Mongo Gill Hole (SE 092635), Lancaster Hole (SD 664807), Pool Sink (SD 677807), Darnbrook Pot (SD 885710) and Dowkabottom Cave (SD 951689) following training in vertical caving techniques. In addition, loggers were placed in Link Pot (SD 668803) and the entrance chamber in Dowkabottom Cave in March 2004, following sightings of torpid bats in these locations. Initial selection of sites and logging locations within sites were made on the basis of anecdotal sightings of bats in winter (various pers. comm.) and the presence of droppings (pers. obs.). Locations of logging sites are shown in Figure 2.4.

Figure 2.4 Locations of sites where winter logging was conducted

2.3.3.5 Recaptures and movements between sites

All bats captured in this study were ringed. Bats have also been ringed in summer, mainly in Wharfedale (by members of the Leeds University Bat Research Group) and in Lancashire (by researchers from Central Science Laboratory), as part of other studies. Recapture of these bats provided limited but valuable information on movement between sites.

2.4 Results

2.4.1 Seasonal changes in bat activity at caves

Activity was recorded throughout the active season (March - October) at all four sites (Link Pot, Bar Pot, Cherry Tree Hole and Dow Cave), although the exact pattern of seasonal change differed between sites (figure 2.5).

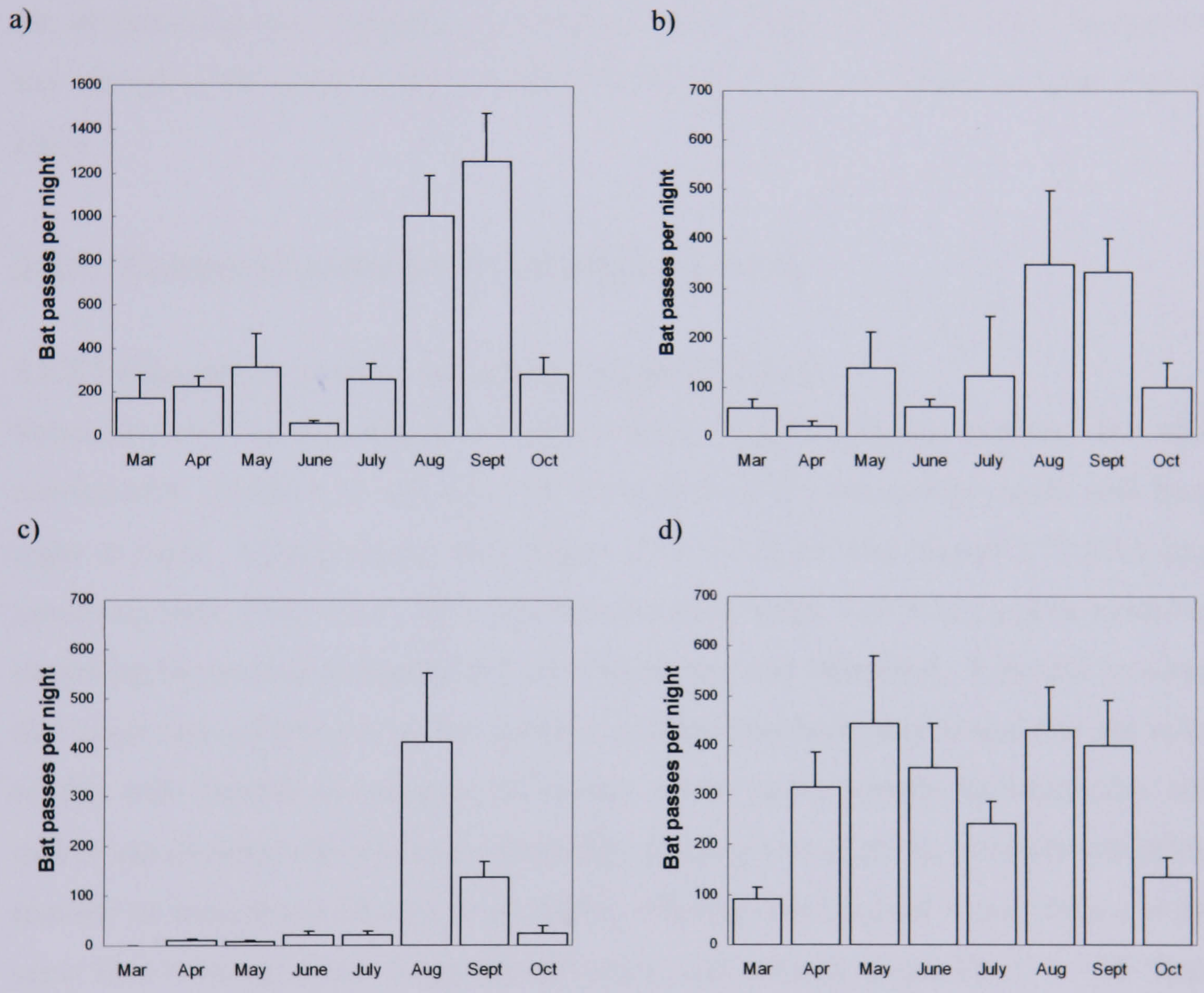

Figure 2.5 Mean number of bat passes per night during each month of the active season (March - October) 2005 at a) Link Pot, b) Bar Pot, c) Cherry Tree Hole and d) Dow Cave. Error bars are standard error of the mean. Note the difference in scale between Link Pot and the other three sites.

At Link Pot, the highest levels of activity were recorded during the autumn when bats are mating and preparing to enter hibernation. The mean number of bat passes per night during the autumn was 1000 in August, 1250 in September, but declined to 300 in October. Consistent with emergence from hibernation and a potential period of spring swarming, monthly mean activity levels of 200-300 bat passes per night were recorded in the spring (March - May). Activity levels were lowest during the summer with a monthly mean of just 58 bat passes per night in June. Bar Pot showed a similar seasonal trend to Link Pot, although overall activity levels were considerably lower. Activity at Cherry Tree Hole was mostly confined to the main period of the autumn swarming season (August - September). Dow Cave was unusual in that activity levels in the spring matched or exceeded those observed in the autumn (April/May mean was 300 - 450 bat passes per night; August/September mean was 400 - 430 bat passes per night). Activity also remained relatively high into June/July suggesting that this site in particular may function as a summer roost. Data from 2004 (not shown) are less complete but show similar trends, although activity was higher in April than in May.

2.4.2 Temporal patterns in swarming activity

2.4.2.1 Changes in activity across the swarming season

Swarming activity was observed between early August and late October, but with considerable variation in activity over the course of the swarming season and from night to night. Using logging data (figure 2.6) and catch data (figure 2.7) from four swarming sites: Dow Cave, Ease Gill, Bar Pot and Cherry Tree Hole, a peak period of swarming between mid-August and mid-September was identified. It should be noted that catch data underestimate the number of individual bats present and that the scale of this will depend on trapping efficiency which varies greatly between sites and makes site comparisons difficult. Similarly, logging counts are an overestimate of the number of individuals (Rivers *et al.* 2006), although this method is not subject to the same bias between sites. Trapping efficiency was highest at Bar Pot (~5 - 10 times more bat passes logged than bats caught) and was also high at Dow Cave (~10 - 50 times more). Trapping efficiency appeared to be lowest at Link Pot and Cherry Tree Hole where logger counts were ~25 - 100 times higher than the number of bats caught. These numbers are approximations using data from across the season. Logging data are therefore comparable between sites but capture data are not.

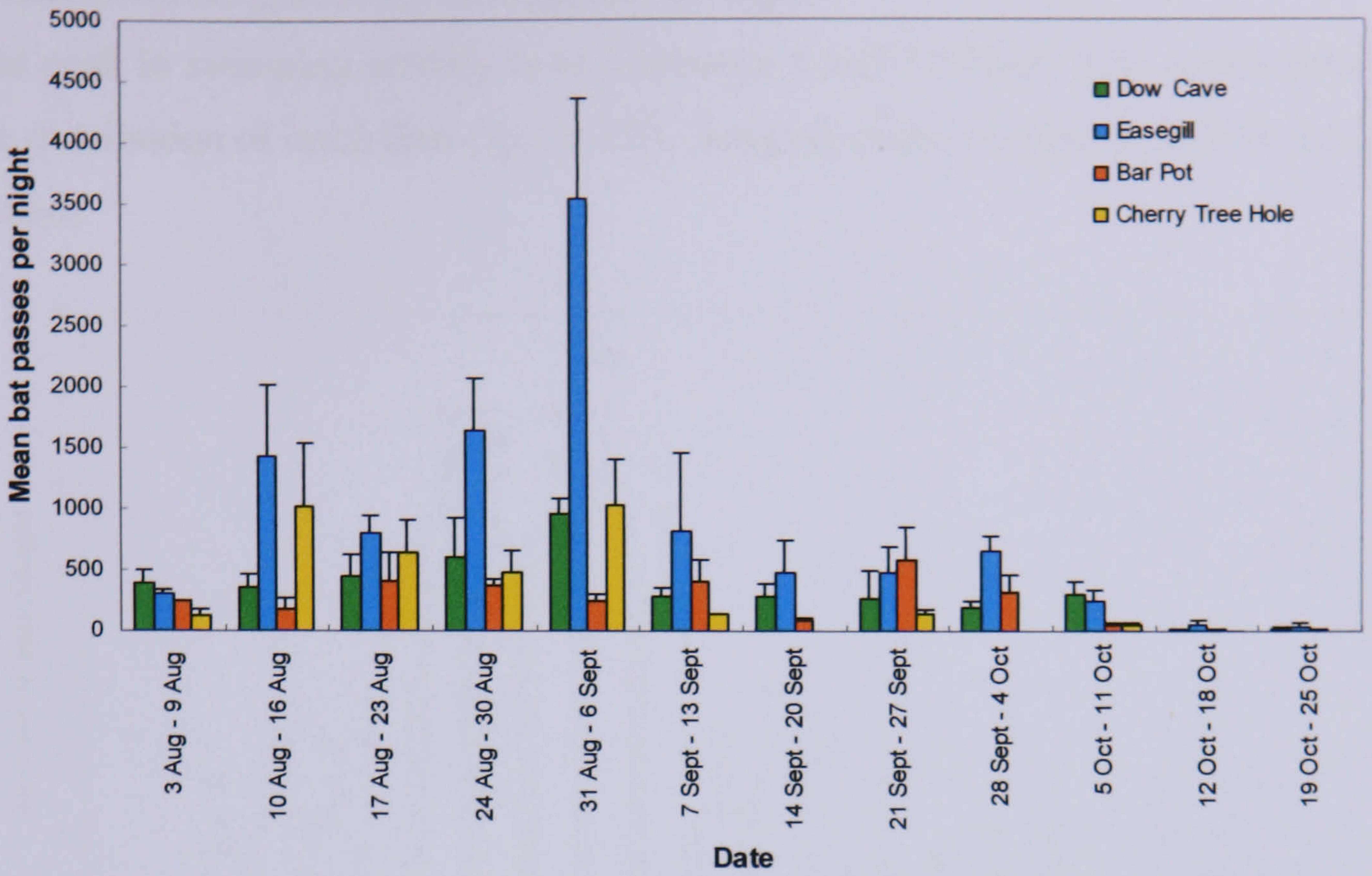

Figure 2.6 Change in activity across the swarming season at four swarming sites: mean number of bat passes logged per night across the swarming season (2003-5). Error bars are the standard error of the mean, values without error bars signify that there was only one logging event in that time period.

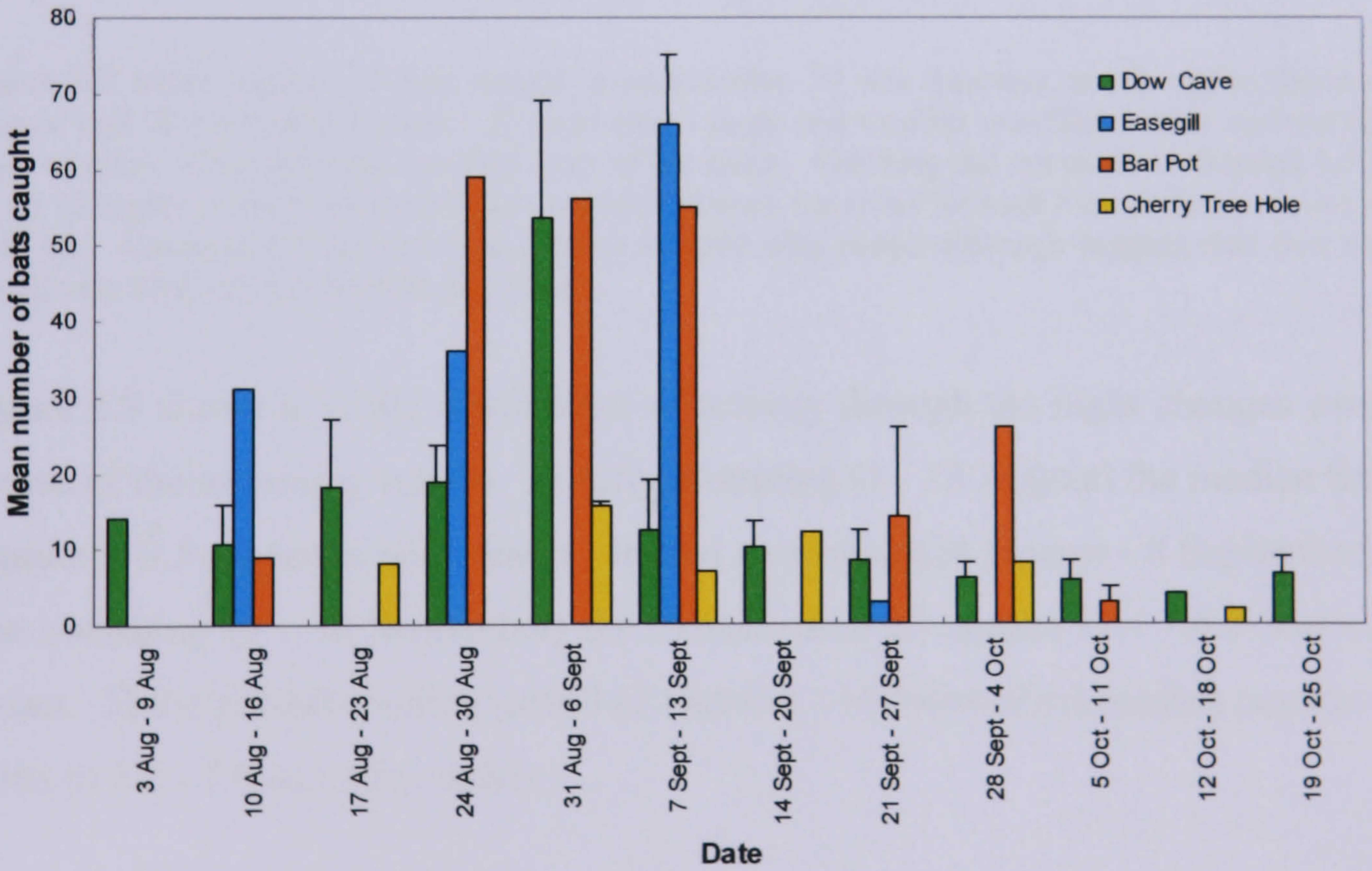

Figure 2.7 Change in activity across the swarming season at four swarming sites: mean number of bats caught per night across the swarming season (1996; 2000-5). Error bars are the standard error of the mean, values without error bars signify that there was only one capture event in that time period.

2.4.2.2 Swarming activity throughout the night

The peak in swarming activity occurs between 3 and 5.5 hours after sunset based on the distribution of catch data (figure 2.8). Logging plots (not shown) follow the same pattern.

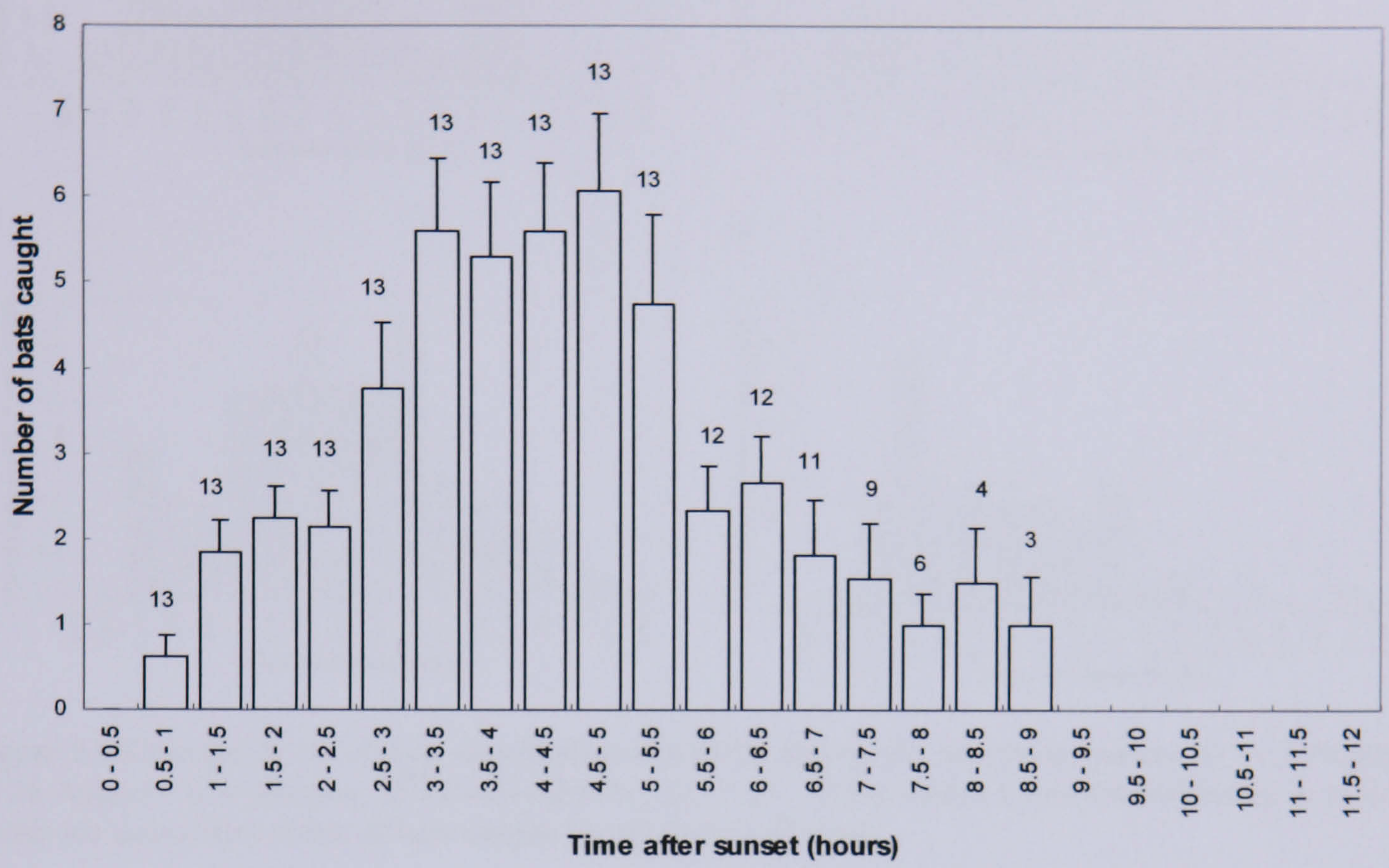

Figure 2.8 Mean number of bats caught in consecutive 30 min intervals on 13 nights (between 16 August and 28 September) where >25 bats were caught and weather conditions were optimal (i.e. no wind or rain). Error bars are standard error of the mean. Catching did not continue beyond 5.5 hours on all 13 nights so the number of nights used to calculate the mean for each time period is shown above each bar. Catching did not continue beyond 9 hours after sunset although logging data (not shown) reveal very little activity beyond this point.

Figure 2.9 shows how the distribution of activity through the night changes over the course of the swarming season. In early swarming (1 - 23 August) the median time of capture is 3.5 - 4 hours after sunset. In mid swarming (24 August - 8 September) and late swarming (9 - 30 September) the median time of capture is 4 - 4.5 hours after sunset. In the pre-hibernation period (1 October - 10 November) median capture time shifts to 2.5 - 3 hours after sunset.

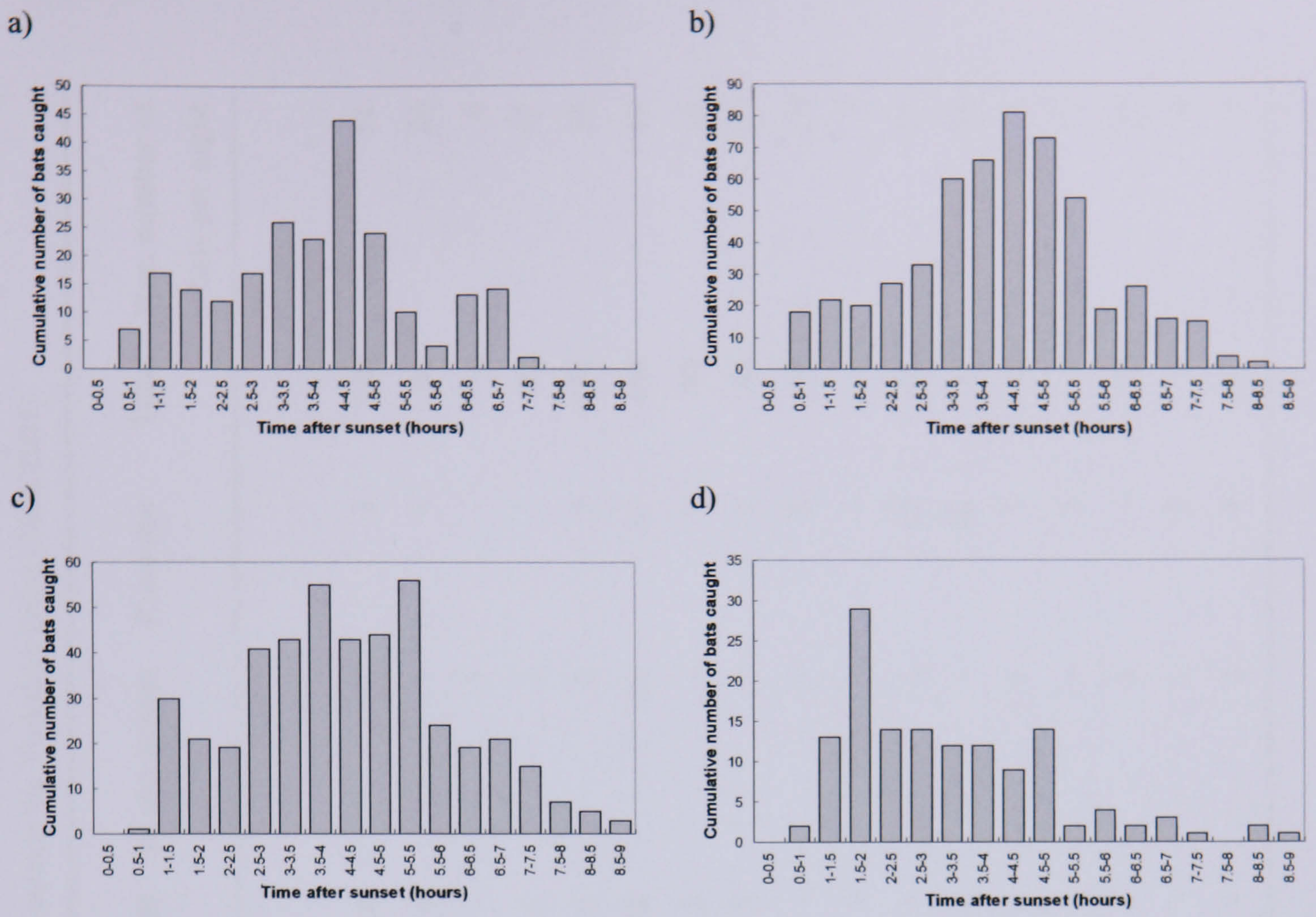

Figure 2.9 Changes in the nightly distribution of activity during the swarming season: a) 1-23 August, b) 24 August - 8 September, c) 9-30 September, d) 1 Oct - 10 November (note the difference in scale). These are cumulative totals of bats caught for all sites in all years.

2.4.3 Patterns in swarming bats

Capture data for all sites are shown in table 2.1 which includes a break down of species caught as well as the total number of captures, number of capture nights and the maximum number of bats caught on a single night. Trapping efficiency certainly varied between sites although this was not directly quantified (see section 2.4.2.1). Although high logging counts were obtained for many sites, the nature of the entrance at some (e.g. Langcliffe Pot) meant that capture success was low. In the case of some of the large potholes, capture was not even attempted.

Table 2.1 Captures at underground sites in the Yorkshire Dales (1996 - 2005) and at 3 underground sites outside the Yorkshire Dales (2004/2005)

Site	**Grid reference**	**Number of catch nights**	***M. nattereri***	***M. daubentonii***	***M. brandtii***	***M. mystacinus***	***P. auritus***	**Total**	**Max. number of bats per night**
Ease Gill:									
Link Pot	SD 668803	**4**	93	15	18	4	8	**138**	**51**
Mistral Hole	SD 666803	**3**	33	5	4	-	4	**46**	**24**
County Pot	SD 675806	**1**	3	2	-	1	-	**6**	**6**
Wretched Rabbit	SD 675806	**1**	6	-	4	1	1	**12**	**12**
Bar Pot	SD 752724	**9**	189	35	5	6	3	**238**	**59**
Dow Cave	SD 984743	**34**	185	148	58	36	27	**454**	**69**
Cherry Tree Hole	SD 882704	**8**	53	19	3	-	9	**84**	**16**
Buckden Gavel Mine	SD 955782	**5**	23	20	3	-	5	**51**	**27**
Manchester Hole	SE 100764	**4**	31	8	-	-	2	**41**	**24**
Goyden Pot	SE 100762	**2**	-	2	-	-	-	**2**	**2**
Hell Hole	SE 066623	**11**	34	12	-	1	15	**62**	**15**
Gill Head Mine	SE 066621	7	3	12	-	-	16	31	**11**
Craven Lime Works	SD 823663	**4**	30	2	-	3	7	**42**	**15**
Victoria Cave	SD 837650	**2**	2	-	-	-	5	7	7
Browgill Cave	SD 800777	7	8	16	3	6	-	**33**	**13**
Birkwith Cave	SD 804770	**3**	2	3	-	-	2	7	**4**
Yordas Cave	SD 705801	7	15	10	-	2	4	**31**	7

Site	Grid reference	Number of catch nights	*M. nattereri*	*M. daubentonii*	*M. brandtii*	*M. mystacinus*	*P. auritus*	Total	Max. number of bats per night
Gatekirk Cave	SD 745787	**2**	1	1	-	-	-	**2**	**2**
Scoska Cave	SD 915724	**8**	5	5	5	7	5	**27**	**10**
Stonelands Cave	SD 920733	**1**	-	2	-	-	1	**3**	**3**
Dowkabottom Cave	SD 951689	**1**	-	1	-	-	2	**3**	**3**
Langcliffe Pot	SD 996711	**1**	-	2	-	-	-	**2**	**2**
Fosse Gill Cave	SD 948744	**1**	-	2	-	-	-	**2**	**2**
Ibbeth Peril I	SD 742864	**1**	1	-	-	-	-	**1**	**1**
Sedbusk Quarry	SD 876912	**3**	6	-	-	9	1	**16**	**8**
Low Houses Adit	SD 976969	**1**	2	2	-	-	-	**4**	**4**
Burtersett Adit	SD 897888	**1**	3	-	-	-	-	**3**	**3**
Sites outside the Yorkshire Dales									
Roudsea Wood Cave (south Cumbria)	SD 332825	**4**	25	1	-	1	33	**60**	**22**
Withcall Tunnel (north Lincolnshire)	TF 262820	**1**	24	9	-	-	13	**46**	**46**
Gt. Blakenham Mine (Suffolk)	TM 108492	**1**	64	26	-	-	-	**90**	**90**

Table 2.1 contd.

2.4.3.1 Species composition

Whilst some minor sites (e.g. Scoska Cave, Browgill Cave) showed a diverse species mix, those sites where large numbers of bats were caught (Easegill, Bar Pot and Cherry Tree Hole), were dominated by *M. nattereri* (60-80%, see figure 2.10). Dow Cave appears to be the exception, with a species composition less dominated by *M. nattereri*, and a higher proportion of *M. daubentonii* relative to the other sites. However, this site difference may be exaggerated by a greater number of capture events at Dow Cave early in the season relative to the other sites (assuming a seasonal change in species composition, see below).

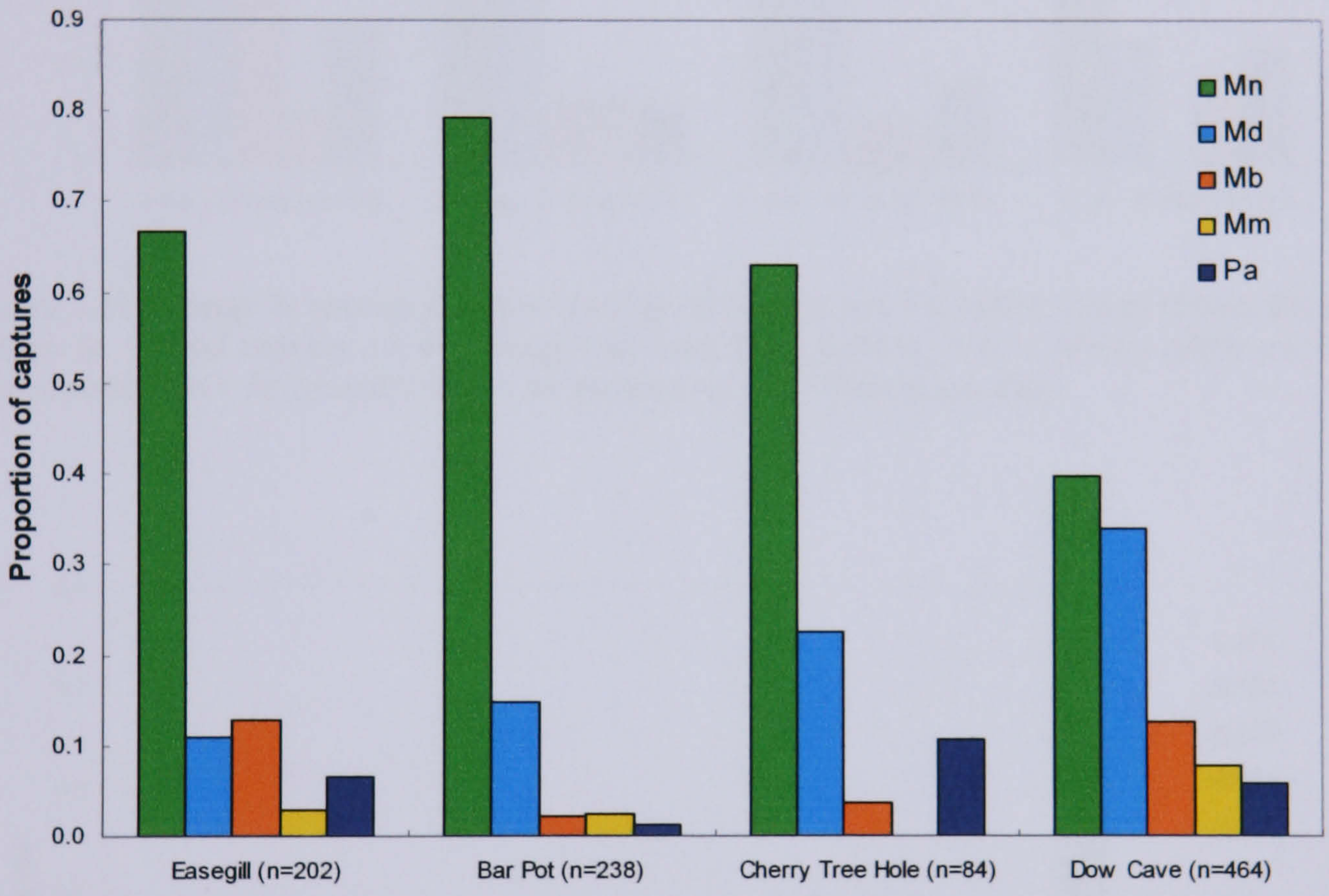

Figure 2.10 Species composition (over the whole swarming season) at four swarming sites (n = total number of bats caught at each site). Mn = *Myotis nattereri*, Md = *M. daubentonii*, Mb = *M. brandtii*, Mm = *M. mystacinus*, Pa = *Plecotus auritus*.

For all sites combined (figure 2.11) and at Dow Cave (figure 2.12) there is at transition from greater species evenness to a species composition dominated by *M. nattereri* as the season progresses. Whilst the seasonal change in species composition at Dow Cave broadly reflects that seen at all sites, the proportion of *M. daubentonii* remains relatively high at this site until the end of September. In addition, Dow Cave accounts for an appreciable proportion (67%) of all captures made between 1 - 23 August.

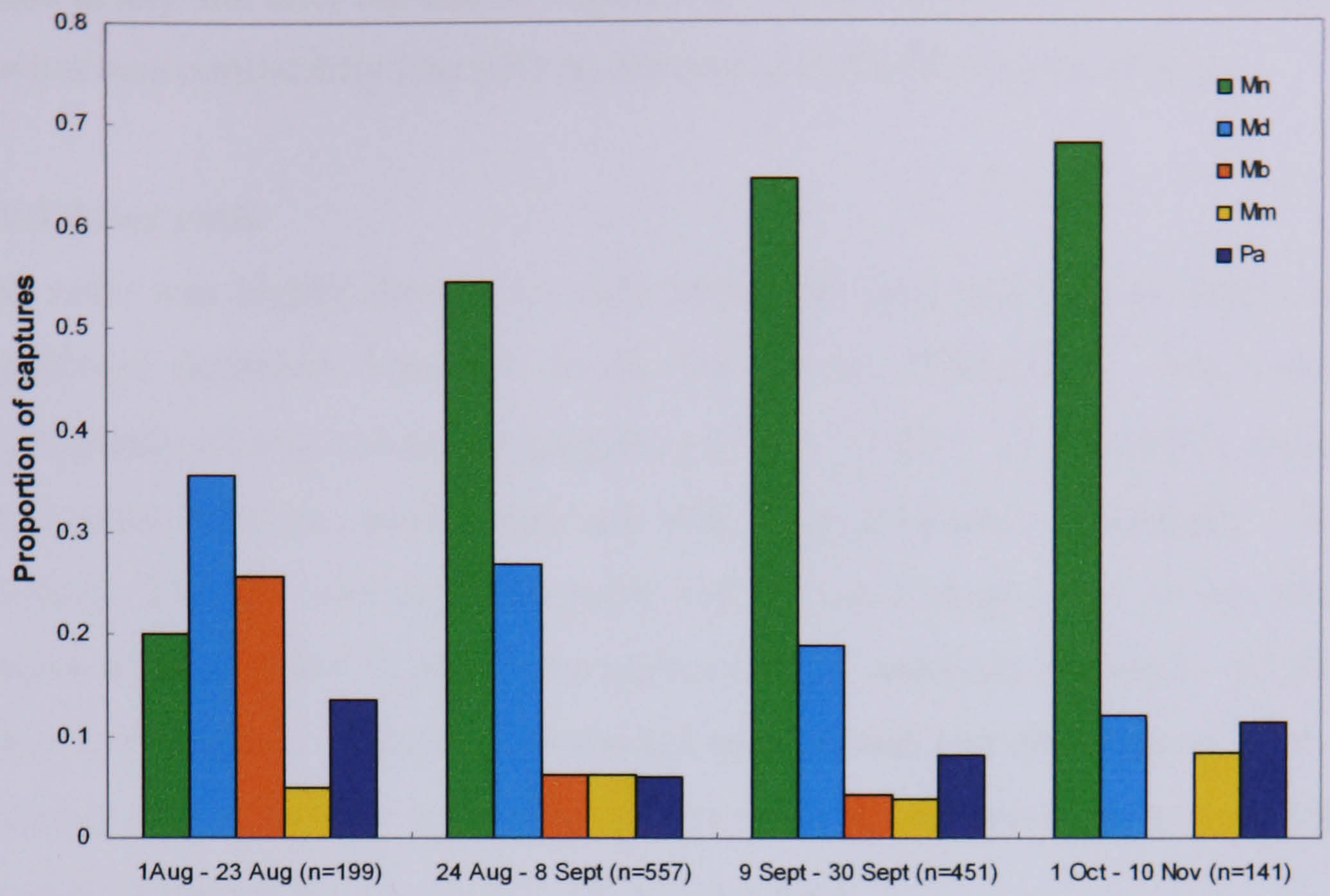

Figure 2.11 Change in species composition (for all sites in the Yorkshire Dales) across the swarming season (n = total number of bats caught in each time period). Mn = *Myotis nattereri*, Md = *M. daubentonii*, Mb = *M. brandtii*, Mm = *M. mystacinus*, Pa = *Plecotus auritus*.

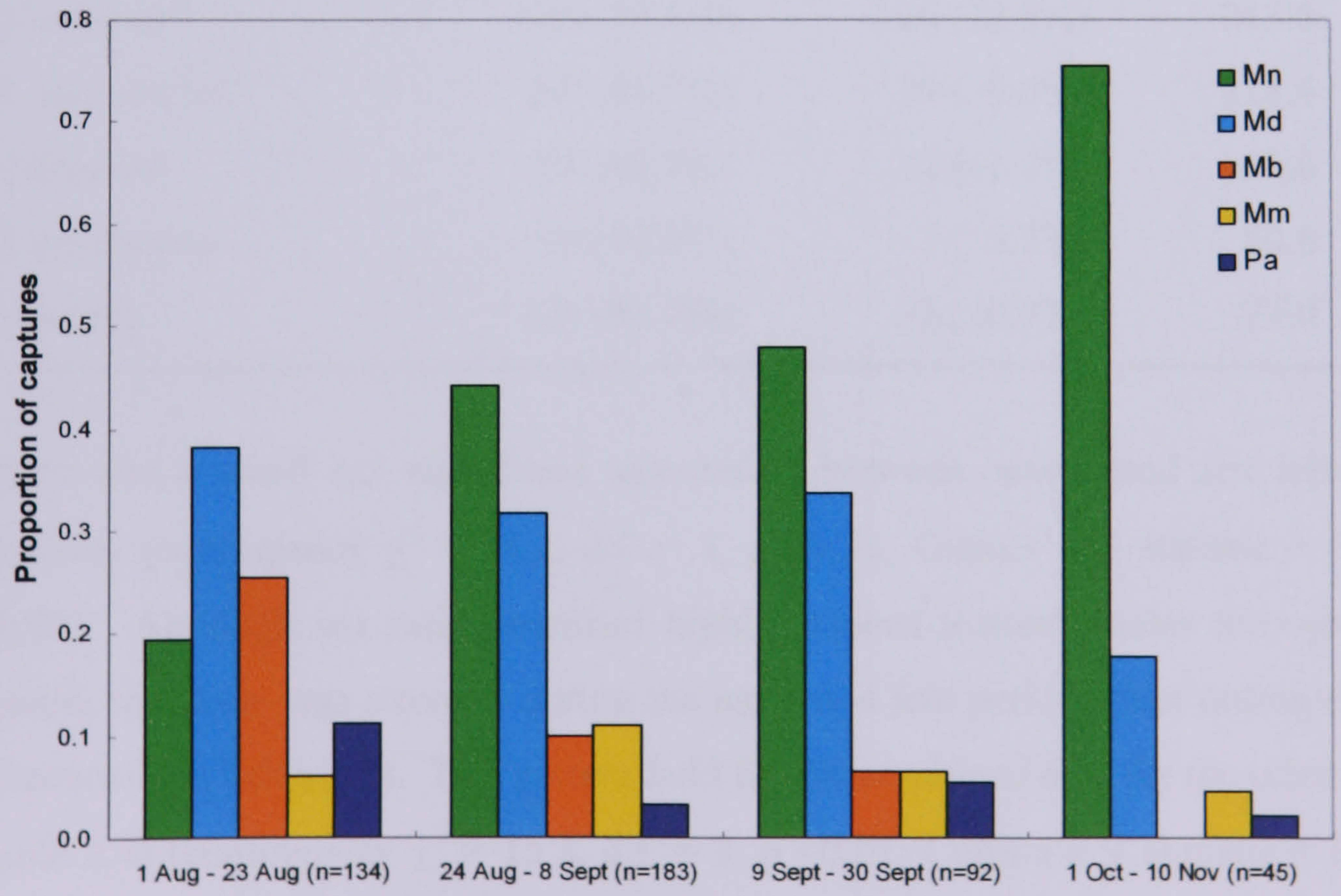

Figure 2.12 Change in species composition at Dow Cave across the swarming season (n = total number of bats caught in each time period). Mn = *Myotis nattereri*, Md = *M. daubentonii*, Mb = *M. brandtii*, Mm = *M. mystacinus*, Pa = *Plecotus auritus*.

The proportion of *M. brandtii* decreases sharply through the season, with no captures made at any site after the end of September. The proportion of *M. mystacinus* and *P. auritus* was consistently low with no apparent pattern of seasonal change.

2.4.3.2 Sex ratio

Sex ratio was highly skewed towards males (all sites and capture dates), showing significant departure from 1:1 in all five species (Table 2.2). Sex ratio varied significantly among species (contingency $\chi^2 = 41.9$, d.f. = 4, $p < 0.001$), although the association between species and sex was weak (Cramer's V statistic = 0.177, $p < 0.001$). The sex ratio in *M. nattereri* was the most likely cause of this association (males accounted for 77.1% of the captures of *M. nattereri* and 88.3 - 91.0% in the other four species). This was confirmed by a second test that showed there was no variation in the sex ratio across the species when *M. nattereri* was excluded from the analysis (contingency $\chi^2 = 0.731$, d.f. = 3, p = n.s.).

Table 2.2 Male skew in the sex ratio of all five species captured (d.f. = 1, *** $p < 0.001$). Values were significant after a sequential Bonferroni correction

Species	**Male**	**Female**	χ^2	**p**
M. nattereri	560 (77.1%)	166 (22.9%)	213.8	***
M. daubentonii	295 (91.0%)	29 (9.0%)	218.4	***
M. brandtii	91 (88.3%)	12 (11.7%)	60.6	***
M. mystacinus	69 (90.8%)	7 (9.2%)	50.6	***
P. auritus	104 (89.7%)	12 (10.3%)	73.0	***

There was a small but significant association between season and sex ratio in *M. nattereri* (contingency $\chi^2 = 14.2$, d.f. = 3, $p < 0.01$; Cramer's V statistic = 0.140, $p < 0.01$). Although sex ratio remained highly skewed towards males throughout the season, the skew was stronger during the early and late periods than during the peak of swarming (Table 2.3). This pattern held for the combined data for the other species (table 2.4) (contingency $\chi^2 = 15.3$, d.f. = 3, $p < 0.01$; Cramer's V statistic = 0.107, $p < 0.01$) - the expected values in some cells of the contingency table were too low (<5) to treat these species separately.

Table 2.3 Association between time of season and sex ratio in *M. nattereri*

Time period	Male	Female
1 Aug - 23 August	35 (87.5%)	5 (12.5%)
24 Aug - 8 Sept	217 (72.8%)	81 (27.2%)
9 Sept – 30 Sept	222 (76.0%)	70 (24.0%)
1 Oct – 10 Nov	86 (89.6%)	10 (10.4%)

Table 2.4 Association between time of season and sex ratio in all species excluding *M. nattereri*

Time period	Male	Female
1 Aug - 23 August	178 (89.9%)	20 (10.1%)
24 Aug - 8 Sept	453 (81.6%)	102 (18.4%)
9 Sept – 30 Sept	361 (80.0%)	90 (20.0%)
1 Oct – 10 Nov	127 (90.1%)	14 (9.9%)

2.4.3.3 Age composition

Age composition was highly skewed towards adults in all species (Table 2.5). There was a significant association between species and age composition (contingency χ^2 = 83.1, d.f. = 4, p <0.001, Cramer's V statistic = 0.249, p <0.001). Skew towards adults was most pronounced in *M. nattereri* and *P. auritus*.

Table 2.5 Adult skew in the age composition of all five species captured (d.f. = 1, *** p <0.001 ** p <0.01). Values were significant after a sequential Bonferroni correction

Species	Adult	Juvenile	χ^2	p
M. nattereri	682 (93.9%)	44 (6.1%)	560.7	***
M. daubentonii	286 (88.3%)	38 (11.7%)	189.8	***
M. brandtii	81 (78.6%)	22 (21.4%)	33.8	***
M. mystacinus	51 (67.1%)	25 (32.9%)	8.9	**
P. auritus	113 (98.3%)	2 (1.7%)	107.1	***

In *M. nattereri* the skew towards adults was consistent in both males and females (Table 2.6), i.e. there was no significant difference in the sex ratio according to age (contingency χ^2 = 0.582, d.f. = 1, p = n.s.). The expected frequencies in some cells of the contingency table were too low (<5) to test this association in the other species individually and it was felt they could not be combined due to the association between species and age composition.

Table 2.6 Consistent sex ratio in both adults and juveniles in *M. nattereri*

Age	**Male**	**Female**
Adults	524 (76.8%)	158 (23.2%)
Juveniles	36 (81.8%)	8 (18.2%)

Too few juveniles were caught to look for an association between season and age composition.

2.4.4 Assessment of hibernacula

2.4.4.1 Visual surveys

The results of visual winter surveys carried out are summarised in Table 2.7. Swarming activity (based on logging - see Chapter 3) and the species caught during swarming are included for comparison where applicable. The maximum number of torpid bats recorded in any one visit was 15 in Link Pot (19 February, 2005). The highest levels of swarming activity are also observed at this site with logging counts exceeding 9000 bat passes per night during the peak period and catches exceeding 50 (including all 5 species). All 4 *Myotis* species were recorded in hibernation at this site, but not *Plecotus auritus*. Up to 8 bats (*Myotis* and *Plecotus* species) were recorded in Dowkabottom Cave which showed moderate levels of swarming activity (up to 534 bat passes per night). 11 bats (10 of which were *M. brandtii* or *M. mystacinus*) were recorded in Buckden Gavel Mine on 15 January, 2005. Although this site was not logged, up to 27 bats (mainly *M. nattereri*, *M. daubentonii* and *P. auritus*) have been caught here during swarming. No more than 4 torpid bats were recorded in a single visit to any other site, including those that showed high levels of swarming activity such as Dow Cave. Not a single torpid bat was recorded in either Bar Pot or Cherry Tree Hole despite nightly logging counts during swarming of up to 1422 and 1998 bat passes, respectively.

Table 2.7 Summary of winter visual surveys at underground sites in the Yorkshire Dales (2002-2005) and an indication of swarming activity at each site, where applicable (- indicates that no logging/catching took place at these sites). Limited winter activity refers to logging arousals (see section 2.4.4.2)

Site	**Grid Reference**	**Swarming activity (based on logging)**	**Species caught during swarming**	**Bat signs/torpid bats in winter**
Link Pot	SD 668803	Very high	All 5 species	Up to 15 torpid bats (*Myotis* spp.); droppings; limited winter activity
Cherry Tree Hole	SD 882704	High	4 species (not *M. mystacinus*)	1 bat in flight in late winter; droppings; limited winter activity
Dow Cave	SD 984743	High	All 5 species	Up to 4 torpid bats (*Myotis* spp.); droppings; limited winter activity
Bar Pot	SD 804770	High	All 5 species	Droppings; limited winter activity
Pool Sink	SD 677807	High	-	3 bats (*M. nattereri*) in flight (2 heard mating) in late winter; droppings
Dowkabottom Cave	SD 951689	Moderate	*M. daubentonii*, *P. auritus*	Up to 8 torpid bats (*Myotis* spp. and *Plecotus auritus*)
Aygill Caverns	SD 663818	Moderate	-	Droppings
Death's Head Hole	SD 668791	Moderate	-	2 torpid bats (*Myotis* spp.); droppings
Mistral Hole	SD 666803	Moderate	4 species (not *M. mystacinus*)	Droppings
Gavel Pot	SD 666791	Moderate	-	1 torpid bat (*P. auritus*); droppings
Browgill Cave	SD 800777	Low	4 species (not *P. auritus*)	1 torpid bat (*M. nattereri*)
Scoska Cave	SD 915724	Low	All 5 species	Up to 3 torpid bats (*Myotis* spp. and *P. auritus*); droppings
Hell Hole	SE 066623	Low	4 species (not *M. brandtii*)	2 torpid bats (*Myotis* spp. and *P. auritus*)
Lost John's Cave	SD 670786	Low	-	1 bat (*M. nattereri*) in flight in mid winter; droppings
Jubilee Cave	SD 837655	Low	-	1 torpid bat (species unknown)
Darnbrook Pot	SD 885710	Absent	-	1 torpid bat (*M. daubentonii*); droppings
Sell Gill Holes	SD 811743	Absent	-	1 bat (*M. nattereri*) in flight in mid winter
Great Douk Cave	SD 747770	Absent	-	1 torpid bat (*P. auritus*)
Lancaster Hole	SD 664807	-	-	Droppings
Mongo Gill	SE 092635	-	-	3 torpid bats (*M. brandtii/mystacinus*); droppings
Notts Pot I	SD 671778	-	-	Droppings
Buckden Gavel Mine	SD 955782	-	4 species (not *M. mystacinus*)	Up to 11 torpid bats (*Myotis* spp.)

On three occasions, two bats were found hibernating together and on one occasion a group of four was observed. All other bats were observed roosting singly and most were in crevices. Droppings were found in dry chambers but would have been washed away in sections of stream passage. Bats were observed in flight during daylight hours on three occasions in mid-late winter. On 30 March, 2004, two *M. nattereri* were observed flying in Pool Sink during the middle of the day. They subsequently crawled into a crevice in the roof and audible, intense vocalisations (presumably related to copulation) were heard.

Hibernating bats were recorded on 6 visits to Link Pot between mid-February and mid-May 2005. A thorough search of the entrance chamber (Hylton Hall), and several subsidiary chambers and passages, was carried out. Counts ranged between 8 and 15 bats. Initially, bats were dispersed throughout the inspected area but from the end of March onwards, almost all bats were observed close to the foot of the entrance pitch and in an adjacent side chamber.

Three bats found hibernating had rings on: one *Myotis* bat (*M. brandtii* or *M. mystacinus*) in Dow Cave; one *M. nattereri* in Link Pot; and one *M. daubentonii* also in Link Pot. Unfortunately, the bats were not in a position where the rings could be read.

2.4.4.2 Automated logging of arousal activity

With a few exceptions, automated logging recorded no arousal activity. Very low levels of activity were recorded on a limited number of nights in March/April inside Cherry Tree Hole, Dow Cave, Bar Pot and Link Pot. However, given the activity levels observed at the entrances at this time of year (see section 2.4.1), it is difficult to separate arousal activity inside the cave from the activity of bats that may be visiting from other sites.

2.4.5 Recaptures and movements between sites

1327 captures have been made at underground sites in the Yorkshire Dales (1996; 2000-2005). This total includes recaptures but does not include bats that were caught but not ringed (ringing of all 5 species did not start until the commencement of this project in autumn 2002). Only two recaptures, from a total of 239, involve the movements of bats between separate underground sites (Table 2.8).

Several bats have been recorded moving between underground sites in the Dales and summer sites to the south and west of the Park. Maximum distances were 17 km for *M. nattereri* (Table 2.9) and 32 km for *M. daubentonii* (Table 2.10). Long term ringing of *M. daubentonii* in Wharfedale shows that bats captured several times clearly move back and forth between summer and nearby underground sites, both within and between years (Table 2.11, see also Figure 2.13).

(In Tables 2.8; 2.9; 2.10; and 2.12, grid references are given for summer locations. Grid references for underground sites can be found in Table 2.1)

Table 2.8 Movement of bats between separate underground sites

Ring number	Species	Sex	Age at ringing	Date and location of ringing	Date and location of recapture	Distance between sites (km)
U8771	*M. nattereri*	Male	Adult	16.09.03 Cherry Tree Hole	27.09.05 Craven Lime Works	7
Y4140	*M. nattereri*	Female	Adult	07.09.03 Manchester Hole	22.10.03 Dow Cave	12

Table 2.9 Bats (*M. nattereri*) ringed at a swarming site: Bar Pot, and recaptured at a maternity roost: Long Preston Church (SD 837580)

Ring number	Sex	Age at ringing	Date of ringing	Date of recapture	Distance between sites (km)
Y4160	Female	Adult	28.09.03	12.06.06	17
Y9872	Female	Adult	27.08.05	12.06.06	17

Table 2.10 Movement of bats (*M. daubentonii*) between summer roosts in Lancashire and underground sites in the Yorkshire Dales

Ring number	Sex	Age at ringing	Date and location of ringing	Date and location of recapture	Distance between sites (km)
Y2081	Male	Juvenile	29.07.03 Denny Beck (SD 503646)	24.09.04 Yordas Cave	25
Y9129	Male	Adult	24.09.04 Yordas Cave	07.07.05 Lancaster (SD 485574)	32
Y4120	Female	Adult	04.09.03 Browgill Cave	10.06.06 Caton Lune (SD 522646)	31

Table 2.11 Recaptures of bats (*M. daubentonii*) moving between summer locations and underground sites in Wharfedale/Littondale/Coverdale. R/F refers to a summer roost or foraging site, UG refers to an underground site. Full names and grid references of sites are given in Table 2.12.

Ring number	**Sex**	**Age at ringing**	**Dates and locations of captures**			**Distance between sites (km)**
				R/F	**UG**	
U3510	Male	Adult	27.08.01		BGM	1.8
			10.07.05	BB		
			06.09.05		BGM	
T5725	Male	Adult	23.07.01	KB		6.0
			07.08.01	KB		
			21.09.01		BGM	
U8752	Male	Adult	27.09.02		DOW	5.3
			06.07.04	BB		
T6182	Male	Adult	10.07.00	KB		2.6
			11.09.01		DOW	
			26.07.02	KB		
T6185	Male	Adult	14.07.00	KB		2.6
			17.06.01	KB		
			29.08.01		DOW	
U7912	Male	Juvenile	27.09.02	KB		2.6
			10.10.02		DOW	
U8959	Male	Juvenile	04.09.03	KB		2.6
			06.06.04	KB		
			14.06.04	KB		
			20.07.05	KB		
			10.08.05	KB		
			25.09.05		DOW	
U8977	Male	Adult	06.06.04	KB		2.6
			17.09.04		DOW	
			04.09.05		DOW	
			25.09.05		DOW	
U8998	Male	Adult	14.06.04	KB		2.6
			04.09.05		DOW	
T5631	Male	Adult	27.08.00		DOW	2.6
			29.08.02		DOW	
			02.09.02	KB		
Y4185	Male	Juvenile	30.09.03		DOW	2.6
			06.06.04	KB		
Y6942	Male	Juvenile	05.09.04		DOW	2.6
			17.08.05		DOW	
			04.09.05		DOW	
			10.10.05	KB		
T0675	Male	Adult	10.07.97	GB		10.6
			27.08.00		DOW	
			02.06.04		DOW	
			05.09.04		DOW	
			17.09.04		DOW	
T5401	Male	Adult	15.07.99	GB		10.6
			27.08.00		DOW	
			14.08.01		DOW	
			26.06.02	GB		
			24.07.02	GB		
			29.08.02		DOW	
Y9143	Male	Adult	30.09.04		DOW	10.6
			28.08.05	GB		

Ring number	Sex	Age at ringing	Dates and locations of captures			Distance between sites (km)
				R/F	UG	
Y9379	Male	Juvenile	11.08.05	ADD		27.3
			04.09.05		DOW	
Y9144	Male	Juvenile	30.09.04		DOW	20.4
			06.07.05	COV		
U8952	Male	Juvenile	04.09.03	KB		3.8
			03.09.04		DKB	
U3669	Male	Adult	17.07.02	LINT		7.8
			17.09.03		LG	
T5750	Male	Adult	01.08.01	ARN		1.9
			07.09.04		STONE	
U8978	Male	Adult	08.06.04	ARN		1.9
			07.09.04		STONE	
U8925	Male	Adult	15.06.03	ARN		1.9
			15.06.03		STONE	

Table 2.11 contd.

Table 2.12 Full names and grid references of sites referred to in Table 2.11.

Abbreviation	Full name of site	Grid reference
BB	Buckden Bridge	SD 940773
KB	Kettlewell Bridge	SD 967723
GB	Grassington Bridge	SD 997638
ADD	Addingham Low Mill	SE 091492
COV	Coverbridge	SE 144870
LINT	Linton Falls	SE 003633
ARN	Arncliffe	SD 934720
BGM	Buckden Gavel Mine	
DOW	Dow Cave	
DKB	Dowkabottom Cave	
LG	Langcliffe Pot	
STONE	Stonelands Cave	

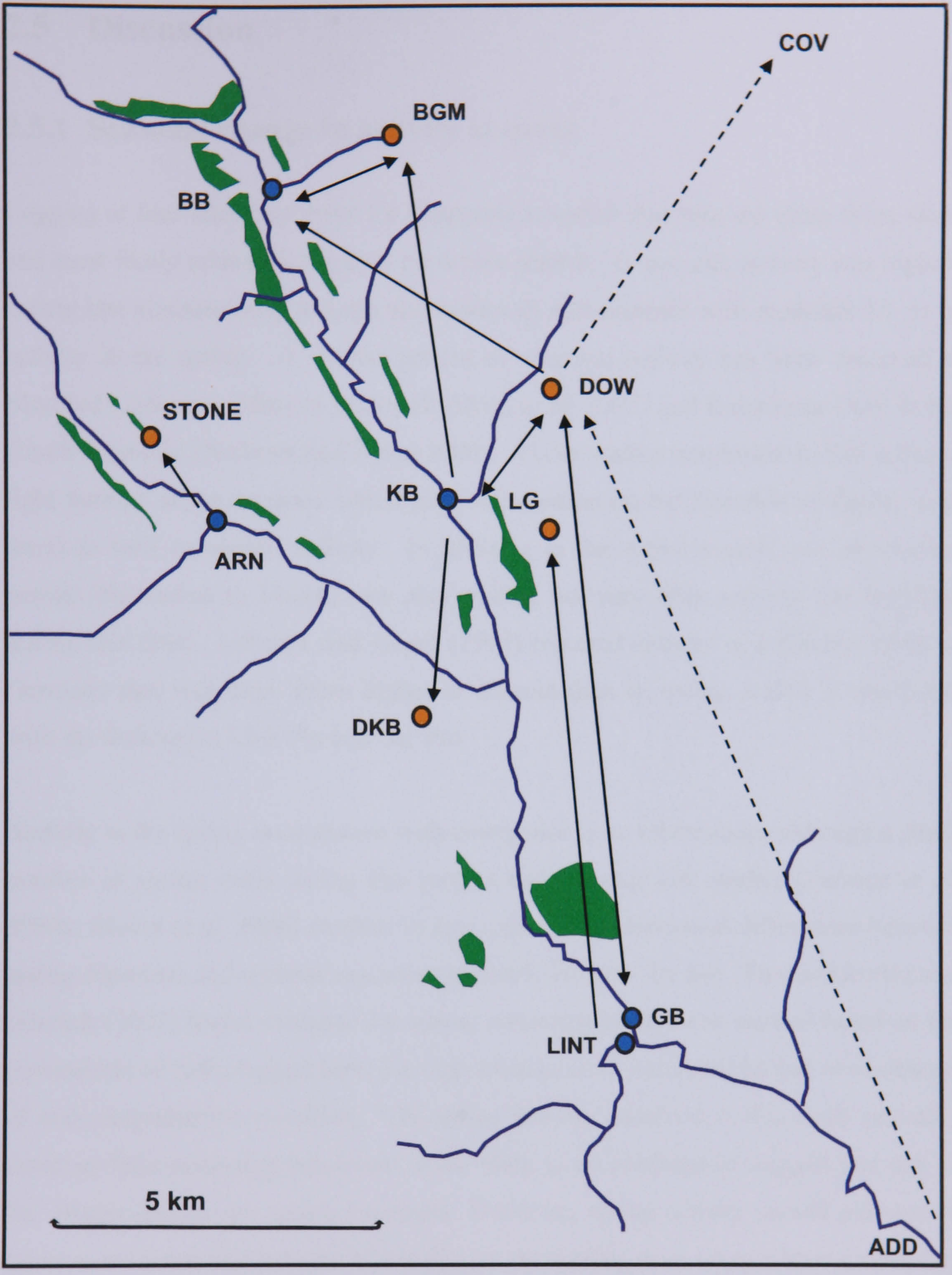

Figure 2.13 Movement of ringed *M. daubentonii* between summer sites and underground sites in Wharfedale/Littondale/Coverdale. See Table 2.12 for details of movements and Table 2.13 for site codes. (Rivers are shown in blue, patches of woodland in green. Red dots indicate swarming sites, blue dots are summer roosts/foraging. Dashed lines indicate movements to/from sites outside the Upper Wharfedale study area).

2.5 Discussion

2.5.1 Seasonal change in activity at caves

Logging at four sites important for swarming revealed that bats are using these sites, and most likely others, throughout the active season. In general, activity was highest during late summer/early autumn and lowest in mid-summer with moderate levels of activity in the spring. A similar pattern of seasonal activity has been observed at Mønsted Limestone Mine in Denmark (Degn *et al.* 1995) and Katerinska Cave in the Czech Republic (Berkova and Zukal 2006). These studies employed double infrared light barriers at the entrance which gave information on the direction of flights (see later) as well as overall activity. In addition to the active season, the hibernation period (November to March) was also studied but very little activity was recorded during this time. Lubczyk and Nagel (1995) reported activity at a hibernaculum in Germany that was three times higher in autumn than in spring, which is consistent with the findings at Link Pot and Bar Pot.

Activity in the spring is consistent with emergence from hibernation, although a small number of spring visits during this project and in other UK studies (Parsons *et al.* 2003a; Rivers *et al.* 2006) resulted in few captures. Behavioural differences between spring departure and autumn swarming probably account for this. Furmankiewicz and Górniak (2002) found evidence for spring swarming in *Plecotus auritus* based on the movements of radio tagged bats, the high incidence of vocalisations and observations of male reproductive condition. The spring activity observed in this study probably involves little swarming behaviour, since there is no evidence to suggest that any of the *Myotis* species are spring swarmers. However, spring activity should perhaps be taken as evidence that bats are hibernating at these sites, even when winter monitoring suggests otherwise.

Low levels of activity were maintained throughout the summer, although Dow Cave clearly differs from the other sites in that activity between April and June was comparable to that seen in August and September. One possible explanation is that Dow Cave acts as a transitional/satellite roost for male bats, particularly *M. daubentonii*. Because male bats are not under the same energetic constraints as females during the breeding season (Kurta *et al.* 1989: Wilkinson and Barclay 1997), they can remain within hibernacula for longer and indeed utilise these colder sites right through the summer. Furmankiewicz and Górniak (2002) found catches of *M. daubentonii* in spring and autumn to be comparable and Lubczyk and Nagel (1995), using a camera triggered by an infrared light barrier, recorded twice as many flights of *M. daubentonii* in April as in autumn. It should be noted however that the latter study commenced in September and may have missed the autumn peak for this species. A peak of activity between mid-May and mid-June at Mønsted Mine was distinct from the earlier departure peak and involved no net influx/efflux (Degn *et al.* 1995). This was attributed to a large transitory population of male *M. daubentonii* (Degn 1989). *M. daubentonii* accounts for a higher proportion of swarming captures at Dow Cave than at the other swarming sites in the Dales, a trend that is consistent across the season. The cave is linked by a watercourse to the River Wharfe where several substantial male/mixed roosts of *M. daubentonii* have been identified and intensively studied (Senior *et al.* 2005). Captures at these roosts and radiotelemetry revealed that roosts were not occupied continuously through May - September and that roost switching occurred often. Although none of the bats in the main study were tracked to a cave, an adult male *M. daubentonii* caught over the river in neighbouring Littondale (15.06.2003) was radio tagged and tracked back to Stonelands Cave 1.9 km away. In addition, an *ad hoc* summer visit to Dow Cave (02.06.2004) resulted in the capture of ten bats, all adult males (data not shown). Six of these were *M. daubentonii*, four of which had been caught at Dow Cave in the autumn in previous years and all six were recaptured at Dow Cave in subsequent swarming seasons. One of the recaptures was ringed as an adult at Grassington Bridge in 1997 but has never been caught there since.

All four sites showed high levels of activity in the autumn, consistent with swarming behaviour. Temporal changes and other swarming patterns are discussed in section 2.5.2.

Automated logging does not provide information on species and sex of the bats present, so discussion as to the precise function of these sites over the course of the year is somewhat speculative. However, they provide a clear indication that underground sites are important to hibernating species all year round and this should be taken into account when planning conservation measures and population studies.

2.5.2 Swarming patterns

A direct and quantitative comparison between swarming studies is difficult due to the variation in survey techniques used (e.g. automated logging, catching) and catching protocol (number and frequency of catches, time in the season, configuration and efficiency of traps). However, common patterns to swarming can be found and differences between studies may be related to geographical variation.

2.5.2.1 Changes in activity through the swarming season

Swarming activity was recorded from the beginning of August to the end of October with a peak in activity between mid-August and mid-September. These findings are consistent with other swarming studies both in the UK (Parsons *et al.* 2003a; b), continental Europe (e.g. Degn *et al.* 1995; Furmankiewicz and Górniak 2002) and North America (e.g. Fenton 1969; Cope and Humphrey 1977). Rivers *et al.* (2006) reported a later peak in swarming activity (mid-September to mid-October) at a group of caves ~60 km east of the Yorkshire Dales. These sites are on average 200 m lower in altitude than swarming sites in the Dales and are sheltered by coniferous plantations. By contrast, swarming sites in the Dales are in exposed upland areas where low, nightly temperatures and windy conditions later in the season may discourage swarming activity (Parsons *et al.* 2003b; Berkova and Zukal 2006).

Several studies have identified phases during the swarming season which relate to the composition of bats and behaviour exhibited (e.g. Hall and Brenner 1968; Fenton 1969; Thomas *et al.* 1979; Schowalter 1980; Degn *et al.* 1995). In the first phase bats visit for a few hours only, 'swarming' in and around the entrances. An equal number of in-flights and out-flights are recorded and few bats remain within the site. The second phase involves a net influx and the gradual build up of a torpid population. Overall activity levels are lower during this second phase. Changes related to the sex ratio and age composition are discussed in section 2.5.2.4.

2.5.2.2 Changes in activity through the night

The peak in swarming activity during the night was 3 - 5.5 hours after sunset based on capture times. The late peak in activity several hours after sunset, in both this and other studies (e.g. Parsons *et al.* 2003b; Rivers *et al.* 2006), is consistent with the idea that bats are visiting and leaving within the night. Bats may spend time foraging elsewhere before swarming: Fenton (1969) found that 90% of bats caught at a mine in Ontario during August had distended stomachs. Alternatively, or in addition, bats may travel substantial distances in order to swarm.

The change in nightly activity patterns across the season observed in this study may be explained by differences in behaviour. A shift towards an earlier peak in activity late in the season is consistent with an increasing population of bats within hibernacula and the arrival of bats that are roosting locally having dispersed from their summer sites. Late in the season when foraging conditions are likely to be sub-optimal, bats increasingly use torpor to lower energy expenditure rather than maintaining or increasing foraging activity to build fat reserves (Altringham 2003). Fenton (1969) found that only 20% of bats caught in September showed signs of having fed. A skew towards early activity in October could therefore be a combination of (1) emergence of a resident hibernating population, (2) arrival of bats from nearby autumn roosts and (3) a reduction in foraging prior to arrival. Degn *et al.* (1995) observed a peak influx of bats in the three hours prior to sunrise late in the season but this has not been reported elsewhere. The skew towards earlier activity in the first part of the swarming season is not so pronounced but is perhaps more difficult to explain. It could be that opportunistic swarming by local populations accounts for activity at this time and bats from more distant sites only arrive in any number during 'peak' season.

2.5.2.3 Species composition

The species composition at swarming sites is inevitably related to the species present in the area and to a degree, their local density. All swarming species present in the north of England (*M. nattereri*, *M. daubentonii*, *M. brandtii*, *M. mystacinus* and *P. auritus*) were caught at all the major sites in the study. Other European species known to swarm but not present in the study area include *M. bechsteinii*, *M. emarginatus*, *P. austriacus* and *Barbastellus barbastellus* (Horácek and Zima 1978;

Bauerová and Zima 1988; Weidinger 1994; Furmankiewicz and Górniak 2002: Kerth *et al.* 2003). *M. nattereri* and *M. daubentonii* are more common and widespread than *M. brandtii* and *M. mystacinus* (Harris *et al.* 1995) and this is reflected in a higher proportion of captures of the former species overall (53% and 24% of the total respectively). *M. nattereri* and *M. daubentonii* were also the most common species (36.5% and 31.6% of the total respectively) caught during a study in the south of England (Parsons *et al.* 2003a). In a study of swarming sites in the North York Moors (Rivers *et al.* 2006), 79.1% of captures were *M. nattereri*, although this high proportion may be in part a reflection of few catches early in the season when species diversity is higher (see below). However, it is also possible that more than one mating strategy may be operating in these species (Senior *et al.* 2005) and the relative importance of swarming as a mating event may vary.

In addition to the five 'swarming' species, a small number of *Pipistrellus pipistrellus* were also caught (five at Dow Cave, three at Browgill Cave, and one at the Craven Lime Works and Victoria Cave). Although *P. subflavus*, a North American species, hibernates underground (Sandel 2001; Briggler and Prather 2002) and has been shown to exhibit swarming behaviour (Davis 1964; Hall and Brenner 1968; Mumford and Whitaker 1975), *P. pipistrellus* has a different mating system based on territorial defence and song flight display (Gerell 1985; Lundberg and Gerell 1986; Lundberg 1990). The incidence of *P. pipistrellus* at swarming sites and in underground hibernacula in Europe appears to differ between populations (Kretschmar and Heinz 1995; Sendor *et al.* 2000). No *P. pipistrellus* were observed during winter surveys in this study and it was assumed that the small numbers caught in the autumn were foraging in the vicinity of the cave entrance.

A change in species composition over the course of the swarming season has frequently been documented (e.g. Lubczyk and Nagel 1995; Parsons *et al.* 2003a). The early peak of *M. brandtii* and *M. daubentonii* in this study is consistent with observations from Box Mine in south west England (Parsons *et al.* 2003a) and Stolec Mine in Poland (Furmankiewicz and Górniak 2002). Also observed elsewhere is the increase in activity of *M. nattereri* later in the season (Lubczyk and Nagel 1995; Furmankiewicz and Górniak 2002) and a point where it dominates the species composition (Parsons *et al.* 2003a).

2.5.2.4 Sex ratio and age composition

The skew in sex ratio towards males that was observed in this study has also been widely reported in swarming studies elsewhere in the UK (Parsons *et al.* 2003a; Rivers *et al.* 2006), in continental Europe (e.g. Furmankiewicz and Górniak 2002; Kerth *et al.* 2003) and North America (e.g. Davis and Hitchcock 1965; Fenton 1969; Humphrey and Cope 1976).

Although an apparent paucity of females during what has been described as a mating event (Fenton 1969; Thomas *et al.* 1979; Kerth *et al.* 2003; Veith *et al.* 2004; Rivers *et al.* 2006) seems at first to be counter intuitive, the male bias can actually be explained by intersexual differences in behaviour during this time. Males have more to gain from maximising their number of matings than females and should therefore visit swarming sites more often or stay around longer. Recapture rates are higher amongst males than females (Rivers *et al.* 2006; N.M. Rivers, A.M. Glover, J. Furmankiewicz, R.K. Butlin and J.D. Altringham, in prep) and individual males may be caught several times within one season (data not shown).

A male biased sex ratio could lead to an increase in intrasexual competition among males, opportunities for female mate choice and intense sexual selection (Emlen and Oring 1977). However, mating during swarming has been cited as random and promiscuous (Thomas *et al.* 1979) and Wai-Ping and Fenton (1988) found that body mass and skeletal size of male *M. lucifugus* had no influence on their chance of obtaining copulations. In addition, male *M. lucifugus* show no signs of competitive behaviour such as territoriality or courtship display (Barclay *et al.* 1979; Thomas *et al.* 1979). They do emit vocalisations during copulation, but these are believed to pacify struggling females rather than attract them (Barclay and Thomas 1979). Despite the lack of behavioural evidence for female mate choice in *M. lucifugus*, there is genetic evidence (DNA fingerprinting) that indicates a skew in paternity towards particular males/male lineages (Watt and Fenton 1995). Females can mate multiply and store sperm, and the potential role of sperm competition in determining successful fertilisations should not be overlooked.

In addition to a real sex ratio bias, intersexual differences in chasing behaviour in and around the entrances may result in differential capture success, thus exaggerating the true sex ratio.

The degree of male bias varies between species (both within and between studies), although non significant departures from unity can often be attributed to small sample sizes amongst rarer species. The sex ratio of *M. nattereri* in this study (77.1% males) is comparable with those obtained in other studies (71-75.8% males: Furmankiewicz and Górniak 2002; Parsons *et* al. 2003a; Rivers *et al.* 2006). The sex ratio is even more biased towards males in the other four species (~90%) but this is not reflected elsewhere in the UK (~60-80%: Parsons *et* al. 2003a; Rivers *et al.* 2006). It may be the case for some species that in upland areas, like the Yorkshire Dales, differences in behaviour between the sexes are more pronounced. Ruedi *et al.* (1989) report a strong male bias (82 - 96% males) at a swarming site in the Jura Mountains, Switzerland which is situated at 1500 m a.s.l.

Whilst the sex ratio remained heavily skewed towards males throughout the season, the proportion of females was highest during the peak of swarming (late August and September). Kerth *et al.* (2003) observed a similar pattern in *M. bechsteinii* - the proportion of females caught was at its highest (25%) in mid-August to mid-September, when overall numbers were at their peak. This is consistent with the idea that males 'hang around' throughout the season in an attempt to maximise mating success, whereas females are more selective and may visit swarming sites less frequently.

A sex ratio close to unity was expected among juveniles given that only a small percentage of bats reach sexual maturity in their first year (Racey and Entwistle 2000). However, the male bias was evident in both adults and juveniles. Again this is consistent with observations for *M. bechsteinii* (Kerth *et al.* 2003).

2.5.3 Assessing hibernation sites

If the assumption is made that swarming 'hot spots' and important hibernation sites are frequently synonymous then it is clear that visual surveys are not a quantitative way of assessing hibernacula, but are they even useful qualitatively? The fact that not a single torpid bat was ever observed at two of the most important swarming sites identified (Bar Pot and Cherry Tree Hole) suggests not. In an English Nature database of almost 1,000 hibernacula, both *Myotis* species and natural underground sites are grossly underestimated relative to their abundance (Altringham 2003). For species that arouse frequently during hibernation e.g. *Rhinolophus ferrumequinum*, automated logging can be used to monitor known sites (e.g. Park *et al.* 1999). However, it appears to be an ineffective method for identifying or monitoring hibernating *Myotis* species in large and complex underground sites. The best method currently available to identify underground sites important to *Myotis* bats in the UK (and perhaps elsewhere in Europe) is to monitor activity at entrances during the swarming period. This rests on the unproven, but logical assumption that bats hibernate in the sites where they swarm.

2.5.4 Seasonal movements

The high fidelity shown to a single underground site, by all species, was also observed by Parsons and Jones (2003) and Rivers *et al.* (2006). This emphasises the conservation significance of these sites. Not only are they used by large numbers of bats from an extensive catchment, but each of these populations is probably reliant on a single site.

Ringing studies (e.g. Griffin 1945; Humphrey and Cope 1976; Rivers *et al.* 2006) report distances between summer roosts and swarming sites/hibernacula that range from 10 km to 500 km. However, it is not clear from these studies whether recaptured bats make the journey from summer roost to cave and back again in one night or whether movements are unidirectional and staged. The break up of colonies at the end of summer does not signify that bats are moving towards hibernation sites straight away. Instead, they may remain dispersed within their summer range where familiarity with foraging areas and alternative roosts is beneficial. Radio tracking studies (Parsons and Jones 2003; Furmankiewicz 2004) have shown that bats will travel at least 35 km in a single night, sometimes remaining at a cave for only one or two nights before returning to their roost. Patterns in autumn movements will no doubt be determined by the distance between summer range and suitable hibernacula. Short, 'back and forth' autumn movements in swarming species are at one end of a continuum. At the other end are the annual long-distance journeys made by migratory species (see Fleming and Eby 2003). Different species will fall on various points along this continuum. In addition, variation in climate and geography probably leads to intraspecific differences in the pattern of autumn movements.

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Chapter 3: Cave Characteristics Determining Bat Activity in the Autumn

3.1 Abstract

Between 2002 and 2005, 53 cave entrances were logged for autumn swarming activity using automated echolocation call loggers. In a multiple regression model chamber development and hydrology were the best predictors of autumn activity and together could explain 45% of the variance. Activity was significantly positively correlated with chamber development and negatively correlated with the amount of water the cave carried. The addition of entrance orientation and the degree of shelter at the entrance to the model explained a further 10% of the variance. Activity was not correlated with entrance size, altitude or 'connectivity' to the nearest foraging, roosting and summer habitat. Since virtually all of the caves are located on open moorland and 'connectivity' was not an important variable, bats appear to be attracted by the characteristics of the cave itself and the surrounding habitat is less important.

3.2 Introduction

Understanding the relationship between habitat characteristics and the presence or abundance of an animal is important for several reasons, two of which are particularly pertinent in this study:

- It allows us to make predictions about the function of those habitats in the animal's life cycle.
- It allows us to focus survey and conservation efforts on those places likely to be of greatest importance.

Temperate bats use a number of reproductive delays (reviewed in Racey and Entwistle 2000) that allow them to mate in the autumn prior to hibernation and commence gestation the following spring. In many temperate vespertilionid species, particularly in the genus *Myotis*, large numbers of bats visit underground sites during the autumn and 'swarm' in and around the entrances (e.g. Fenton 1969; Parsons *et al.* 2003a; Rivers *et al.* 2006). Both behavioural and genetic studies (e.g. Barclay and Thomas 1979; Thomas *et al.* 1979; Kerth *et al.* 2003; Kerth and Morf 2004; Veith *et al.* 2004; Rivers *et al.* 2005; Furmankiewicz and Altringham 2006; N.M. Rivers, A.M. Glover, J. Furmankiewicz, R.K. Butlin, J.D. Altringham in prep.) support the idea that this swarming behaviour is linked to mating. The requirements of a mating site will be dependent on aspects of the mating strategy in place (e.g. male display, territorial defence and female mate choice). Shelter and predation risk may also be important, as may location, if bats travel from distant roosts.

The consensus is that important swarming sites are also likely to be important hibernacula, in many cases (e.g. Rivers *et al.* 2006; Furmankiewicz and Altringham 2006), and it may be that the suitability of a site for the purpose of hibernation is a major factor determining autumn activity. There are two reasons why we would expect autumn activity to be high at important hibernation sites:

- Swarming is likely to involve the assessment of hibernacula (Davis and Hitchcock 1965) and information transfer between adults and juveniles regarding the location of hibernation sites (Fenton 1969; Humphrey and Cope 1976).

- Male distribution during the mating season is likely to be a reflection of female dispersion (Altringham 1996). In species which rely on underground sites for hibernation, gathering at these sites may present the best opportunity for males to locate potential mates.

The habitat requirements of a hibernation site will relate to microclimate (temperature, humidity, airflow), the level of disturbance and provision of suitable substrate/crevices for roosting. Swarming also involves seasonal movements between summer roosts and underground sites and site selection may be influenced by the ease with which bats can disperse to and locate sites. It has also been proposed that swarming sites are ‘stop-overs’ on migration routes (Fenton 1969), although there is no direct evidence to support this.

Conservation and management strategies often rely on being able to model habitat use. This is particularly important where habitats/sites are under threat and funding/time is insufficient for adequate survey.

The aim of this chapter is to relate cave characteristics to bat activity during the swarming season. These characteristics relate to the internal environment of the cave, the nature of the cave entrance and its location in the landscape. The model developed will be used to focus survey and conservation effort in the Yorkshire Dales National Park but the overall patterns could be applied to other cave-rich karstic regions and are relevant to our understanding of swarming ecology in its broadest sense.

The primary questions to be addressed in this chapter are:

- Which cave characteristics are correlated with bat activity?
- Which characteristics should be included in a regression model to predict bat activity?
- Are these results affected by the measure of bat activity used in the analysis (i.e. mean/maximum nightly activity)?

3.3 Methods

3.3.1 Study area and period

The study area was the Yorkshire Dales in the north of England (see Chapter 1). Specifically the study considered 53 cave entrances located within the Great Scar Limestone area (Figure 3.1).

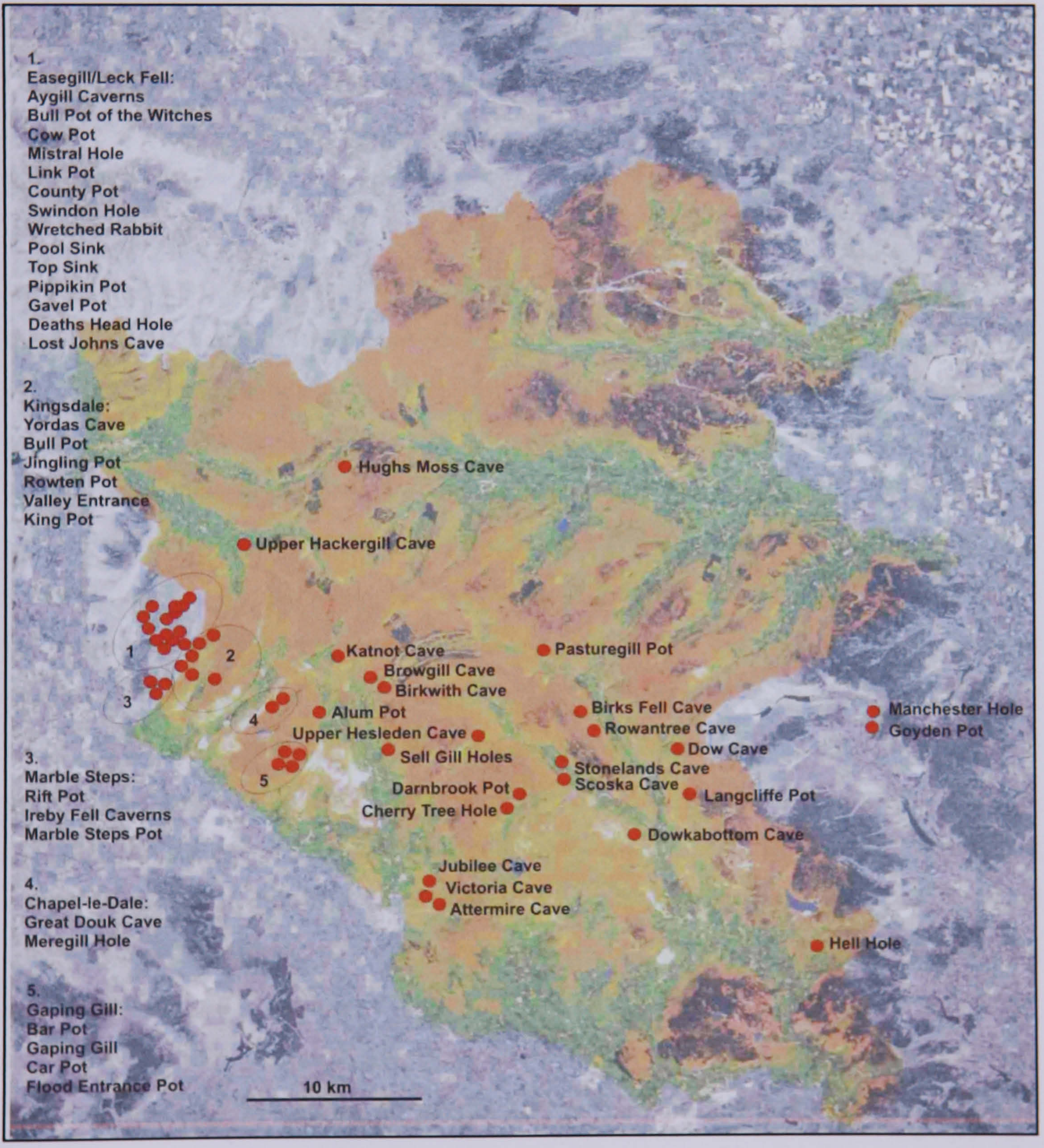

Figure 3.1 Location of 53 caves logged autumn 2003-2005.

Bat activity at cave entrances was logged between mid-August and early October 2003-2005. The study period was timed to coincide with the peak period of swarming (see Chapter 2) to reduce bias due to seasonal variation.

3.3.2 Cave selection and variation

The caves sampled included several entrances into the Easegill Caverns system which, at over 50 km in length, is the most extensive cave system in the UK. Also included were several entrances into the Gaping Gill system on Ingleborough. The UK's deepest natural shaft (from the surface) and the largest natural chamber are to be found in this cave system. Excluding the Easegill system, cave lengths ranged from 60 m to 10.8 km.

In the Yorkshire Dales the Carboniferous limestone is made up a small number of thick beds that together are 200 m thick. It has undergone little folding and tilting, so with only a few localised exceptions, the bedding remains horizontal. The caves are formed and continuously modified by the dissolution of the limestone by rainwater, which enters the rock through the horizontal bedding planes and vertical faults. The water initially cuts small, smooth-walled stream passages either as roughly circular, water-filled phreatic tubes, or tall, narrow, only partially filled vadose canyons, with most of the development between major bedding planes taking the form of vertical pitches in the faults. These passages can grow to substantial dimensions: phreatic tubes can exceed 5 m diameter and vadose canyons may be more than 20 m high, but rarely more than 3 m in width. Major faults can play a significant role in determining the path of a cave and it is often through breakdown around these faults that major chambers develop. Most of the Dales caves are still active and form part of large underground drainage systems. The volume of water flowing through an active cave is determined by changes in rainfall and the water table and can vary considerably, and often rapidly. Nevertheless, some caves rarely take more than a small stream whilst others frequently flood to the roof over much of their length. Many caves have extensive fossil sections, long since abandoned by the water. Smaller caves may be simple in structure but the larger systems are frequently complex, containing sections with a wide range of dimensions and stages of development. A single cave can therefore contain horizontal, high-roofed, meandering stream passages interrupted by tortuous crawls, large breakdown chambers, partially filled with boulders and vertical pitches.

Entrance size varies greatly - many cave entrances are a tight squeeze for even the smallest caver, whilst the entrance to Victoria Cave is 12 m wide at ground level and 9m high.

The caves' entrances range in altitude between 200 m and 500 m a.s.l. so all can be regarded as being situated in upland habitats. The habitats around the caves range from semi-improved/unimproved pasture in the valley bottoms, to moorland and rough pasture at higher altitudes. A small number of caves are situated in woodland patches, but these patches are typically small and fragmented. Of those cave entrances that are situated on exposed moorland, a significant number are sheltered by being within shakeholes of varying sizes. Large trees and shrubs have established themselves in the shelter provided by many shakeholes.

Although the primary aim of this study was to identify the cave characteristics that influence swarming activity, a secondary aim was to identify key sites for further study (see chapters 2 and 5). For this reason, only sites deemed to be 'potentially attractive' to swarming bats (in the broadest sense) were selected. There are many cave systems in the Dales that flood suddenly and completely and they were not considered suitable. Initially, only caves >100m in length were considered, although Jubilee Cave (90m) was included in 2004 and Jingling Pot (60m) in 2005. This elimination process still left a large and diverse shortlist of sites.

3.3.3 Measuring bat activity

Bat activity was measured using an automated system based on the detection of echolocation calls (detailed in Chapter 2). It is worth emphasising again that the logging counts produce an index of activity based on number of bat passes and not a count of the number of individual bats present.

Each site was logged for a minimum of three consecutive nights during settled weather (low wind and rain), so as to reduce the variance in activity caused by weather (see Parsons *et al.* 2003b). If conditions deteriorated significantly during a logging session, the sites were logged again, either in the same season or in a subsequent one. Data from the aborted session were not used.

The data were exported from Gemini Logger Manager to Microsoft Excel, where total counts of bat passes on each night were calculated. Bat activity can show large nightly variation (Parsons *et al.* 2003b; Rivers *et al.* 2006, Chapter 2) so mean activity may underestimate a site's potential. For this reason two values of activity were generated for each site:
(i) Mean nightly total
(ii) Maximum nightly total

3.3.4 Assessing/measuring cave characteristics

The criteria for selecting cave characteristics were:
(i) They had to be characteristics that could be reliably measured or estimated using published cave surveys, extensive ground truthing and Ordnance Survey maps.
(ii) There had to be a biological basis for assuming that they could influence swarming activity.

- Cave length (metres)

  This is the length of the cave system that is accessible to a human, without having to dive a sump (a point in the cave where the roof lowers to meet the water, so that the passage ahead is flooded). These were the adjusted values obtained from the Northern Caves Guides (Brookes *et al.* 1988; 1991; 1994), with minor corrections based on more recently published surveys.
- Degree of chamber development (high/low)

  A simple categorisation was used, with caves being classified as either with our without major chamber development. High means the presence of large and/or numerous chambers.
- Hydrological activity (dry/wet)

  These went from fossil systems that transport no running water, through those with small streams, to those which carry substantial streams and those occasionally flooding through part of their length. Caves were classified as either primarily dry or wet.

- Entrance size (large/small)

  Classified as large (>2 m in both planes) or small (< 2m in both planes).

- Entrance orientation (horizontal/vertical)

  This refers to the entrance only, the division between horizontal and vertical being defined by whether or not the bat could fly into the cave with little or no change in flight path.

- Degree of shelter at the entrance (exposed/sheltered)

  Classified as exposed (no shelter, the cave entrance being exposed to the wind and rain) or sheltered (considerable shelter from the elements at the cave entrance).

- Altitude (metres a.s.l.)

- Connectivity (remote/accessible)

  Sites classified as remote had none or few connecting landscape elements. Sites classified as accessible were those close to low altitude habitat (suitable for foraging or summer roosts) and/or directly connected to it by major linear landscape elements such as sheltered valleys.

3.3.5 Statistical analysis

A forward stepwise multiple regression analysis was performed in SPSS, with the following assumptions:

(i) Predictor variables (i.e. cave characteristics) must be quantitative or, if categorical, have only two categories.

(ii) The outcome variable (i.e. bat activity) should be quantitative, continuous and unbounded.

3.4 Results

A sample plot of raw data from a logger is shown in Figure 3.2. Data for mean activity, maximum activity and cave characteristics are summarised in Table 3.1.

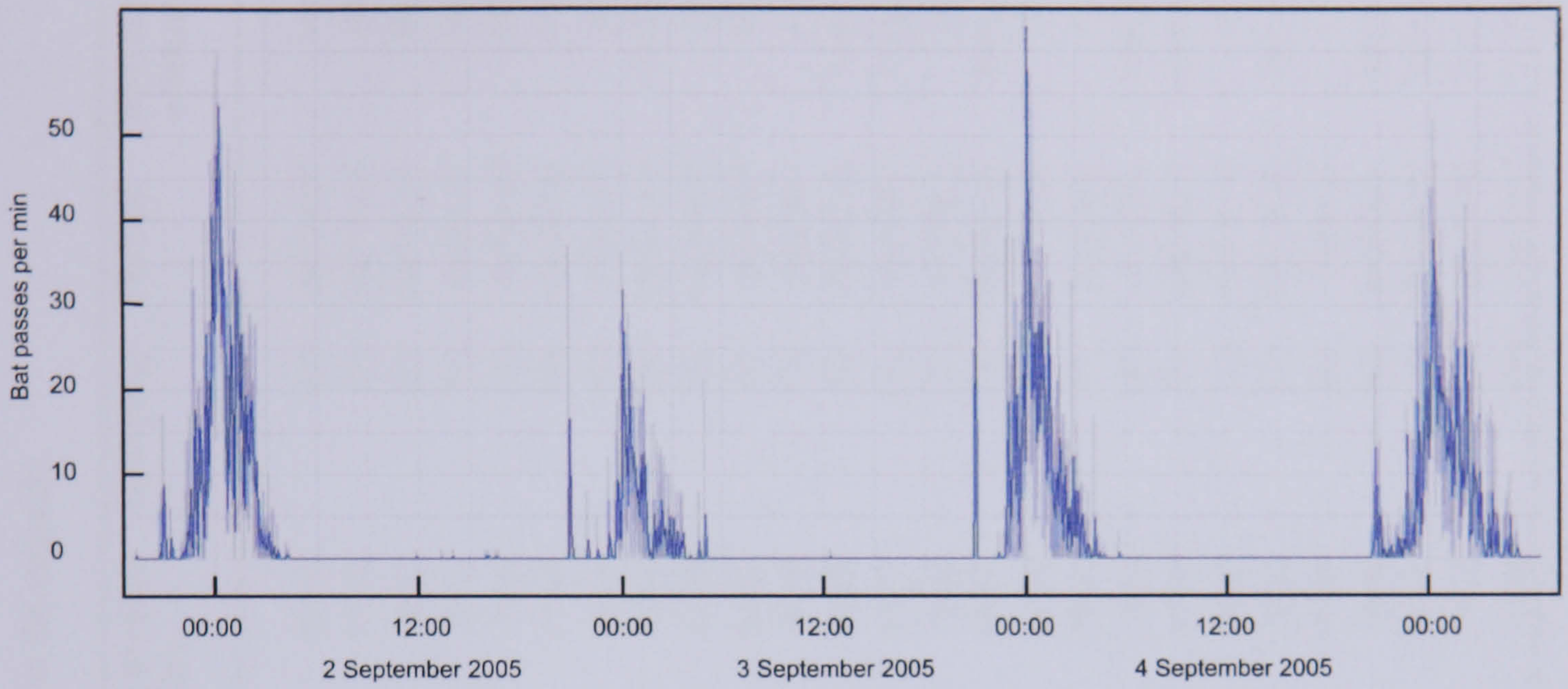

Figure 3.2 Raw plot of bat passes per minute over four nights at Rift Pot (see Table 3.1) showing early peak due to the dusk emergence of a small number of bats from the cave, followed by the late and sustained activity due to swarming. 00:00 = midnight, 12:00 = noon.

3.4.1 Which factors are correlated with bat activity?

Preliminary analysis showed that the assumption of normal distribution of residuals after regression analysis was not met, so all analyses were carried out on $\log_{10}$ transformed data. Log_{10} bat activity (both mean and maximum number of bat passes per night) was positively and significantly correlated with cave length, degree of chamber development and shelter at the entrance. The correlation between bat activity and the level of hydrological activity was negative and significant. There was no significant relationship between bat activity and entrance size, connectivity and entrance orientation. Correlation coefficients and their significance values are given in Table 3.2.

Table 3.1 Activity (mean and maximum) is expressed in bat passes per night. Other parameters are defined in the methods.

Site	Grid reference	Mean activity	Max activity	Length (m)	Chambers	Altitude (m)	Entrance Size	Hydrology	Shelter	Entrance orientation	Connectivity
Link Pot	SD 668803	4894	9309	52000	high	276	small	dry	sheltered	vertical	accessible
Rift Pot	SD 679769	3584	4632	2720	high	365	small	dry	sheltered	vertical	remote
Cherry Tree Hole	SD 882704	1100	1998	1200	high	467	small	dry	sheltered	vertical	remote
Cow Pot	SD 664808	1025	2957	52000	high	305	large	dry	exposed	vertical	accessible
Bull Pot of the Witches	SD 662813	1014	1376	2500	high	293	large	dry	sheltered	vertical	accessible
County Pot	SD 675806	945	2769	52000	high	314	small	dry	sheltered	vertical	accessible
Langcliffe Pot	SD 996711	928	1216	9600	low	488	small	wet	exposed	vertical	remote
Dow Cave	SD 984743	744	1195	3700	high	320	large	dry	sheltered	horizontal	accessible
Bar Pot	SD 804770	645	1422	10800	high	395	small	dry	sheltered	vertical	accessible
Rowten Pot	SD 698780	489	1119	260	low	363	small	dry	exposed	vertical	remote
Dowkabottom Cave	SD 951689	448	534	670	high	387	small	dry	exposed	vertical	remote
Pool Sink	SD 677807	401	1183	52000	high	323	small	dry	sheltered	horizontal	accessible
Aygill Caverns	SD 663818	295	846	2410	high	295	small	dry	exposed	horizontal	accessible
Deaths Head Hole	SD 668791	261	387	720	high	341	large	dry	exposed	vertical	remote
Swindon Pot	SD 675805	246	852	52000	low	320	small	dry	sheltered	horizontal	remote
Mistral Entrance	SD 666803	184	638	52000	high	267	small	dry	sheltered	vertical	accessible
Flood Entrance Pot	SD 751724	174	390	10800	high	396	small	wet	exposed	vertical	accessible
Victoria Cave	SD 837650	168	236	180	high	439	large	dry	exposed	horizontal	remote
Wretched Rabbit Entrance	SD 675806	145	724	52000	high	319	small	dry	sheltered	horizontal	accessible
Gavel Pot	SD 666791	113	288	1970	high	323	large	dry	sheltered	vertical	remote
Browgill Cave	SD 800777	100	230	850	high	329	large	dry	sheltered	horizontal	accessible
Bull Pot	SD 702787	88	198	200	low	358	small	dry	exposed	vertical	remote
Stonelands Cave	SD 920733	86	170	180	low	280	large	dry	sheltered	horizontal	accessible
Scoska Cave	SD 915724	78	116	1500	low	285	large	dry	sheltered	horizontal	accessible
Hell Hole	SE 066623	77	161	210	low	280	small	dry	exposed	horizontal	remote
Alum Pot	SD 775756	75	77	500	low	347	large	wet	exposed	vertical	remote

Site	Grid reference	Mean activity	Max activity	Length (m)	Chambers	Altitude (m)	Entrance Size	Hydrology	Shelter	Entrance orientation	Connectivity
Ireby Fell Caverns	SD 673773	71	201	4900	low	396	small	wet	sheltered	vertical	remote
Manchester Hole	SE 100764	63	88	500	high	222	small	wet	exposed	horizontal	accessible
Yordas Cave	SD 705801	58	86	210	high	312	small	wet	sheltered	horizontal	accessible
Lost Johns Cave	SD 670786	54	135	6000	high	354	small	wet	exposed	horizontal	remote
Car Pot	SD 755725	48	48	800	low	396	small	wet	sheltered	vertical	remote
Pippikin Pot	SD 667800	47	117	52000	low	326	small	dry	exposed	horizontal	remote
Attermire Cave	SD 841642	42	70	180	low	427	small	dry	exposed	horizontal	remote
Birkwith Cave	SD 804770	41	109	550	low	338	small	wet	sheltered	horizontal	accessible
Darnbrook Pot	SD 885710	39	52	340	low	455	small	wet	exposed	vertical	remote
Sell Gill Holes	SD 811743	39	96	500	low	347	large	wet	exposed	vertical	remote
Jubilee Cave	SD 837655	36	104	90	low	376	large	dry	exposed	horizontal	remote
Katnot Cave	SD 780774	35	102	670	low	297	small	dry	sheltered	horizontal	accessible
Great Douk Cave	SD 747770	22	46	910	low	343	large	dry	sheltered	horizontal	remote
Gaping Gill	SD 751727	15	33	10800	high	396	large	wet	exposed	vertical	remote
Pasturegill Pot	SD 905793	5	15	760	low	356	small	wet	exposed	vertical	remote
Jingling Pot	SD 699784	4	7	60	low	378	large	dry	exposed	vertical	remote
Rowantree Cave	SD 938755	3	10	860	low	343	small	dry	sheltered	vertical	accessible
King Pot	SD 708774	2	3	5000	low	378	small	wet	exposed	vertical	remote
Meregill Hole	SD 740757	2	3	4600	low	396	small	wet	exposed	vertical	remote
Top Sink	SD 680811	2	7	52000	low	349	small	dry	exposed	vertical	accessible
Birks Fell Cave	SD 931769	1	1	3600	low	362	small	wet	exposed	horizontal	remote
Goyden Pot	SE 100762	1	4	3600	high	210	large	wet	sheltered	horizontal	accessible
Hughs Moss Cave	SD 788901	1	1	1100	low	503	small	wet	exposed	horizontal	remote
Marble Steps Pot	SD 680771	1	1	1270	low	384	large	wet	exposed	vertical	remote
Upper Hackergill Cave	SD 740861	1	1	820	low	198	small	dry	exposed	horizontal	accessible
Upper Hesleden Cave II	SD 864739	1	1	600	low	337	small	dry	exposed	horizontal	accessible
Valley Entrance	SD 698774	1	1	4200	low	268	small	wet	exposed	horizontal	accessible

Table 3.1 contd.

Table 3.2 Pearson correlation coefficients between cave characteristics and mean/ maximum nightly activity. (p (one tailed) * <0.05, ** <0.01, *** <0.001, n.s. not significant)

Cave characteristic	**r (mean bat activity)**		**r (maximum bat activity)**	
Cave length	0.29	*	0.36	**
Chamber development	0.56	***	0.56	***
Hydrological activity	-0.43	**	-0.45	***
Entrance size	0.02	n.s	0.01	n.s
Entrance orientation	0.21	n.s	0.20	n.s
Shelter at the entrance	0.38	**	0.42	**
Altitude	0.03	n.s	-0.01	n.s
Connectivity	0.09	n.s	0.13	n.s

3.4.2 Which multiple regression model best describes the data?

The models which best explain the variation in both mean and maximum bat activity between sites are summarised in Table 3.3. Between them, the degree of chamber development, the level of hydrological activity and entrance orientation could explain 48 and 50% of the variation in bat activity as measured by mean and maximum activity respectively (Table 3.3). When maximum activity was used as the dependent variable, the addition of degree of shelter meant that the model could explain a total of 55% of the variation in activity.

Adding predictors to the model increased the variance that could be explained but decreased the accuracy of the model (Table 3.3, F-ratio). The models can be cross-validated by comparing R^2 with the adjusted R^2. The two values are very similar indicating that the models generalise well.

Table 3.4 shows the relative importance of each cave characteristic at each stage of the model. The b-value (B) provides an indication of the degree to which each cave characteristic affects bat activity if the effects of the other characteristics in the model are held constant. The relative importance (in standard deviation units) of each cave characteristic is shown by the standardised b-value (β).

Table 3.3 Summary of the multiple regression models for mean and maximum bat activity. (p * <0.05, ** <0.01, *** <0.001)

Step	**Cave characteristics included in the model**	**R**	**R^2**	**Adjusted R^2**	**F change$_{df}$**	**Sig F change**	**F ratio**
Mean bat activity							
1	Chamber formation	0.56	0.31	0.30	$22.9_{1,51}$	***	22.9
2	Chamber formation, hydrological activity	0.65	0.42	0.40	$9.4_{1,50}$	**	18.0
3	Chamber formation, hydrological activity, entrance orientation	0.69	0.48	0.45	$5.6_{1,49}$	*	15.0
Maximum bat activity							
1	Chamber formation	0.56	0.32	0.30	$23.5_{1,51}$	***	23.5
2	Chamber formation, hydrological activity	0.67	0.44	0.42	$11.5_{1,50}$	**	19.9
3	Chamber formation, hydrological activity, entrance orientation	0.71	0.47	0.47	$5.4_{1,49}$	*	16.2
4	Chamber formation, hydrological activity, entrance orientation, shelter	0.74	0.55	0.51	$5.2_{1,48}$	*	14.5

Table 3.4 Coefficients of the final regression models for mean and maximum bat activity.

	B	SE B	β
Mean bat activity			
Constant	0.53	0.56	
Chamber development	1.02	0.22	0.49
Hydrological activity	-0.75	0.22	-0.36
Entrance orientation	0.50	0.21	0.25
Maximum bat activity			
Constant	0.04	0.67	
Chamber development	0.95	0.22	0.44
Hydrological activity	-0.72	0.23	-0.33
Entrance orientation	0.56	0.21	0.26
Shelter at the entrance	0.52	0.23	0.24

Outliers were identified as any cases with a standardised residual outside the range of -2 to 2. Only one site (Langcliffe Pot, residual 2.5) fell outside this range. Diagnostic statistics were used to confirm that neither this site nor any other had an undue influence on the model (Barnett and Lewis 1978; Cook and Weisberg 1982; Stevens 1992; Field 2005).

There was no significant multicollinearity between cave characteristics in the models (Myers 1990; Menard 1995), the assumptions of linearity and homoscedasticity were met and the residuals in the model were normally distributed. Durbin-Watson tests (Durbin and Watson 1951) showed that the residual terms of any two observations were independent.

3.5 Discussion

Logging bat activity over three or more consecutive nights revealed considerable variation in activity from night to night. This is consistent with the findings of other swarming studies (e.g. Parsons *et al.* 2003a; b; Rivers *et al.* 2006) and is demonstrated by the discrepancy between mean nightly activity and maximum nightly activity (see Table 3.1). Despite this discrepancy, the measure of activity used had only a small effect on the single correlations and the regression model. Swarming activity was suppressed by low temperatures and high rainfall in a study in south east England (Parsons *et al.* 2003b) but the relationship between environmental variables and swarming activity is neither marked nor straight forward. Berkova and Zukal (2006) found no effect of temperature on activity during the swarming period. The time and energy spent finding a suitable hibernation site and securing matings must be balanced against the need to build/maintain fat reserves to survive the winter. Parsons *et al.* (2003b) suggest that when foraging conditions are good (warm, dry, still), bats are able to 'attain a positive energy budget rapidly', enabling them to make energetically expensive visits to swarming sites. Occasionally however, on nights like these, very low activity was observed in this study and so there may be occasions when bats take full advantage of optimal foraging conditions in preference to swarming. On marginal nights, faced with limited foraging success, bats may choose to swarm instead. Alternatively, bats can save energy by remaining torpid in the roost. Environmental conditions on the night is just one factor and the choice a bat makes will depend as much on past experience from previous nights and present physiological status.

Bat swarming activity was greatest in caves with extensive chamber development and low hydrological activity. Shelter at the entrance and entrance orientation may be important but to a lesser degree. Entrance size, altitude and location in the landscape did not appear to be important factors to swarming bats. In the following sections the functional significance of these results will be explored.

3.5.1 Factors related to hibernation

3.5.1.1 Microclimate

Microclimate (temperature, humidity and airflow) is perhaps the most important factor determining the distribution of bats both within and amongst underground sites (Twente 1955; Kallen 1964; Ransome 1968; Nagel and Nagel 1991; Thomas and Cloutier 1992). In general, hibernating bats require a cold, stable, humid environment in which to hibernate, but the precise requirements vary between species (Webb *et al.* 1996). Fluctuations in microclimate over the course of the winter can lead to an increase in arousal activity and 'internal migration', and the causes and consequences of arousals have been the subject of much study (e.g. Daan and Wichers 1968; Kuipers and Daan 1970; Ransome 1971; Daan 1973; Speakman and Racey 1989; Park *et al.* 2000). Arousals are energetically expensive and should be avoided if their sole purpose is to move to a more suitable location in response to changes in microclimate. However some species, e.g. *Rhinolophus ferrumequinum*, will feed in the winter when conditions are favourable, so it is important for these species to select sites where fluctuations in cave temperature convey information about the environment outside and hence foraging opportunities.

Although microclimate was not measured directly in this study, several cave characteristics that were assessed do influence temperature, humidity and airflow. The structure of an underground site, both at the entrance and internally, will affect air flow which in turn will affect temperature and humidity. In non-dynamic systems with no air flow, cave temperature approximates to mean annual temperature outside. In the case of the Yorkshire Dales this is 8 - 10 °C. Such sites are thermally stable but are often too warm for hibernation. In dynamic systems, convection currents arise from the temperature differential between the internal cave environment and the exterior. In winter, cold air is drawn into the cave at ground level, is warmed and flows out at roof level. This effect is reversed in the summer. This pattern is relatively simple in adits, tunnels and horizontal caves with one entrance, although the extent of the convection current will depend on the length of the system and the size of the passage. Sites that are structurally complex have equally complex patterns of air flow. Single vertical shafts and caves on an incline (the highest point of the cave being the entrance) can act as cold air sumps. High roofed chambers beyond the entrance can retain warm air pockets. Multiple entrances at different levels give rise to air currents that lower humidity.

Bat activity in this study was highest at the entrances to complex systems containing one or more substantial chambers. Tuttle and Stevenson (1977) suggest that structural complexity in large caves contributes to thermal complexity and stability. Larger caves (in terms of length and passage height) support greater numbers of hibernating bats e.g. *Pipistrellus subflavus* (Briggler and Prather 2002); several North American *Myotis* species (Raesley and Gates 1987); and *Eptesicus fuscus* (Raesley and Gates 1987). Large chambers will provide a wider range of temperatures allowing bats to select the most favourable microclimate without having to move between sites. They will also provide more crevices than sections of stream passage, allowing bats to find thermally stable pockets buffered from air currents.

The *Myotis* and *Plecotus* species using caves in the study area have been recorded torpid in hibernacula at temperatures between -3.0 °C and +10.4 °C (Webb *et al.* 1996, and references therein). On average, they are found at temperatures several degrees lower than the mean annual temperature (e.g. Daan and Wichers 1968), where the internal structure of the cave in relation to the position of the entrance results in areas that act as cold air sumps. There was a weak relationship between bat activity and the orientation (horizontal or vertical) of the entrance. Nine out of the ten sites recording the highest levels of activity (see Table 3.1) have vertical entrances and the relationship is weak possibly because a large number of sites with vertical entrances are less suitable hibernacula on the basis of other factors. Although Dow Cave (which was the eighth highest in terms of autumn activity) is classified as a horizontal entrance in caving terms, the entrance is on a downward incline (dropping about 15 m in the first 30 m), which demonstrates how our classification of entrance orientation is an over-simplification in terms of patterns of air flow.

Other factors that may influence patterns of airflow and internal temperature are the size of the entrance and the degree of shelter. Small entrances and a high degree of shelter will buffer the effects of external temperature fluctuations, particularly in entrance areas. Species that arouse relatively frequently and forage opportunistically during the winter (see Park *et al.* 2000) rely on cues from the external environment and may select sites with larger entrances (Raesley and Gates 1987). The species present in this study arouse relatively infrequently (Daan 1970; 1973) and no relationship between bat activity and entrance size was observed. There was a positive, weak (but significant) relationship between bat activity and the degree of shelter. Whilst a high degree of shelter may protect entrances from wind in these otherwise exposed upland areas, and serve to moderate airflow, its impact is probably greater in relation to swarming behaviour (see later).

Bat activity was negatively correlated with the level of hydrological activity. Sites with a tendency to undergo flooding events were considered unsuitable for hibernation and were not investigated. Relative humidity was high (90-100%) in all sites where it was measured (data not shown) including those with extensive fossil sections. This is typical of large limestone caves (Daan and Wichers 1968) and there is no reason to believe that there is a relationship between hydrological activity and relative humidity that will impact on bats. Similarly, bats that arouse in order to drink (see Kallen 1964; Speakman and Racey 1989; Thomas and Cloutier 1992) should be able to do so in all sites regardless of hydrological activity. Pools of standing water are available in all sites and beads of condensation often form on the cave walls and on the bats themselves (pers. obs.). Van Nieuwenhoven (1956) and Daan (1973) observed bats licking condensation from their fur during arousal episodes.

The impact of hydrological activity on hibernating bats could be brought about by the way it influences air flow, and subsequently temperature (Bögli 1980). Air is moved forward by flowing water as a result of friction. Through sections of phreatic passage, air movement occurs by means of suction at the start point and an increase in pressure at the end. The extent of air movement will depend on passage size and gradient, water volume and velocity, and overall structural complexity of the site. During the winter, water carried into the cave will have a cooling effect which could be seen as beneficial to bats seeking low cave temperatures. However, high volumes of water can interact with cave structure to create strong air currents. Such air movements may increase evaporative water loss, even in sites with high humidity (Kallen 1964), and initiate costly arousals (Tinkle and Patterson 1965).

3.5.1.2 Disturbance

Human disturbance, both deliberate and unintentional, can cause catastrophic declines in populations of cave-dwelling bats (Rabinowitz and Tuttle 1980; Weigel and Weigel 1998). Tuttle (1977) has attributed declines of up to 95% in some populations to human disturbance, although in reality other factors are likely to contribute. Hibernating populations and breeding colonies are most sensitive to the effects of disturbance (Sheffield *et al.* 1992; Johnson *et al.* 1998; Mann *et al.* 2002). Human activities such as caving and tourist visits can potentially expose bats to non-tactile stimuli e.g. torchlight, photographic flash, noise and raised temperatures in enclosed areas (Speakman *et al.* 1991). Thomas (1995) experimentally demonstrated an increase in arousal activity, at a hibernaculum of *Myotis lucifugus* and *Myotis septentrionalis*, in response to non-tactile human disturbance.

By and large, caves in the Yorkshire Dales are inaccessible to tourists as many require specialist equipment and knowledge of vertical techniques in order to gain access. However, the area is popular with cavers and only a handful of sites have locked barriers restricting access. A permit system operates in some areas (e.g. Easegill and Leck Fell) but caving traffic can be substantial at popular sites. Several caves with a low technical rating are used by outdoor pursuits groups and both group size and the frequency of visits can be considerable. Given the effects of disturbance, it is perhaps surprising that many of the key swarming sites identified in this study (e.g. Easegill, Bar Pot and Dow Cave) are amongst the most heavily visited by cavers. Bats may select extensive sites, with high roofed chambers and deep crevices that provide a refuge away from human induced stimuli.

Speakman *et al.* (1991) suggest that one source of natural non-tactile stimuli within hibernacula is water noise. This is consistent with the observation in this study that bat activity declined with increasing levels of hydrological activity. (It should be noted that the sound produced by water has ultrasonic components.) Many *Plecotus auritus* fold their ears beneath their wings during torpor and Coles *et al.* (1989) suggest that this reduces the bats' sensitivity to aural stimuli and thus prevents unnecessary arousal.

3.5.1.3 Winter foraging

If winter foraging is important to hibernating bats in this study, selection of low altitude sites and/or sites with a high degree of connectivity to potential foraging areas may have been expected. No such relationship was found. With the possible exception of *P. auritus* (see Hays *et al.* 1992), none of the species in this study are known to feed during arousal (see Daan 1973, and references therein). Species that forage on mild nights in winter e.g. *Rhinolophus ferrumequinum* show synchronicity with sunset in the timing of arousals and the maintenance of a 24 hour circadian rhythm (Ransome 1968; Park *et al.* 2000). Winter foraging appears to be less important in *Myotis* species (Daan 1970) and arousals appear random in their timing (Daan 1973). Thomas (1993) reported a lack of synchronicity with sunset and a free-running circadian rhythm in a hibernating population of *M. lucifugus*, *M. septentrionalis* and *Eptesicus fuscus*.

3.5.2 Factors related to swarming behaviour

During swarming, bats chase each other in, out, and around the entrances of underground sites (e.g. Fenton 1969; Rivers *et al.* 2006). Many chases involve two bats but can involve more (up to 10 bats, pers. obs.). These chases persist once inside and have been observed to lead to copulation (Thomas *et al.* 1979). Night scope observations at several key swarming sites demonstrated the ease with which bats chased each other in and out of extremely narrow vertical entrance pitches (pers. obs.). Neither individual bats, nor chases appear to be impeded by these entrances and there was no relationship between the level of swarming activity and entrance size in this study. However, the placement of grilles at the entrances to sites does influence swarming behaviour (Pugh and Altringham 2005; Spanjer and Fenton 2005) and this is discussed in Chapter 6.

The amount of chasing behaviour taking place outside a cave can be substantial and it is probable that the immediate area around the entrance acts as a 'swarming arena'. The entrances to several major swarming sites identified in this study (e.g. Bar Pot, Cherry Tree Hole and Rift Pot) are situated at the bottom of large shakeholes. These shakeholes, which are surrounded by exposed moorland, provide a sheltered area for social interactions to occur. There was a weak correlation between bat activity and the degree of shelter, although several sites with a high degree of exposure (e.g. Langcliffe Pot and Dowkabottom Cave) also showed high bat activity. Shelter at the entrance may not be so important if caves provide a substantial 'swarming arena' inside. Caves with large chambers may be selected, in part, for this reason and weather conditions could influence the relative amounts of chasing occurring inside and outside a cave. Attempts to investigate this with infra-red video recording proved unsuccessful due to the difficulties of illuminating large chambers.

The function of chases, and indeed the mating system in place during swarming are poorly understood. The large chambers (and complex cavities) present in caves with high activity could provide suitable locations for male display and copulations. In *Miniopterus minor*, McWilliam (1990) reported aggressive competition amongst males for access to a dome in the ceiling of a cave during the mating season. Many of these males showed fidelity to the dome both within and between years. Copulations were not observed but the males were older and heavier than those roosting elsewhere in the cave, perhaps reflecting their dominant status. In *Artibeus jamaicensis*, dominant males occupy solution cavities in the roofs of limestone caves and actively defend the harem that roosts with them (Ortega and Arita 1999; 2000). These solution cavities are not considered a limited resource in larger caves, which support more harem groups than smaller caves. In a population of *Macrotus californicus*, males defended locations within an abandoned mine, that were distinct from their usual roosting sites (Berry and Brown 1995). From these locations, males sought to attract females by wing flapping and vocalisations. Male *Rhinolophus ferrumequinum* also establish mating territories in underground sites which they may hold over successive years (Ransome 1991). Territorial males are visited by females from several different colonies (Rossiter *et al.* 2000a; b).

Swarming in relation to mating behaviour has been best studied in *Myotis lucifugus* (see Barclay and Thomas 1979; Barclay *et al.* 1979; Thomas *et al.* 1979). Swarming males were observed making looping flights around drill holes in the roof of a mine, occasionally landing. This behaviour attracted the attentions of other bats including females, although the number of flights performed had no bearing on the number of females attracted and no other self-advertisement displays (including vocalisations) were evident. 77% of copulations observed took place in these drill holes although males did not appear to defend these sites. Thomas *et al.* (1979) noted that copulations on exposed walls were often terminated through dislodgement of the female by other bats and suggest that the drill holes offer protection against such disruptions. Only three copulations were observed during the course of this study, but all took place in deep crevices. In the case of Cherry Tree Hole, mating took place in a crevice in the wall of the shakehole in which the entrance was situated.

3.5.3 Factors related to movement to caves

Quantitative analysis of the relative importance of different habitats to bats has concentrated predominantly on summer use and foraging (e.g. Walsh and Harris 1996a; b). *R. ferrumequinum* hibernate in sites close to summer roosts (Ransome 1991) and may select sites on the basis of winter foraging opportunities. Large scale analysis of habitat types surrounding caves was not undertaken in this study and was probably unwarranted: (1) because *Myotis* species do not tend to forage in winter; (2) because the distances bats are known to travel between summer habitats and swarming sites greatly exceed the distance between sites; (3) there is little variation between sites other than fine scale habitat features such as linear landscape elements. This approach is supported by Raesley and Gates (1987), who looked at cave structure and microclimate as well as surrounding habitat and found the latter to be unimportant.

Because it is probable that bats disperse from summer habitats in the lowlands to swarming sites in this upland area, altitude and connectivity via linear features were considered. Limpens and Kapteyn (1991) proposed that linear features (e.g. water courses, tree lines, hedgerows) provide shelter from wind and are used as landmarks for orientation (either by echolocation or vision). In this study there was no significant relationship between bat activity and either altitude or connectivity. Several major swarming sites (most notably Cherry Tree Hole, Dowkabottom Cave and Rift Pot) have no connecting linear features leading to them, which raises the question: how do bats locate these sites?

Bats use echolocation and vision in short-range navigation (Altringham and Fenton 2003) but the sensory cues that facilitate long-distance movements are poorly understood. Orientation with respect to post-sunset glow, celestial cues, magnetic fields and predictable patterns of atmospheric disturbance have all been put forward as possible mechanisms (Griffin 1970; Buchler and Childs 1982; Holland *et al.* 2006). Wilkinson and Fleming (1996) suggest that migrating *Leptonycteris curasoae* use topographical features such as the Pacific coastline and the Sierra Madre mountain range to navigate. Although dry valleys were included as a connecting feature in this study, it is possible that more subtle topographical features were overlooked.

The fact that bats are recaptured at sites over successive years suggests they maintain a form of spatial memory that enables them to relocate sites. This does not answer the question of how they locate these sites in the first place. Bats, particularly juveniles, may follow conspecifics to hibernacula, and there is some evidence from ringing that males arrive at swarming sites in coalitions that persist between years (N.M. Rivers, A.M. Glover, J. Furmankiewicz, R.K. Butlin and J.D. Altringham in prep.). The entrances to Link Pot and Cherry Tree Hole were only opened up by caving digs in the last 40 years and yet they support large swarming populations. Link Pot is in the same valley system as several other identified swarming sites and its discovery is likely to have been an incidental result of bats swarming close by. However, Cherry Tree Hole is one of the most isolated sites: high altitude, no connecting features and several kilometres from any riparian/woodland habitat. It seems improbable that bats would be present in the vicinity of this cave unless there is an element of active 'prospecting' for sites. Information transfer between conspecifics could then lead to the adoption of newly discovered sites.

In summary, swarming activity is best predicted by features of a cave itself rather than of the habitat surrounding it. The importance of these features can be explained by a combination of direct evidence and an understanding of the behavioural and physiological needs of the bats. The regression model can explain 45% of the variation in bat activity with just two parameters, the degree of chamber formation and the level of hydrological activity, with entrance orientation and shelter explaining a further 10%. This knowledge is being used in the search for as yet unidentified swarming sites in the Yorkshire Dales and beyond.

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Chapter 4: Species Recognition of Echolocation Calls using Time Domain Signal Coding and Artificial Neural Networks

4.1 Abstract

Time-expanded echolocation calls were recorded from four species of *Myotis* bat: *Myotis brandtii, M. daubentonii, M. mystacinus* and *M. nattereri*. Time Domain Signal Coding (TDSC) was used to characterise entire passes or single chirps. TDSC characterises a signal by the shape of its amplitude envelope. A number of algorithms were used to encode the calls and artificial neural networks (ANNs) were trained to recognise a subset of the encoded calls. A second subset of known calls was then used to test the ability of these networks to correctly assign the calls to species. Despite the preliminary nature of this study, the networks trained and tested using single chirps produced results comparable to those obtained by spectral analysis for two of the four species tested. TDSC has the advantage that is computationally undemanding, making it a potential starting point for an automated analysis system for real time bat call identification.

4.2 Introduction

4.2.1 Echolocation in bats

A simple definition of echolocation, provided by Altringham (1996), is 'the analysis by an animal of the echoes of its own emitted sound waves, by which it builds a sound-picture of its immediate environment'. Echolocation in bats (see Neuweiler 2000) and cetaceans (see Au 1993) involves the generation of ultrasonic sounds, that is those above the range of human hearing (typically >18 kHz). Other taxa, such as cave-dwelling birds (Fullard *et al.* 1993), use lower frequency sound. Ultrasonic sounds are also used in communication in several mammalian taxa including bats, cetaceans, rodents and some marsupials (Sales and Pye 1974).

A major disadvantage of echolocation is the restricted range over which it works. As sound travels away from a point source, it attenuates (decreases in amplitude) so that returning echoes are much weaker than the emitted sound. This occurs by two processes: geometric attenuation and atmospheric absorption (Lawrence and Simmons 1982) and partly explains the relationship between echolocation call design, wing morphology and foraging strategy (Norberg and Rayner 1987).

There are four basic properties of bat calls (Altringham 2003): (1) Frequency - bats use high frequency calls. In the majority of bats most of the energy is found between 20 and 100 kHz, but the full range extends from 12 to over 200 kHz). Although high frequency sounds are more rapidly attenuated (via atmospheric absorption) than those of lower frequency, they are important for target discrimination (Neuweiler 1998). This is because reflections from prey are strongest when the wavelength of the call does not exceed prey size; (2) Intensity - bats typically use high intensity calls (up to 130 dB at 10 cm). High intensity calls help to compensate for the effects of attenuation; (3) Duration - most bat calls are short (<5 ms to ~50 ms). Short calls are used by bats whose echolocation processing mechanism is intolerant of pulse/echo overlap (Neuweiler 1998). Species that use longer calls have auditory mechanisms that work with overlapping signals; (4) Structure - call structure is a complex continuum between species and can vary considerably from pulse to pulse in some species. However, it is convenient to classify call components as fundamentally either constant frequency (CF) or frequency modulated (FM). Most species have calls with both components so calls fall into categories variously labelled FM (narrow- and broadband), quasi-CF and CF-FM-CF (Figure 4.1).

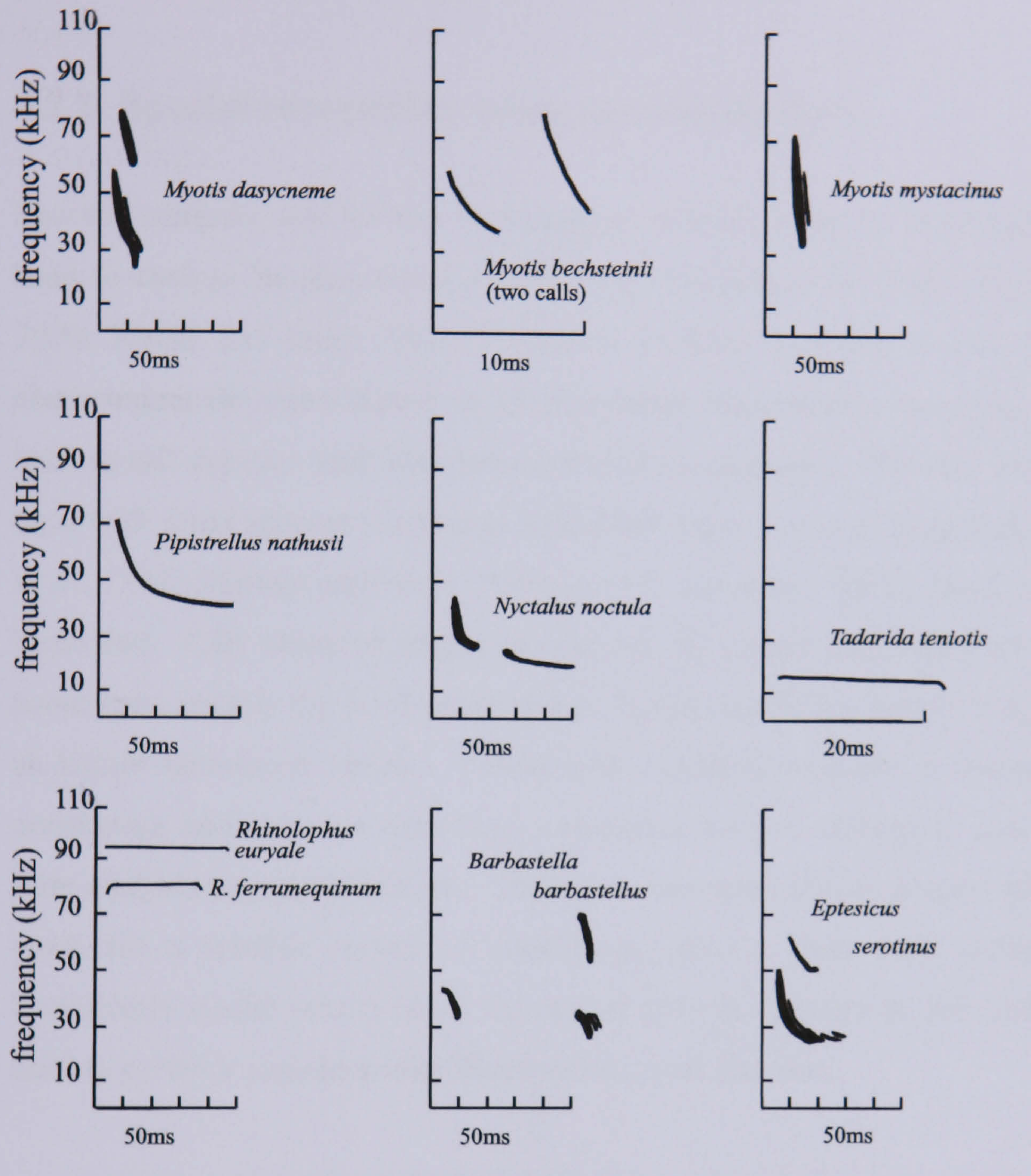

Figure 4.1 Echolocation calls from a range of species to show variation in call structure. From Altringham (1996).

Differences in the complexity and flexibility of echolocation calls between species have been used in the past to construct cladograms intended to reflect bat phylogeny (Simmons and Stein 1980). Recent molecular studies (e.g. Teeling *et al*. 2000; 2002) have proposed major revisions to bat systematics and in light of this, the evolution of echolocation in bats has also been re-evaluated (Jones and Teeling 2006). Examples of convergent evolution of acoustic features and adaptive radiation in echolocation call design are highlighted in this review and demonstrate how 'perceptual challenges imposed by the environment can often override phylogenetic constraints' (Jones and Teeling 2006). Because call structure is highly functional and crosses taxonomic boundaries, identification of bats by their calls is fraught with difficulties, made worse by the considerable call plasticity seen in many species.

4.2.2 Species recognition using spectral analysis

Spectral analysis, also known as frequency domain analysis, is the method currently used to analyse bat echolocation calls (e.g. Vaughan *et al.* 1997; Parsons and Jones 2000; Russo and Jones 2002). Spectral analysis uses fast fourier transforms and characterises the signal based on its component frequencies. Temporal characteristics such as call duration and inter-pulse interval are also used. Spectral analysis has been used with some success to identify individual species or species groups (e.g. Vaughan *et al.* 1997; Parsons and Jones 2000; Russo and Jones 2002), but it is far from the ideal tool. Call variation within species can be greater than that between species in some bats, notably the widespread genus *Myotis*, and it is possible to misidentify bats at higher taxonomic levels. Furthermore, spectral analysis is complex and time-consuming and requires significant computing power, making it unsuitable for real time analysis and identification. There is a real need in both conservation and survey work for a reliable means of identifying species from their echolocation calls. Particularly useful would be an automated system that can be left unattended in the field to perform real-time identification and store the data.

4.2.3 Time Domain Signal Coding

Time domain signal coding (TDSC), as a bioacoustic species identification tool, has been successfully applied to 13 species of British Orthoptera and 10 species of woodland bird in Japan (Chesmore 2001). It is a technique derived from time encoded speech (TES) which was developed for the compression of speech for digital transmission (King and Gosling 1978). Unlike spectral analysis, TDSC is a purely time domain approach and characterises a signal by the shape of its amplitude envelope. The signal is divided into epochs (segments between zero-crossings in amplitude). Each epoch is described by its duration (D) in samples and its shape (S) which is determined by the number of positive minima or negative maxima (see

Figure 4.2). This can give rise to a large number of different D-S pairs which are then mapped non-linearly onto a smaller set of representative codes (typically 25 - 30). The resulting codebook is specific to the group of sounds (i.e. taxa) under investigation and must be generated by manual examination of the distribution of D-S pairs.

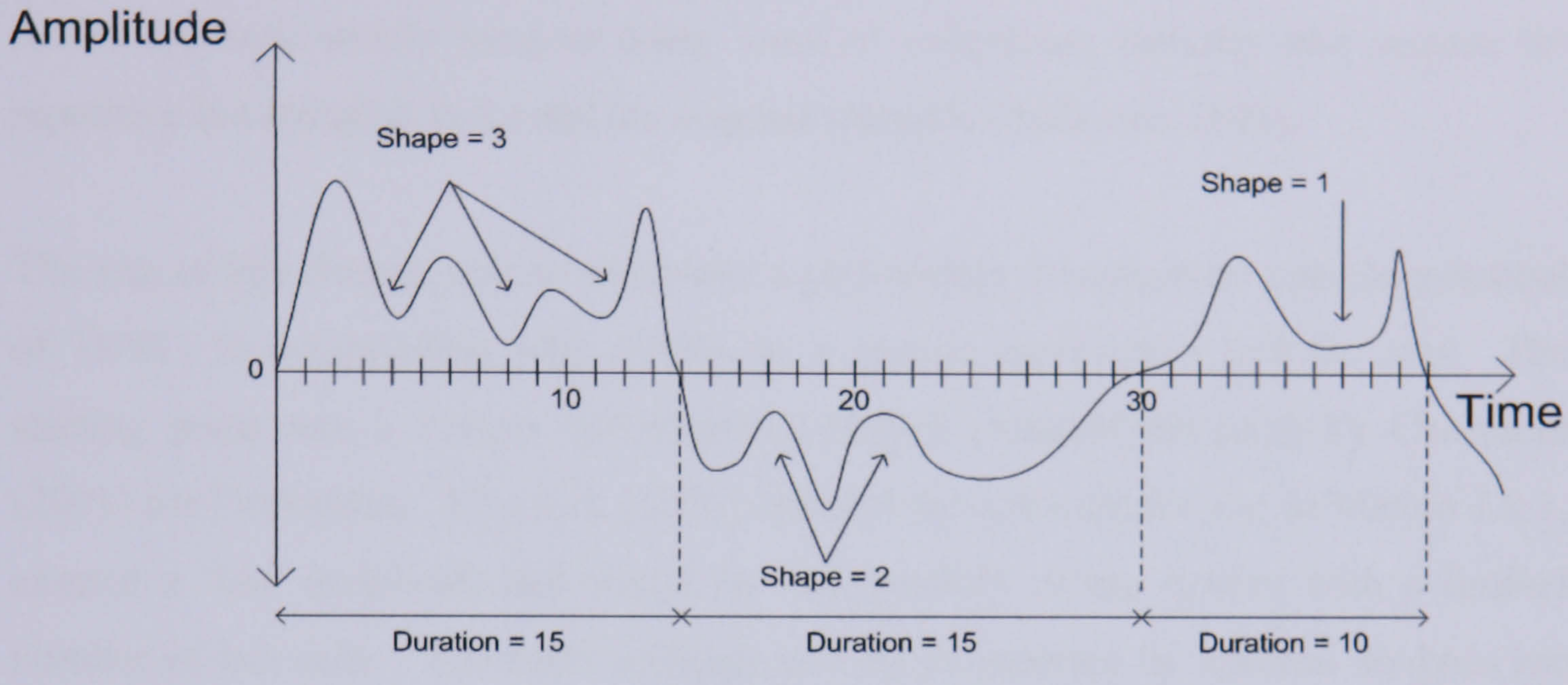

Figure 4.2 TDSC waveform characterisation. Waveform with three epochs: the first has duration D = 15 and shape S = 3, the second and third are D15,S2 and D10,S1. From Haydock (2005).

Calls may be analysed using one of several encoding techniques: (1) S-matrix: a 1-dimensional histogram of the frequency of occurrence of codes; (2) A-matrix: a 2-dimensional histogram describing the occurrence of pairs of codes occurring in succession; (3) Energy S/A matrix: incorporates information on signal amplitude using matrix scaling (Swarbrick 2001).

There are two reasons for wishing to apply TDSC to bat calls: (1) Given the limitations of spectral analysis, TDSC offers a completely different analytical approach; (2) TDSC is computationally easy, and if successful it could be implemented in small, automated systems for use in the field, particularly in conjunction with neural networks.

4.2.4 Artificial Neural Networks

An artificial neural network (ANN) is a computer program that is designed to work in a fashion analogous to neuronal networks in the brain. The network learns to perform tasks from a set of training patterns, which alter the relative strengths of the interconnections between nodes in the network. In a sufficiently complex network, each training pattern will lead to a unique set of interconnections, known as weights. ANNs are now widely used in many areas of commerce, industry and science for repetitive but complex tasks and for original research (Anderson 1995).

The aim of this chapter was to undertake a preliminary investigation into the potential of TDSC, in combination with ANNs, as a species recognition tool for bats. The starting point was a system developed in Matlab (MathWorks.com) by Chesmore (2001) for Orthoptera. Haydock (2005) updated the software for use in Matlab 7.0.1, created a 'bat' codebook and tested the functionality of the system with a limited number of bat calls. The most difficult species to separate by spectral analysis are those in the genus *Myotis* (e.g. Parsons and Jones 2000). Since we were catching four species of *Myotis* at swarming sites in large numbers, a library of calls from known individuals could be obtained. These species would be a robust test of the capabilities of TDSC and if successful, we would have the beginnings of a valuable logging device for monitoring swarming bats.

The primary questions to be addressed in this chapter are:

- Can both individual chirps and bat passes be utilised in ANN training and subsequent species identification?
- Which of the available encoding techniques works best in training the ANN and in subsequently discriminating calls?
- What is the discriminatory power of this technique and how does this compare with studies using conventional spectral analysis?

4.3 Methods

4.3.1 Collection and preparation of echolocation recordings

Echolocation calls from bats caught at swarming sites in 2005 were recorded at the point of release. Recordings (3 s duration, 10x time expanded) were made using a Pettersson D-980 time expansion ultrasound detector (Pettersson, BatSound.com) and a Sony Walkman minidisc recorder. Recordings were downloaded into BatSound Pro v3.32 (Pettersson, BatSound.com) and saved as WAV files. To convert the BatSound WAV files into a format that was compatible with the encoding and classification program, the files were converted to a 'PCM signed 16 bit, mono' format in Goldwave v5.12 (Goldwave.com). For each bat, a file of the central 5-15 chirps in a pass was saved. More than half of these contained 10 chirps or more. In addition, each of these chirps was saved as a separate file. In selecting each chirp, the aim was to remove any echo without truncating the call.

For each species, two subsets of good quality (high amplitude and signal:noise ratio) passes/chirps were selected: one subset for training the artificial neural network, and one subset for testing it, from different individuals. The subset for training consisted of two passes (one pass from each of two individuals) and six chirps (three chirps from each of the same two individuals). The subset for testing consisted of 10 passes (one pass from each of 10 individuals) and 100 chirps (10 chirps from each of the same 10 individuals).

Subsequent encoding and classification of calls were carried out in Matlab v7.0.1 release 14 (The MathWorks.com).

4.3.2 Encoding the calls by time domain signal coding (TDSC)

All calls (passes and chirps) were encoded systematically in batches using each of the coding techniques available (Table 4.1). The general codebook was designed to include the shapes commonly encountered in animal sounds and was used in previous work (e.g. Chesmore 2001). Since this may not include all those found in the bat chirps, Haydock (2005) created a bat codebook after examining passes from a sample of *Myotis* bats. A Matlab program identified and counted Duration-Shape pairs and placed them in a matrix which was used to manually create a bat codebook (Table 4.2).

Table 4.1 Encoding techniques. The terms are explained in section 4.2.3.

Codebook	Algorithm	Matrix	Matrix scaling
General	Original	S-matrix	Basic
General	Original	S-matrix	Energy
General	Original	A-matrix	Energy
General	New	S-matrix	Basic
General	New	S-matrix	Energy
General	New	A-matrix	Basic
General	New	A-matrix	Energy
Bat	Original	S-matrix	Basic
Bat	Original	S-matrix	Energy
Bat	Original	A-matrix	Energy
Bat	New	S-matrix	Basic
Bat	New	S-matrix	Energy
Bat	New	A-matrix	Basic
Bat	New	A-matrix	Energy

Haydock (2005) also made minor changes to the original encoding algorithms, to increase consistency, hence the use of both original and new algorithms (Table 4.1). The basic A-matrix was not available using the original algorithm for either codebook

Table 4.2 The bat codebook of 121 shapes. D = duration, S = shape. The terms are explained in section 4.2.3.

	S0	S1	S2	S3	S4	S5
D1	C1					
D2	C2					
D3	C3					
D4	C4					
D5	C5	C21				
D6	C6	C22				
D7	C7	C23				
D8	C8	C24				
D9	C9	C25				
D10	C10	C26				
D11	C11	C27	C44			
D12	C12	C28	C45			
D13	C13	C29	C46			
D14	C14	C30	C47			
D15	C15	C31	C48			
D16	C16	C32	C49			
D17	C17	C33	C50	C67		
D18	C18	C34	C51	C68		
D19	C19	C35	C52	C69		
D20	C20	C36	C53	C70		
D21		C37	C54	C71		
D22		C38	C55	C72		
D23		C39	C56	C73		
D24		C40	C57	C74	C89	
D25		C41	C58	C75	C90	
D26		C42	C59	C76	C91	
D27		C43	C60	C77	C92	
D28			C61	C78	C93	
D29			C62	C79	C94	C108
D30			C63	C80	C95	C109
D31			C64	C81	C96	C110
D32			C65	C82	C97	C111
D33			C66	C83	C98	C112
D34				C84	C99	C113
D35				C85	C100	C114
D36				C86	C101	C115
D37				C87	C102	C116
D38				C88	C103	C117
D39					C104	C118
D40					C105	C119
D41					C106	C120
D42					C107	C121

4.3.3 Training the artificial neural network (ANN)

The files of encoded 'training' calls were concatenated into a series of multiple training files (two training files for each encoding technique: one containing passes, the other containing chirps). Each training file was used to train a network where the goal for convergence (root mean squared (RMS) error of observed v. expected outputs) was 10^{-6} after a maximum of 2000 epochs. The networks were three-layered, i.e. they had a single hidden layer and used a back-propagation algorithm with momentum. Further details can be found in Chesmore (2001). In the first instance 20 hidden nodes were selected, increasing to 30 nodes and then 40 until convergence occurred. If there was still no convergence at 40 nodes, the training regime was abandoned. Only networks with convergence were saved for subsequent testing.

4.3.4 Classifying calls and testing the ANN

Each neural network was tested for its reliability in discriminating calls using encoded testing files (passes or chirps as appropriate to each individual network). For this, the encoding techniques used in both the training of the network and in the testing had to be identical.

4.4 Results

Table 4.3 summarises the call parameters for the four species determined by spectral analysis, using BatSound Pro v3.32, (1024 point FFTs with a Hamming window). These values are very similar to those reported in the literature (e.g. Parsons and Jones 2000). F_{START} is the highest frequency, at the beginning of the call. F_{END} is the lowest frequency, recorded at the end of the call. F_{MAXE} is the frequency containing the maximum energy and was determined from a power spectrum. Also given are call duration and the time interval between calls.

Table 4.3 Summary of call parameters determined from spectral analysis.). Mb = *Myotis brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*.

	F_{START}	F_{END}	F_{MAXE}	**Duration**	**Inter-pulse interval**
	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)	Mean ± s.d. (range)
Mb n = 25	99.0 ± 9.9 (84-119)	31.8 ± 3.9 (26-40)	54.8 ± 4.9 (45-65)	4.5 ± 0.5 (3.7-5.3)	76.5 ± 9.3 (59.7-91.6)
Md n = 25	88.7 ± 6.6 (74-99)	31.3 ± 2.1 (28-34)	53.2 ± 3.2 (48-58)	4.8 ± 1.1 (3.4-6.7)	73.6 ± 10.5 (58.5-97.5)
Mm n = 25	99.4 ± 17.4 (76-124)	29.4 ± 1.7 (27-33)	54.0 ± 8.2 (46-72)	4.6 ± 0.6 (3.8-5.7)	73.5 ± 10.9 (47.6-91.5)
Mn n = 25	115.9 ± 17.6 (78-142)	20.5 ± 2.5 (15-29)	57.0 ± 10.1 (44-81)	4.9 ± 1.4 (2.8-8.1)	67.6 ± 15.8 (42.1-95.5)

Table 4.4 summarises the results of training using the various permutations of codebook, algorithm, matrix and training files used. Several patterns emerged: firstly, none of the training regimes using S-matrices or energy S-matrices led to convergence. The bat codebook performed better than the general codebook. Training regimes using both general and bat codebooks converged with chirps from a single individual (both A- and energy A-matrices), but when a second individual was introduced, only the use of the bat codebook led to convergence. This was true only for the new algorithm, regimes using the old algorithm converged in only one case. Interestingly, the only case in which using passes led to convergence was in a regime using the old algorithm.

Table 4.4 The results of training on all permutations of codebook, algorithm, matrix and training files used

Matrix	Codebook	Algorithm	ANN on 2 passes (2 individuals)	ANN on 6 chirps (1 individual)	ANN on 6 chirps (2 individuals, 3 chirps from each)
S-matrix	General	New	No convergence	No convergence	No convergence
A-matrix	General	New	No convergence	Converged: 20 nodes (399 epochs)	No convergence
Energy S-matrix	General	New	No convergence	No convergence	No convergence
Energy A-matrix	General	New	No convergence	Converged: 20 nodes (664 epochs)	No convergence
S-matrix	General	Old	No convergence	No convergence	No convergence
Energy S-matrix	General	Old	No convergence	No convergence	No convergence
Energy A-matrix	General	Old	No convergence	No convergence	No convergence
S-matrix	Bat	New	No convergence	No convergence	No convergence
A-matrix	Bat	New	No convergence	Converged: 20 nodes (398 epochs)	Converged: 30 nodes (1746 epochs)
Energy S-matrix	Bat	New	No convergence	No convergence	No convergence
Energy A-matrix	Bat	New	No convergence	Converged: 20 nodes (991 epochs)	Converged: 30 nodes (471 epochs)
S-matrix	Bat	Old	No convergence	No convergence	No convergence
Energy S-matrix	Bat	Old	No convergence	No convergence	No convergence
Energy A-matrix	Bat	Old	Converged: 20 nodes (601 epochs)	No convergence	No convergence

Following training, three networks were therefore considered suitable for testing:

- The network trained on energy A-matrices using the bat codebook, the old algorithm and bat passes.
- The network trained on energy A-matrices using the bat codebook, the new algorithm and single chirps from two individuals.
- The network trained on A-matrices using the bat codebook, the new algorithm and single chirps from two individuals.

The results from these three networks are shown in Tables 4.5 - 4.7. The networks provide not the probability of correct identification but the relative confidence of recognition for each species. The criterion for an identification (whether correct or incorrect) was that this confidence exceeded 0.9 for only one species. Any passes or chirps that did not meet this criterion were left unclassified. Reduction in this threshold led to more calls being classified but an increase in misclassifications (data not shown). However, the rate of misclassification did not increase rapidly.

The results from training and testing on passes were not encouraging (Table 4.5). A large proportion of the passes remained unclassified and classification of the remaining calls was poor. Classification was better using single chirps, and the A-matrices (Table 4.6) gave better results than the energy A-matrices (Table 4.7): more calls were classified and a greater percentage of these were classified correctly. The highest correct classification rates were obtained for *M. daubentonii* (81%) and *M. nattereri* (69%).

Table 4.5 Classification of bat passes using the network trained on passes (Energy A-matrices, bat codebook, old algorithm). Mb = *Myotis brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*.

		True species:			
		Mb	**Md**	**Mm**	**Mn**
Classification:	**Mb**	2	1	3	0
	Md	0	3	1	0
	Mm	2	2	2	1
	Mn	0	0	0	3
	Unclassified	6	4	4	6

Table 4.6 Classification of bat chirps using the network trained on chirps (A-matrices, bat codebook, new algorithm). Mb = *Myotis brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*. Data are numbers/percentage of all chirps classified correctly (n = 100 chirps per species) and in parentheses the percent of classified calls classified correctly.

		True species:			
		Mb	**Md**	**Mm**	**Mn**
Classification:	**Mb**	19 (35)	7 (9)	33 (53)	2 (3)
	Md	16 (30)	64 (81)	18 (29)	10 (16)
	Mm	18 (33)	8 (10)	6 (10)	8 (12)
	Mn	1 (2)	0 (0)	5 (8)	44 (69)
	Unclassified	46	21	38	36

Table 4.7 Classification of bat chirps using the network trained on chirps (Energy A-matrices, bat codebook, new algorithm. Mb = *Myotis brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*. Data are numbers/percentage of all chirps classified correctly (n = 100 chirps per species) and in parentheses the percent of classified calls classified correctly.

		True species:			
		Mb	**Md**	**Mm**	**Mn**
Classification:	**Mb**	19 (46)	23 (40)	18 (40)	3 (6)
	Md	4 (10)	28 (50)	6 (14)	2 (4)
	Mm	14 (34)	3 (5)	6 (14)	16 (30)
	Mn	4 (10)	3 (5)	14 (32)	32 (60)
	Unclassified	59	43	56	47

4.5 Discussion

The spectral characteristics of the calls were within the range published for the species (Parsons and Jones 2000) and show that there is considerable overlap in most parameters. Only *M. nattereri* shows any degree of differentiation, with a lower F_{END}, a character that makes it the species most reliably identified using spectral analysis.

The essential first step in the use of ANNs is to successfully train the networks. It is perhaps not surprising that none of the training attempted using S-matrices or energy S-matrices led to convergence, since they contain only limited information relative to the A-matrices. It was also expected that the bat codebook would perform better than the general codebook, as observed. Since it contains 121 codes specifically based on bat echolocation calls, compared to the 28 codes of the general codebook, it will be better able to describe bat calls in this abbreviated form. Finally, the new algorithm performed better than the old, since it had been modified to improve its ability to identify minima and maxima (Haydock 2005). Although many of the networks did not converge, those that did were those that logic suggests would be most likely to perform best. Convergence rates could have been improved, perhaps by making the convergence goal less stringent or increasing the number of epochs allowed. However an examination of the plots of RMS error during training suggested this might not be the case and that more fundamental changes to the networks may be necessary.

It is difficult to assess the true performance of the networks trained on passes due to the low sample size, but the high misclassification rates were nevertheless discouraging. The inclusion of the long inter-pulse intervals with their associated background noise and echoes is probably a major contributory factor.

The better performance of A-matrices over energy A-matrices was not expected, since the latter should provide a more detailed characterisation of the chirps. The poorer performance of the energy A-matrices may be due to unpredictable changes in amplitude in the chirps: due to interference, head turning by the bat, or sound emission itself. The low classification rates in both cases may be improved by training with more files than were available in this study.

The best classification rates obtained in this study were for *M. daubentonii* (81%) and *M. nattereri* (69%). They compare favourably with those obtained by spectral analysis and subsequent discriminant function analysis (54% and 96% respectively) or neural network analysis (75% and 90%), by Parsons and Jones (2000). TDSC performed better in the case of *M. daubentonii* but was worse at identifying *M. nattereri*.

TDSC was more successful with Orthoptera and bird calls (Chesmore 2001) than with bats. This is perhaps due to their function in communication, for which species specificity is clearly desirable. The function of echolocation calls is essentially the same in all species, so convergence rather than divergence is expected, making species identification difficult.

Despite the limited scope of this study, the results suggest that TDSC merits further investigation. Training with more files, experimentation with parameters in the existing networks, and the design of new networks may all improve performance. Whether it will be possible to get satisfactory results for *M. brandtii* and *M. mystacinus* remains to be seen.

It is very probable that TDSC will be able to identify many other species with considerable accuracy, given the results obtained from spectral analysis. Even if it proves to be no better than spectral analysis, the prospect of real time identification and automated loggers based on TDSC makes the research worth pursuing.

4.6 References

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Chapter 5: Genetic Diversity and Differentiation in Swarming Populations of *Myotis nattereri*

5.1 Abstract

The caves of the Yorkshire Dales National Park are potentially an important assemblage of underground bat sites, but prior to this study nothing was known about the ecology of cave use. Between 2002 and 2005, 53 cave entrances were logged for swarming activity using automated echolocation call loggers. Logging was followed up by trapping at caves with significant autumn bat activity. All bats caught were ringed, identified to species and sexed. 3 mm diameter wing biopsies were taken for genetic analysis.

Genetic diversity of *Myotis nattereri*, based on an analysis of six microsatellite loci, was high in swarming populations suggesting high gene flow and large effective populations. Most of the genetic variation was explained by variation among individuals within populations, but some was due to differences between swarming areas and by differences between populations within swarming areas. Isolation by distance was found between sites across six swarming areas between Cumbria and Sussex, but not between sites in the Yorkshire Dales alone. The differentiation observed was more logically explained on the basis of local geography and probable catchment areas.

5.2 Introduction

The population structure of a species can be defined as 'the spatial variation in density and genetic composition of it individuals' (Hewitt and Butlin 1997). Population structure is determined by a combination of past events (e.g. range expansion and contraction in response to climatic or environmental fluctuations) and present processes (e.g. dispersal patterns and mating strategies).

Two important components of population structure are genetic diversity within populations and differentiation between populations. Genetic diversity is important for adaptation to environmental change, and loss of diversity may result in reduced reproductive fitness and offspring survival (Rossiter *et al.* 2001; Frankham *et al.* 2002). Genetic diversity is typically high in large populations of outbreeding animals but may be reduced in small, fragmented populations. Colonisation events and habitat fragmentation can lead to small populations and increase the chance of genetic drift (random changes in allele frequencies) via founder effects and genetic bottlenecks (Lowe *et al.* 2004).

Gene flow is defined as 'the proportion of newly immigrant genes moving into a given population' (Endler 1977) and will reduce the effects of genetic drift. Patterns of gene flow will influence the degree to which populations are differentiated. Gene flow is determined by intrinsic factors such as philopatry, dispersal patterns and mating systems (e.g. McCracken and Gassel 1997; Burland *et al.* 1999; 2001); and extrinsic factors such as environmental discontinuities and physical barriers (e.g. Castella *et al.* 2000; Miller-Butterworth *et al.* 2003).

Molecular genetic techniques are increasingly being adopted in evolutionary and ecological studies of bats (see Burland and Worthington Wilmer 2001 for a review). They range from studies of phylogeny (Teeling *et al.* 2002; 2005), the evolution of echolocation and flight (Teeling *et al.* 2000; Springer *et al.* 2001; Jones and Teeling 2006) and cryptic diversity (Barrett *et al.* 1997; Mayer and von Helversen 2001; von Helversen 2001; Kiefer *et al.* 2002) to studies of colonisation events (Ruedi and Castella 2003; Salgueiro *et al.* 2004) and barriers to gene flow (Castella *et al.* 2000).

A combination of direct methods (field studies) and indirect methods (molecular techniques) is increasing our understanding of bat ecology including patterns of philopatry, dispersal and migration (e.g. Wilkinson and Fleming 1996; Burland *et al.* 2001; Miller-Butterworth *et al.* 2003); mating systems (Rossiter *et al.* 2001; 2005; Heckel and von Helversen 2002; 2003; Vonhof *et al.* 2006); and coloniality and kin selection (Kerth *et al.* 2000; 2002b).

Autumn swarming (reviewed in Chapter 1) describes the aggregation of bats at the entrances to underground sites in the months prior to hibernation. This behaviour has been described for a large number of temperate species in North America and Europe and is particularly prevalent amongst *Myotis* species (e.g. Fenton 1969; Thomas *et al.* 1979; Parsons *et al.* 2003; Rivers *et al.* 2006). In common with other temperate bats, swarming species mate during the autumn (and to some extent through the hibernation period) and use reproductive delays involving sperm storage by both sexes, and delayed fertilisation (Racey and Entwistle 2000). It has been hypothesised that swarming serves two main functions: a) mating and b) information transfer regarding the location of hibernacula (Davis and Hitchcock 1965; Fenton 1969; Humphrey and Cope 1976; Thomas *et al.* 1979; Kerth *et al.* 2003; Kerth and Morf 2004; Veith *et al.* 2004; Rivers *et al.* 2005). Ringing studies (Rivers *et al.* 2006) have revealed that swarming populations are comprised of bats from many summer colonies from large catchment areas. This is consistent with both mating and information transfer hypotheses and these functions are not mutually exclusive.

Genetic studies using maternally inherited markers from mitochondrial DNA (Petit and Mayer 2000; Kerth *et al.* 2003; Veith *et al.* 2004) provide further evidence that swarming/hibernation sites support bats from several colonies. Analyses of paternity in several swarming species (e.g. Burland *et al.* 2001; Kerth and Morf 2004; Senior *et al.* 2005) suggest that mating occurs outside the breeding habitat. The genetic structure of bats from a swarming site and surrounding summer colonies is consistent with the hypothesis that most of the mating occurs at the swarming site (Rivers *et al.* 2005).

The aim of this chapter is to investigate genetic diversity and differentiation in swarming populations of *Myotis nattereri* on different spatial scales: within the cave-rich area of the Yorkshire Dales and between the Dales and other swarming areas in England. Microsatellites (codominant markers consisting of short tandem repeats of two to four nucleotides, embedded in non-coding regions of DNA) were used in this study. Microsatellites are suitable genetic markers for population studies as they are abundant, highly polymorphic and are both easy and reliable to score (Bruford and Wayne 1993; Luickart and England 1999).

The primary questions to be addressed are:

- Do swarming sites show high genetic diversity and low levels of structure consistent with high levels of gene flow and a large effective population size?
- Is the level of genetic diversity at swarming sites related to the size of the population and catchment area inferred from bat activity levels?
- Is there differentiation between geographically distinct swarming areas and do genetic distances between the populations of these areas reflect the degree of geographical separation?
- Do swarming sites within the Yorkshire Dales support small, local and differentiated populations or do the bats make up one panmictic population? In other words, are the Dales a single swarming population or many?
- What can be inferred about the catchment areas of Dales swarming sites and autumn dispersal patterns from the observed population structure?

5.3 Methods

5.3.1 Study sites

Bats (*M. nattereri*) were caught and sampled at nine sites across the Yorkshire Dales (Table 5.1, Figure 5.1). Six of these sites (Easegill, Yordas Cave, Bar Pot, Cherry Tree Hole, Dow Cave and Manchester Hole) are natural, limestone caves of varying length and complexity. Buckden Gavel Mine is a disused lead mine with a complex network of adits and shafts. Troller's Gill is an area of disused lead mines with entrances (including Gill Head Mine) in close proximity to a natural cave, Hell Hole. Recapture data shows that bats frequently move between the sites in Troller's Gill, both within and between nights, therefore they are treated as one site in the analysis. The Craven Lime Works complex incorporates several disused 19th century lime kilns: the large Hoffmann continuous kiln, and three smaller draw kilns.

Table 5.1 Number of bats sampled and successfully genotyped at sites within the Yorkshire Dales

Site	**Grid reference**	**n**
Easegill (4 entrances to the same system):		40
Link Pot	SD 668803	
Mistral Entrance	SD 666803	
County Pot	SD 675806	
Wretched Rabbit	SD 675806	
Yordas Cave	SD 705801	13
Bar Pot	SD 752724	49
Cherry Tree Hole	SD 882704	41
Dow Cave	SD 984743	57
Manchester Hole	SD 100764	31
Buckden Gavel Mine	SD 955782	23
Trollers Gill:		30
Hell Hole	SE 066623	
Gill Head Mine	SE 066621	
Craven Lime Works complex	SD 823663	26

Figure 5.1 Sites sampled in the Yorkshire Dales (figures in parentheses are the number of bats sampled and successfully genotyped). n.b. Manchester Hole and Easegill fall outside the boundaries of the National Park.

In addition, bats were caught and sampled at several sites outside the Yorkshire Dales to allow comparison with other 'swarming areas' (Table 5.2, Figure 5.2).

Table 5.2 Number of bats sampled and successfully genotyped at underground sites in five areas outside the Yorkshire Dales.

Site	Grid reference	n
Ryedale Windy Pits:		
Slip Gill	SE 575835	196
Buckland's	SE 587828	92
Antoft's	SE 582829	136
Ashberry	SE 570850	12
Roudsea Wood Cave	SD 332825	21
Withcall Tunnel	TF 262820	20
Little Blakenham Pit	TM 108492	43
West Sussex:		15
Cocking Tunnel	SU 875170	
Drover's Tunnel	SU 872148	

- The Ryedale Windy Pits, in the North York Moors National Park, are an isolated cluster of natural caves in Corallian Jurassic limestone. Unlike the solution caves of the Dales, the Windy Pits (Slip Gill, Bucklands, Antofts and Ashberry) are formed by movements of the bedrock along faults. The role of these caves, as swarming sites, has been extensively studied since 1997 (Rivers *et al.* 2005; 2006). They are SSSI's on geological, biological and archaeological grounds.
- Roudsea Wood Cave is a low lying, fossil phreatic system situated within Roudsea Wood National Nature Reserve, Cumbria.
- Withcall Tunnel is a disused, brick-built railway tunnel in North Lincolnshire. It is a designated SSSI owing to the large number of bats that hibernate there. Winter counts range from 125 – 250 bats.
- Little Blakenham Pit in Suffolk is a small chalk pit with a 127 m tunnel at the north end. The entrance to the tunnel is gated to prevent disturbance of a wintering bat population (mainly *M. nattereri* and *M. daubentonii*). The site is a SSSI.
- Cocking and Drover's in West Sussex are disused railway tunnels approximately 3km apart (treated as one site in the analysis).

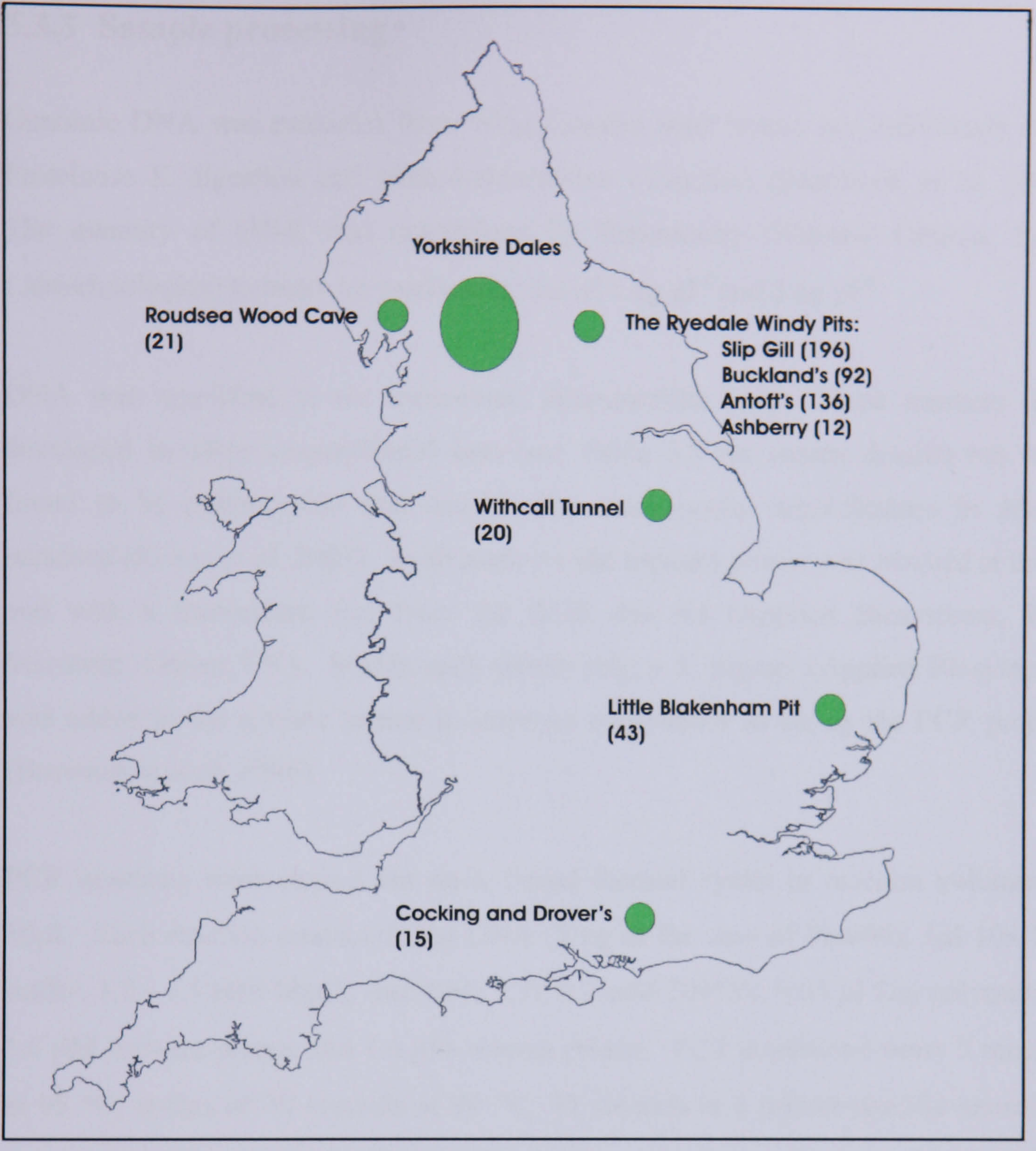

Figure 5.2 Sites sampled outside the Yorkshire Dales (figures in parentheses are the number of bats sampled and successfully genotyped).

5.3.2 Sample collection

Bats were caught at the entrances to underground sites between August and November 2000-2005 (detailed in Chapter 2). A biopsy sample (3mm diameter) was taken from each wing membrane according to Worthington Wilmer and Barratt (1996). Biopsy samples were stored at room temperature in 70-100% ethanol. All catching and processing were carried out under licences from English Nature and the Home Office.

5.3.3 Sample processing

Genomic DNA was extracted from wing biopsies (one biopsy per individual) using Proteinase K digestion and phenol-chloroform extraction (Sambrook *et al.* 1989). The quantity of DNA was determined by fluorometry (Fluostar Optima, BMG Labtechnologies) to make up working stocks of 1 ng μl^{-1} and 5 ng μl^{-1}.

DNA was amplified at six autosomal, microsatellite loci. These markers were developed in other vespertilionid bats (see Table 5.3 for source details) but were found to be polymorphic and suitable for cross-species amplification in *Myotis nattereri* (Rivers *et al.* 2005). In all markers, the forward primer was labelled at the 5' end with a fluorescent dye from the ROX dye set (Applied Biosystems, Web Scientific, Crewe, UK). Within each primer pair, a 5' pig-tail (Applied Biosystems) was added to the reverse primer to improve consistency in sizing the PCR product (Brownstein *et al.* 1996).

PCR reactions were carried out on a Tetrad thermal cycler in reaction volumes of 10μl. Each reaction contained 1ng DNA (5 ng in the case of Paur06), 1μl 10x *Taq* buffer, 1.0 - 1.5 mM $MgCl_2$ (see table 5.3), 0.2 mM DNTPs, 0.05 μl *Taq* polymerase, 1.0 μM forward primer and 1.0 μM reverse primer. PCR conditions were: 3 minutes at 95 °C; cycles of 30 seconds at 94 °C, 30 seconds at a primer-specific annealing temperature, 30 seconds at 72 °C (45 seconds at 72 °C in the case of Paur06); 10 minutes at 72 °C. Touchdown PCR reactions (Don *et al.* 1991) over a primer-specific temperature range (minus 1 °C per cycle) were proceeded by 35 cycles at the final temperature. The touchdown temperature ranges for each primer are given in Table 5.3.

PCR products were run on an ABI3730 48-well capillary sequencer (Applied Biosystems) with an internal size marker (Homebrand ROX). A 'standard' (an individual of known genotype) was included on each gel, to check between gel consistency in sizing alleles. Samples were genotyped using GENEMAPPER v3.7 software (Applied Biosystems).

5.3.4 Statistical analysis

5.3.4.1 Data preparation

In order to include data from the North York Moors sites (genotyped by N.M. Rivers, see Rivers *et al.* 2005), DNA from a subset of this data was amplified and genotyped again. This was to check for size differences due to variation in the PCR conditions, the installation of a new sequencer machine and scoring differences between authors. 48 individuals were selected to include all alleles detected at each locus for that population. Size differences varied between loci but the pattern of size differences was consistent within a locus allowing rescoring of the NYM bats according to a locus-specific conversion factor.

MICROSATELLITE TOOLKIT (Park 2001) was used to check genotype data for errors and to generate 2-digit 'genepop' files which could be converted by the analysis programs. Only individuals that amplified at five or more loci (after re-running samples that failed initially) were used in the analysis. The 'percentage missing data' met the requirements of the statistical analyses i.e. <5%. Yordas Cave and Ashberry were not included in the analyses of inter-population variation and isolation by distance due to small sample sizes.

High levels of gene flow, both within and between populations, were expected in this study, therefore *F*-statistics (Weir & Cockerham 1984) were used in the analyses rather than *R*-statistics (Slatkin 1995) as they perform better in such circumstances (Balloux & Goudet 2002; Balloux & Lugon-Moulin 2002). Where multiple comparisons were made, a sequential Bonferroni correction was applied (Rice 1989).

5.3.4.2 Genetic diversity

Observed (H_O) and expected (H_E) heterozygosities, and departures from Hardy Weinberg equilibrium (HWE) were calculated in ARLEQUIN 3.01 (Schneider *et al.* 2000; Excoffier *et al.* 2006). Null allele frequencies per locus were calculated in CERVUS 2.0 (Marshall *et al.* 1998) and linkage equilibrium was tested for using exact tests in GENEPOP 3.4 (Raymond & Rousset 1995). F_{IS} (the inbreeding coefficient) was calculated for each locus and each population in GENETIX 4.05 (Belkir *et al.* 1996 - 2004).

Differences in genetic diversity between swarming sites was assessed by comparing gene diversities (H_E) per locus. The differences were tested for significance using Kendall's coefficient of concordance, computed in GENSTAT 9.0 (www.vsni.co.uk). Kendall's coefficient of concordance tests whether the ranking of diversity at swarming sites is consistent across loci. A significant value would indicate greater consistency than expected by chance and so real differences in diversity among sites. A non- significant value suggests that sites do not differ.

5.3.4.3 Inter-population variation

An analysis of molecular variance (AMOVA) was carried out in ARLEQUIN 3.01 to assess genetic variation within and between swarming areas. The AMOVA calculates the percentage variance attributed to the following levels of population structure: (i) among individuals within populations, (ii) among populations within swarming areas and (iii) among swarming areas.

Fisher's exact tests (Raymond and Rousset 1995) were conducted in GENEPOP 3.4 to assess genic differentiation (i) between swarming areas and (ii) between populations within the Yorkshire Dales. Pairwise F_{ST} values between populations were calculated in ARLEQUIN 3.01.

5.3.4.4 Isolation by distance

Isolation by distance between all sites was assessed using ARLEQUIN 3.01. $F_{ST}/(1-F_{ST})$ was correlated with the log distance between sites in a Mantel test over a range of permutations (1,000 - 10,000). Isolation by distance among the Yorkshire Dales sites only was also tested by the same method.

Table 5.3 Details of loci used in this study, PCR conditions (TD = touchdown program with temperature range), post PCR dilution factor, diversity statistics (A = number of alleles observed, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient) and source information.

Locus	**Primer fluorescent label**	**$MgCl_2$ concentration (mM)**	**PCR program (cycles)**	**Post PCR dilution**	**Allele size range (bp)**	**A**	**H_O**	**H_E**	**F_{IS}**	**Null allele frequency**	**Source species (and reference)**
B22	6-FAM	1.0	60-50 °C TD (35)	x500	153-164	8	0.72	0.76	0.053	+0.026	*Myotis bechsteinii* (Kerth *et al.* 2002a)
D15	NED	1.0	60-50 °C TD (35)	x500	111-139	15	0.83	0.83	-0.001	-0.002	*Myotis myotis* (Castella and Ruedi 2000)
E24	NED	1.0	60-50 °C TD (35)	x500	216-250	17	0.81	0.87	0.066	+0.034	*Myotis myotis* (Castella and Ruedi 2000)
D9	NED	1.0	63-54 °C TD (35)	x500	127-167	18	0.78	0.88	0.177	+0.062	*Myotis myotis* (Castella and Ruedi 2000)
H29	6-FAM	1.5	64-59 °C TD (35)	x375	169-185	9	0.60	0.65	0.080	+0.042	*Myotis myotis* (Castella and Ruedi 2000)
Paur 06	HEX	1.5	57-51 °C TD (35)	x375	153-175	12	0.66	0.84	0.214	+0.120	*Plecotus auritus* (Castella and Ruedi 2000)

5.4 Results

All six loci were highly polymorphic (8-18 alleles) and expected heterozygosity was high (0.65 - 0.88). There was no significant linkage dis-equilibrium for loci pairs after correcting for multiple comparisons and no consistent departure from HWE with the exception of Paur 06. E24 showed significant departure from HWE in two populations: Manchester Hole and Slip Gill, B22 in one population: Antofts, and D15 in one population: Little Blakenham Pit. The departure from HWE for Paur 06 in eight of the seventeen populations may be due to the presence of null alleles. Below frequencies of 0.2, null alleles are considered uncommon to rare and their effects on the outcome of statistical analyses are negligible (Dakin and Avise 2004). The null allele frequency for Paur 06 (+0.120) is well below this threshold. Diversity statistics for each locus are summarised in Table 5.3.

5.4.1 Genetic diversity

Diversity statistics for all populations are given in Table 5.4. Overall, allelic diversity was high, with the greatest mean number of alleles at sites with large sample sizes. Observed and expected heterozygosities were also high.

Gene diversity (H_E) per locus is given for each population in Table 5.5. No significant difference in gene diversity between sites was detected using Kendall's coefficient of concordance.

Table 5.4 Diversity statistics for all study sites: n = number of individuals sampled and included in the analysis, A = mean number of alleles per locus (± standard deviation). H_O = mean observed heterozygosity per locus (± standard deviation), H_E = mean expected heterozygosity per locus (± standard deviation), F_{IS} = mean inbreeding coefficient per locus (95% confidence intervals).

Area	**Population**	**n**	**A**	**H_O**	**H_E**	**F_{IS}**
Yorkshire Dales	Dow Cave	57	10.33 (3.14)	0.778 (0.023)	0.817 (0.039)	0.047 (-0.001 - 0.052)
"	Buckden Gavel Mine	23	8.50 (3.89)	0.752 (0.037)	0.781 (0.050)	0.038 (-0.031 - 0.063)
"	Manchester Hole	31	8.50 (2.74)	0.698 (0.034)	0.814 (0.028)	0.144 (0.051 - 0.162)
"	Trollers Gill	30	9.00 (3.29)	0.766 (0.032)	0.787 (0.040)	0.028 (-0.107 - 0.048)
"	Cherry Tree Hole	41	9.67 (2.80)	0.692 (0.030)	0.801 (0.036)	0.138 (0.087 - 0.197)
"	Craven Lime Works	26	8.50 (3.02)	0.728 (0.036)	0.795 (0.050)	0.087 (0.035 - 0.119)
"	Bar Pot	49	9.33 (2.50)	0.699 (0.027)	0.779 (0.039)	0.103 (0.046 - 0.120)
"	Yordas Cave	13	7.50 (2.26)	0.791 (0.046)	0.809 (0.044)	0.024 (-0.102 - 0.125)
"	Easegill	40	10.00 (3.29)	0.726 (0.029)	0.822 (0.037)	0.118 (0.055 - 0.140)
North York Moors	Slip Gill	196	10.17 (3.54)	0.741 (0.013)	0.789 (0.039)	0.061 (0.047 - 0.075)
"	Bucklands	92	8.83 (3.25)	0.758 (0.018)	0.797 (0.034)	0.049 (0.017 - 0.086)
"	Antofts	136	9.67 (3.72)	0.739 (0.016)	0.796 (0.035)	0.071 (0.034 - 0.099)
"	Ashberry	12	6.50 (1.97)	0.698 (0.055)	0.797 (0.019)	0.129 (0.020 - 0.132)
Cumbria	Roudsea Wood Cave	21	8.00 (3.16)	0.661 (0.043)	0.768 (0.056)	0.143 (-0.014 - 0.247)
Lincolnshire	Withcall Tunnel	20	7.83 (2.56)	0.644 (0.045)	0.777 (0.038)	0.176 (0.060 - 0.284)
Suffolk	Little Blakenham Pit	43	9.17 (2.93)	0.693 (0.029)	0.831 (0.023)	0.169 (0.084 - 0.202)
West Sussex	Cocking/Drovers Tunnels	15	8.33 (2.25)	0.797 (0.043)	0.840 (0.025)	0.077 (-0.004 - 0.110)

Table 5.5 Gene diversity (H_E) per locus per population

Area	**Population**	**B22**	**D15**	**E24**	**D9**	**H29**	**Paur 06**
Yorkshire Dales	Dow Cave	0.768	0.832	0.887	0.896	0.648	0.869
"	Buckden Gavel Mine	0.677	0.845	0.871	0.903	0.588	0.798
"	Manchester Hole	0.749	0.865	0.874	0.867	0.710	0.816
"	Trollers Gill	0.708	0.754	0.851	0.898	0.650	0.864
"	Cherry Tree Hole	0.767	0.802	0.884	0.891	0.652	0.811
"	Craven Lime Works	0.805	0.787	0.835	0.913	0.562	0.870
"	Bar Pot	0.766	0.760	0.847	0.863	0.604	0.832
"	Yordas Cave	0.686	0.825	0.883	0.914	0.665	0.884
"	Easegill	0.815	0.864	0.884	0.902	0.649	0.817
North York Moors	Slip Gill	0.743	0.825	0.860	0.864	0.614	0.831
"	Bucklands	0.732	0.833	0.865	0.873	0.661	0.815
"	Antofts	0.747	0.835	0.845	0.858	0.641	0.848
"	Ashberry	0.736	0.822	0.823	0.844	0.740	0.815
Cumbria	Roudsea Wood Cave	0.675	0.854	0.848	0.858	0.528	0.844
Lincolnshire	Withcall Tunnel	0.749	0.654	0.866	0.876	0.696	0.823
Suffolk	Little Blakenham Pit	0.802	0.868	0.900	0.850	0.740	0.830
West Sussex	Cocking/Drovers Tunnels	0.802	0.868	0.907	0.857	0.733	0.871

5.4.2 Inter-population variation

The AMOVA shows that most of the genetic variation (98.75%) can be explained by differences between individual bats within populations (Table 5.6). However, a significant amount of the variation is not explained at this level, indicating structure in the overall population. There is low but statistically significant variation among swarming areas (0.81%) suggesting differentiation between geographically distinct areas. There is also low but highly statistically significant variation among populations within swarming areas (0.44%). This is most likely to be due to differences between swarming sites in the Yorkshire Dales (see below) as there is limited scope for differentiation amongst the Windy Pits (see section 5.5), and the other swarming areas are single sites.

Table 5.6 The percentage of genetic variation explained by the different levels of population structure (AMOVA) among swarming bats.

	% of variation	**p (random value ≥ observed value) ± SE**
Among individuals within populations	98.75	0.00000 ± 0.00000
Among populations within swarming areas	0.44	0.00000 ± 0.00000
Among swarming areas	0.81	0.00391 ± 0.00185

P values for the pairwise comparisons of genic differentiation between swarming areas are given in Table 5.7. All comparisons were significant prior to correction for multiple comparisons. Following correction, no significant differentiation was detected between Roudsea Wood Cave in Cumbria and the Yorkshire Dales, perhaps due to the proximity of the Cumbrian site to the western edge of the Dales. Neither was there significant differentiation between the Cumbrian site and Cocking and Drover's tunnels in West Sussex, although this could be due to the relatively small sample sizes at both sites.

Table 5.7 P values calculated for pairwise genic differentiation between swarming areas (significant values after sequential Bonferroni correction are shown in bold).

	Yorkshire Dales	North York Moors	Cumbria	Lincolnshire	Suffolk
North York Moors	**<0.00001**				
Cumbria	0.02282	**0.00020**			
Lincolnshire	**<0.00001**	**<0.00001**	**<0.00001**		
Suffolk	**<0.00001**	**<0.00001**	**<0.00001**	**<0.00001**	
Sussex	**0.00111**	**<0.00001**	0.00583	**0.00054**	**0.00018**

Table 5.8 P values calculated for pairwise genic differentiation between swarming sites in the Yorkshire Dales (top value). Significant values for genic differentiation after sequential Bonferroni correction are shown in bold. Pairwise F_{ST} values are also shown (bottom value).

	Dow Cave	Buckden Gavel Mine	Manchester Hole	Trollers Gill	Cherry Tree Hole	Craven Lime Works	Bar Pot
Buckden Gavel Mine	0.01443 0.00481						
Manchester Hole	**0.00013** 0.00988	**0.00102** 0.01054					
Trollers Gill	0.00682 0.00589	0.01224 0.01748	**0.00001** 0.02634				
Cherry Tree Hole	**0.00001** 0.01109	**0.00198** 0.01625	**<0.00001** 0.01262	**0.00224** 0.01456			
Craven Lime Works	**0.00025** 0.00589	**0.00098** 0.01571	**<0.00001** 0.01889	0.22276 0.00347	0.21221 0.00329		
Bar Pot	**<0.00001** 0.00995	**0.00010** 0.01750	**<0.00001** 0.01732	0.01539 0.00036	0.00651 0.00528	0.45954 -0.00137	
Easegill	**0.00004** 0.00787	**0.00026** 0.01605	**0.00175** 0.00843	0.00392 0.01354	0.01882 0.00678	0.42805 -0.00092	0.03187 0.00663

P values for the pairwise comparisons of genic differentiation between swarming sites in the Yorkshire Dales, and pairwise F_{ST} values, are given in Table 5.8. F_{ST} is a measure of differentiation between sub-populations. Values of 1 indicate complete differentiation, 0 indicates panmixia. F_{ST} values below 0.05 indicate high gene flow and weak population structure. In general, significant comparisons of genic differentiation also showed higher pairwise F_{ST} values and some clear patterns of differentiation were evident. Manchester Hole (in Nidderdale) showed significant differentiation with all other sites. There was no significant differentiation between the two Upper Wharfedale sites: Buckden Gavel Mine and Dow Cave. Easegill, Bar Pot, the Craven Lime Works and Cherry Tree Hole (in the south west of the Park) were not differentiated from one another. All pairwise comparisons between the Upper Wharfedale and south western groups were significant. Troller's Gill (Lower Wharfedale) was not differentiated from any of the sites in the Upper Whafedale or south western groups (with the exception of Cherry Tree Hole). This pattern of differentiation is summarised in Figure 5.3 and is explored in relation to geographical distances, topography and potential catchment areas in section 5.5.

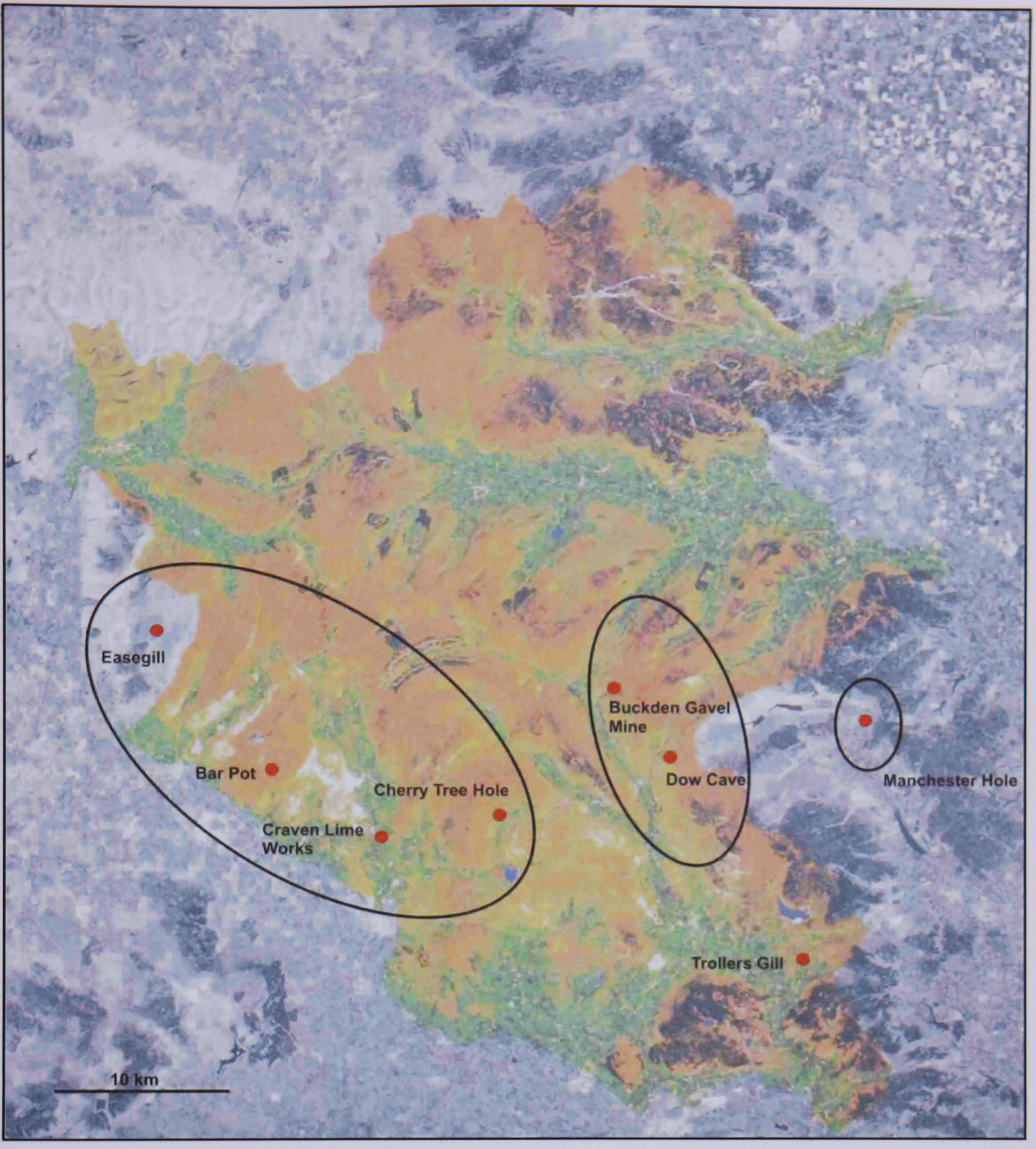

Figure 5.3 Genic differentiation between swarming sites in the Yorkshire Dales. Elipses enclose sites that are not significantly differentiated from one another. All comparisons between enclosed groups were significant. Troller's Gill was not significantly differentiated from any other site with the exception of Manchester Hole and Cherry Tree Hole.

5.4.3 Isolation by distance

There was significant isolation by distance among all populations (Mantel $r = 0.518$, p <0.001, Fig. 5.4). However, there was no significant isolation by distance among populations within the Yorkshire Dales (Mantel $r = 0.164$, $p = 0.211$). The results were consistent for 1,000, 2,000 and 10,000 permutations.

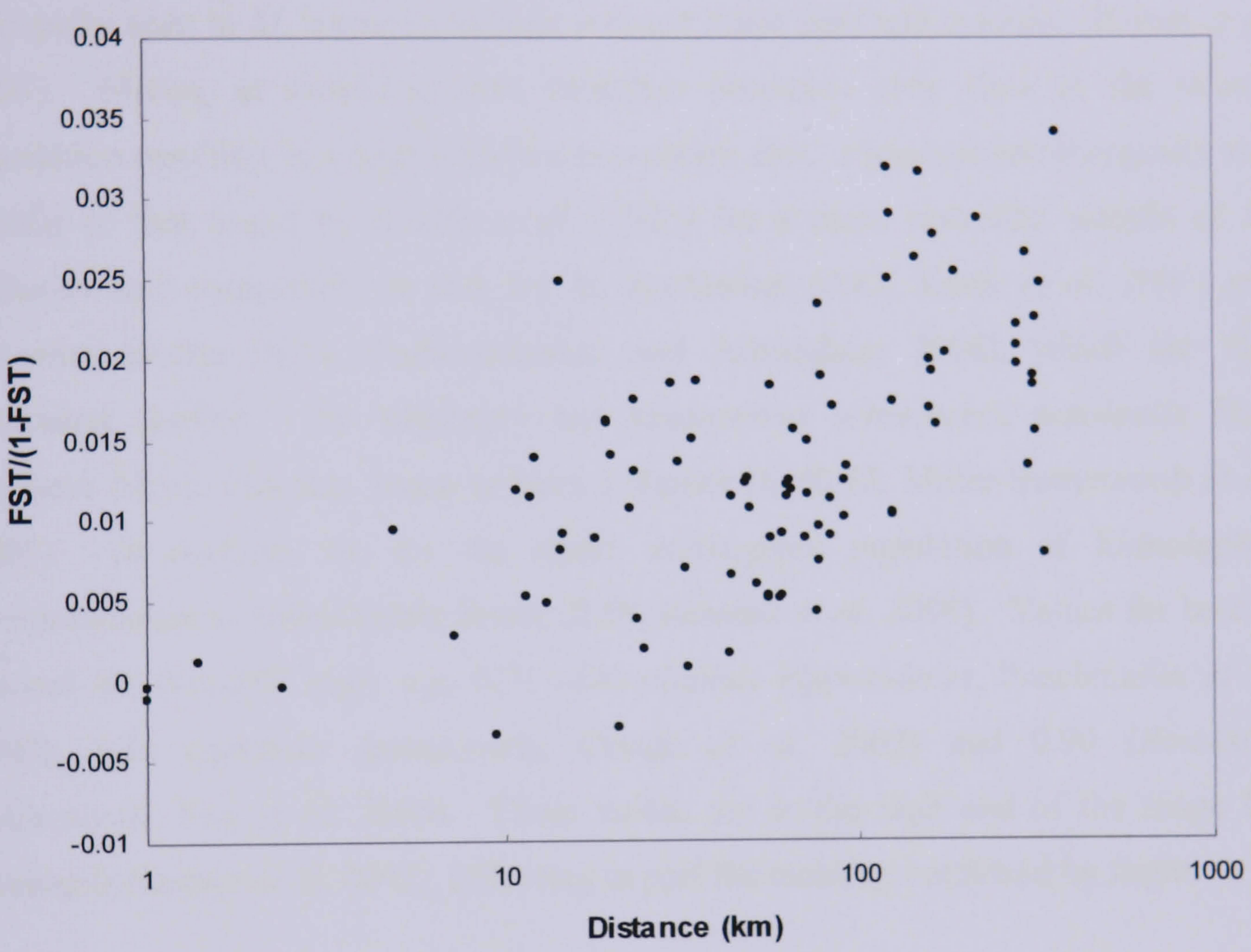

Figure 5.4 Isolation by distance across all sites.

5.5 Discussion

Allelic diversity (A) and expected heterozygosity (H_E) were high in all swarming populations (6.5 - 10.33 and 0.768 - 0.840 respectively). In addition, pairwise F_{ST} values between sites were low (<0.001 - 0.033). These results are consistent with the view that bats from many summer populations meet at swarming sites and that mating at these sites is the most likely cause of this high diversity, given the high degree of philopatry seen in *M. nattereri* to their summer roost sites and colonies (Rivers *et al.* 2006). Mating at swarming sites therefore promotes gene flow in the overall population resulting in a large effective population size. Expected heterozygosity was similar to that found by Rivers *et al.* (2005) for a more restricted sample of *M. nattereri* and comparable to that for *M. bechsteinii* (0.82, Kerth *et al.* 2003) and *Plecotus auritus* (0.76, Furmankiewicz and Altringham 2006), which are also swarming species. The migratory bat *Miniopterus schreibersii natalensis* from southern Africa was also found to have a similar H_E (0.72, Miller-Butterworth *et al.* 2003). In contrast, H_E for the small, endangered population of *Rhinolophus ferrumequinum* is considerably lower (0.58, Rossiter *et al.* 2000). Values for bats in general are typically high, e.g. 0.71 (*Rhinolophus hipposideros*, Puechmaille *et al.* 2005), 0.85 (*Artibeus jamaicensis*, Ortega *et al.* 2002) and 0.90 (*Rousettus leschenaulti*, Hua *et al.* 2006). These values are at the high end of the range for mammals (Garner *et al.* 2005), reflecting in part the mobility conferred by flight.

We interpret the differences in activity recorded using echolocation call loggers (Chapter 3), backed up by proportionally similar differences in the average numbers of bats caught (unpub. data) as a reflection of the size of the swarming populations at different sites. This seems logical if we assume visitation rates and behaviour are similar across sites. We hypothesised that sites with larger swarming populations were likely to show greater diversity. However, no significant difference in gene diversity was detected across sites. This result can be interpreted in two ways: the first is that differences were not detected due to the relatively large number of sites, small number of loci and the small sample sizes for some sites. Alternatively, mating during swarming may be so effective at promoting high gene diversity within individual colonies that between site gene diversity may be small or non-existent.

Rivers *et al.* (2005) found that gene diversity in *M. nattereri* was significantly higher at swarming sites than summer colonies, although the differences were small and could result from differences in sample size. No significant difference in nuclear DNA gene diversity between summer colonies and swarming sites was found in either *Myotis bechsteinii* (Kerth *et al.* 2003) or *Plecotus auritus* (Veith *et al.* 2004). However, both studies found significantly higher mitochondrial DNA gene diversity at swarming sites. Petit and Mayer (2000) report a similar pattern between summer sites and hibernacula in a migrating species, *Nyctalus noctula*. These genetic studies, and ringing studies (e.g. Rivers *et al.* 2006), provide evidence that bats from several colonies visit a swarming site/hibernaculum but provide only indirect evidence that mating occurs here. However, genetic studies on two swarming species, *M. bechsteinii* (Kerth and Morf 2004) and *P. auritus* (Burland *et al.* 2001; Furmankiewicz and Altringham 2006) provide evidence to show that mating occurs outside the breeding habitat.

Observations of behaviour at swarming sites, including a male-biased sex ratio, chasing behaviour and copulation events, provide additional evidence that swarming is an important mating event (e.g. Davis and Hitchcock 1965; Thomas *et al.* 1979; Bauerová and Zima 1988; Rivers *et al.* 2006). The idea that this promotes gene flow between isolated summer populations was proposed prior to the publication of genetic evidence to support it (e.g. by Parsons *et al.* 2003a). Rivers *et al.* (2005) used Wright's (1951) island model to predict the proportion of matings taking place at swarming sites that would explain the observed population structure (F_{ST}) amongst summer colonies in the catchment area. The model suggested that most of the mating did occur at swarming sites and not within the summer colonies, particularly if the effective population size of colonies was low.

Even if mating occurs outside the breeding habitat, it does not necessarily occur during swarming. A number of species have been observed copulating during hibernation (e.g. *Myotis lucifugus*, Thomas *et al.* 1979) and the proportion of inseminated females was reported to increase between November and April in a hibernating population of *P. auritus* (Strelkov 1962). *P. auritus*, also exhibits swarming behaviour in the spring (Furmankiewicz 2004; Furmankiewicz and

Altringham 2006). Several non-swarming, temperate species migrate long distances between their summer roosts and hibernation sites. Males may establish mating territories prior to migration, (e.g. *Pipistrellus nathusii*, Gerell Lundberg and Gerell 1994), mate along migration routes (e.g. *Nyctalus noctula*, Sluiter and van Heerdt 1966; Petit and Mayer 1999) or mate upon arrival at hibernacula (e.g. *Miniopterus schreibersii natalensis*, van der Merwe 1973; Miller-Butterworth *et al.* 2003).

The results of this study show that while gene flow appears to be high in *M. nattereri* (high gene diversity at all sites, low pairwise F_{ST} values between sites), nevertheless there is population structure at both regional and national scales. Whilst most of the genetic variation can be attributed to differences between individuals (98.75%), a small but significant proportion of the variance (0.81%) is due to differences between swarming areas. In addition, 0.44% of the variance was explained by within swarming area variation. This presumably resides in the Yorkshire Dales, since other areas are either single sites, or in the case of the Windy Pits, a small cluster of caves a maximum of 4 km apart and already shown to lack differentiation (Rivers *et al.* 2005). Across all sites, significant differentiation was found amongst swarming areas and a significant correlation between genetic distance (pairwise F_{ST} range = -0.00032 - 0.03298) and geographic distance (Euclidean distance range = 1 - 400 km) was also found. These results are perhaps not surprising given that the distances between most swarming areas exceed the maximum reported dispersal distances for *M. nattereri* between summer colonies and swarming sites (17 km, Chapter 2; 25 km, Parsons and Jones 2003; 63 km, Rivers *et al.* 2006). Whilst these values almost certainly underestimate true maximum dispersal distance, the number of migrants greatly exceeding them is likely to be very small.

The pattern of population structure in the Yorkshire Dales was not explained by geographic distance between sites, but this was not expected. An explanation for the observed pattern of differentiation between sites is more likely to be found in the topography of the landscape and geographic relationships of swarming sites and their catchment areas. These catchment areas are likely to include bats from summer roosts within the same dale as the swarming sites and bats from lowland areas outside the Yorkshire Dales.

Bats commute and forage along linear features (Limpens and Kapteyn 1991), particularly riparian corridors (Walsh and Harris 1996a; b). It seems likely that bats dispersing to swarming sites will do so along river valleys, especially in this upland area where woodland habitat is sparse and fragmented and the high open moorland between the dales offers minimal protection from the elements and few navigational aids. Dispersal and navigation in bats is discussed in Chapter 1, and in relation to the location of swarming sites in Chapter 3.

Manchester Hole, at the head of Nidderdale on the eastern side of the Yorkshire Dales, showed significant differentiation from all other sites. Although this site is less than 15 km from the nearest populations sampled (Buckden Gavel Mine, Dow Cave and Trollers Gill in Wharfedale), moorland habitat up to 700m a.s.l. separates the two valleys. The most probable potential catchment area for Manchester Hole lies to the east of the Dales in the Vale of York. The unit comprising Buckden Gavel Mine and Dow Cave lies in Wharfedale, a narrow, isolated dale. The only access that does not necessitate crossing high ground is to the south and its most probable catchment is the dale itself and the low lying areas along the River Wharfe which flows south-east. This is supported by the movement patterns of ringed *M. daubentonii* described in Chapter 2. In the final unit, Easegill, Bar Pot and the Craven Lime Works are all readily accessible to bats along the Rivers Lune and Ribble to the south-west and the extensive lowland around them. Again, the movements of ringed bats support this (Chapter 2); all recaptures were of bats moving between these swarming sites and lowland summer roosts to the west and south-west.

IBD models based on Euclidean distances between populations assume dispersal through a homogenous environment. In several taxa, studies combining genetic data and landscape elements demonstrate the importance of habitat heterogeneity in dispersal patterns and hence population structure (e.g. Broquet *et al.* 2006a; b). Landscape features had a significant influence on genetic structure of a roe deer (*Capreolus capreolus*) population inhabiting a fragmented landscape (Coulon *et al.* 2004; 2005). A highway and several waterways were barriers to gene flow whereas wooded corridors appeared to aid dispersal. Genetic distance was correlated with 'least cost distance' (the trajectory that maximises the use of wooded corridors) but not Euclidean distance. In a mountainous region, Euclidean distance was not representative of actual dispersal distance in the Pacific jumping mouse (*Zapus trinotatus*) and population structure was better explained by riparian connectivity and elevation (Vignieri 2005). Significant isolation by distance was found in the blotched tiger salamander (*Ambystoma tigrinum melanostictum*), however the fit of the model explaining genetic differentiation was substantially improved by incorporating landscape variables (Spear *et al.* 2005).

Due to the mobility conferred by flight, the study of the influence of habitat heterogeneity on population structure of bat species has focused on significant barriers to dispersal, and hence gene flow, such as sea crossings and mountain ranges.

Significant isolation by distance has been detected in two non-migratory island species of Pteropodidae using highly polymorphic allozyme markers. In *Cynopterus nusantengarra* (Schmitt *et al.* 1995) and *Eidolon helvum* (Juste *et al.* 2000), the patterns of differentiation between populations on different islands are related to sea crossing distances with little 'within island' differentiation. The effects of barriers are not always a simple reflection of distance, nor are they predictable. The population structure of the endemic Azorean bat *Nyctalus azoreum*, reflects its distribution on an isolated group of islands (Salgueiro *et al.* 2004), but genetic diversity was high for an insular species. Both the Alps and the Rhodopes mountains appeared to be significant barriers to the post-glacial expansion of *Myotis myotis* in Europe, but the Pyrenees did not (Ruedi and Castella 2003).

Population structure is also likely to reflect dispersal capabilities. No isolation by distance was detected amongst summer colonies of *M. nattereri* (inter-colony distance = 10 - 100 km) (Rivers *et al.* 2005) but was found amongst summer colonies of *P. auritus* across a similar range (Burland *et al.* 1999). *P. auritus* exhibits a wing morphology and flight pattern consistent with short-range dispersal (Norberg and Rayner 1987). A similar pattern related to dispersal capacity was shown between two vespertilionid species in the Indonesian archipelago (Hisheh *et al.* 2004). *Scotophilus kuhlii*, characterised by strong flight capabilities, did not show differentiation between islands whereas *Myotis muricola*, with weak dispersal ability, showed marked population structure. Across the Chiroptera, migratory species show less population structure than sedentary species (Burland and Wothington Wilmer 2001, and references therein).

This study is the first to investigate population structure among a large number of swarming sites on a regional and national scale. In order to better understand the relationship between the geography of swarming sites and their catchments, we need to identify and sample summer colonies within those catchments. Whilst nuclear DNA markers can tell us something about the genetic structure of populations, a better understanding of intersexual dispersal patterns and gene flow will only come from a study using mitochondrial and/or Y-chromosome markers. Analytical methods for genetic data, providing more detailed information about population structure, gene flow and demographic history, are advancing at an exciting rate. New and emerging analytical techniques that facilitate the study of population structure in the context of the landscape are reviewed in Pearse and Crandall (2004).

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Chapter 6: General Discussion

6.1 Populations, catchments and conservation

Resource distribution is one of the major forces driving population structure: from distribution and density to the social dynamics and dispersal of organisms. Caves are a scarce resource in most parts of the world, so that where caves are present, bats can form enormous aggregations. Many of these aggregations are nursery colonies (in tropical and sub tropical regions), but the same rationale should apply to bats visiting swarming sites if they too are a limiting resource. Rivers *et al.* (2006) have shown that a small cluster of isolated caves (Ryedale Windy Pits) in the North York Moors National Park has a swarming population of about 4,000 *M. nattereri* that are recruited from a catchment area over 60 km in diameter, strongly suggesting that these caves are indeed a scarce and vital resource to the bats of the region. Given the very large number of caves in the Yorkshire Dales it could be argued that swarming sites are unlikely to be in short supply. So, it could be hypothesised that swarming bats would be more evenly distributed across the landscape, with each cave attracting a relatively small number of bats from a localised catchment area. However, the evidence presented in this thesis suggests otherwise. It is clear from the results presented in Chapter 3 that bats are highly selective and the majority of caves attract few bats. Only at a few sites were large numbers of bats present and at these, activity was comparable to that at the Windy Pits. Clearly, despite the abundance of caves in the Dales, individual caves can be as important to the bats of the region as the isolated caves of the North York Moors. This use of a relatively small number of primary mating sites with large catchments ensures that individuals are more likely to meet mates on a given night and also promotes gene flow between colonies.

Is it possible to infer population sizes from the data available? The calculations are only rough, but do yield interesting results. From Table 3.1, the seven most active sites in the Dales had activity levels comparable to that at Slip Gill, the best studied Windy Pit (Rivers *et al.* 2006). Assuming similar visitation rates and behaviour at the sites, then we would expect the Dales caves to be attracting similar numbers of bats. Recapture rates in the Dales (data not shown) were variable, but comparable overall to those at the Windy Pits, again suggesting similar population sizes. Mark-recapture analysis has not yet been carried out on the Dales data. Mark-recapture population analysis for the Windy Pits gives an estimate of 1,400 *M. nattereri* at each cave and both studies have shown virtually no exchange between caves. Together, the seven most active sites in the Dales may therefore support 10,000 *M. nattereri*. Given that there are many more sites supporting smaller but still significant numbers of bats, an estimate of 10,000 *M. nattereri* across the Dales is conservative. If these figures are correct, then these ten caves in the Yorkshire Dales and North York Moors recruit 20% of the estimated English population (Hutson 1993), although the national population is likely to be an underestimate.

The genetic population structure across swarming sites (Chapter 5) indicates some degree of spatial structure in their catchment areas. For swarming sites within the Dales, the size and shape of catchment areas, and their degree of overlap will in part be determined by the landscape, and the position of the caves in this landscape. The Dales appear to have at least three genetically differentiated groups of swarming sites, with catchment areas extending into adjacent lowland areas via the migration corridors of the dales themselves.

Conservation of a species often relies on an ability to identify management units that hold viable populations. For bats, which undertake considerable seasonal movements to meet their complex resource needs, we must identify and protect a large landscape that fulfils all of these needs. In this case, it means swarming sites and summer foraging and roost sites within the protected area of the Park, as well as an extensive area of summer habitat beyond its boundaries.

The patterns emerging in *M. nattereri* will apply to some degree to other species. However, significant species differences are expected. Bats that have evolved slow, manoeuvrable flight as part of their foraging strategy and therefore have low dispersal ability should show greater population differentiation. A comparison between *M. daubentonii* and *M. nattereri* would test this, since both are abundant, but the wing form of *M. nattereri* will make it a slower and less efficient flier. Rarer bats should show greater differentiation, due to greater isolation and lower gene flow between colonies. A comparison of *M. nattereri*, *M. brandtii* and *M. mystacinus* should be informative since all have similar wing form and ecology, but in the north of England increase in rarity in the order given. More interesting and robust comparisons could be made with larger species assemblages over larger areas. To this end, DNA samples from all five swarming species, collected as part of the work for this thesis, will be used in a multi-species analysis with samples from caves across Europe.

The results of this study are already influencing survey and monitoring strategies and conservation policy with regards to bats at underground sites, at a local, national and European level. The following sections of this discussion relate to threats to cave-bat populations, the effects of gating sites and effective methods for survey and monitoring.

6.2 Threats to bats at underground sites

Commercial interests can pose a threat to cave-roosting bats. For example, the development of a cave for tourism inevitably leads to gating, disturbance and altered microclimate. The most destructive threat comes from quarrying which can result in severe damage or even complete loss of a site.

In the UK, the recent Countryside and Rights of Way (CROW) Act 2000 has given the public free access to a great part of the countryside. Landowners, concerned about litigation after accidents at underground sites, have sought to gate or completely block the entrances to many sites. This applies primarily to mines since caves are generally protected, particularly those with conservation designations.

Agricultural and forestry activities pose a number of threats. Agricultural chemicals can enter caves rapidly and in high concentrations by direct entry into the cave system, or more slowly by percolation into the soil. Enhanced drainage for agriculture or forestry can greatly alter cave hydrology, through increased run-off. In areas with livestock, such as the Yorkshire Dales, makeshift barriers are often used to prevent animals entering of falling into caves and mines.

Bats in underground sites have been deliberately disturbed and even killed through vandalism. Unintentional disturbance can also occur due to the activities of outdoor recreation groups, cavers, scientists and conservationists (see Thomas 1995). However, small responsible groups following the Cave Conservation Code (British Caving Association - code under revision) should not have a detrimental impact on bat populations; particularly in large underground systems (see Chapter 3).

If climate change raises the mean annual temperature, then deep cave temperature will rise to the same extent. More extreme temperature fluctuations will be reflected in those at cave entrances. Changes in the timing and intensity of rainfall will alter cave hydrology and hence microclimate. Changes in cave microclimate could make many sites unsuitable, particularly for hibernation.

The solutions to some of these threats, notably climate change, will only be achieved in the long term and with significant changes in policy and political will. There are two achievable courses of action that would further the conservation of cave-roosting bats in the Yorkshire Dales. The first is the replacement of unsuitable barriers at the entrances to caves and mines. The second is further survey and monitoring of underground sites, followed by appropriate protective measures. The remaining sections of this chapter will address some of the practical issues associated with these goals.

6.3 The effects of gating site entrances

Cave gates fall into two categories, those that were built without consideration for bats and those designed to protect bat populations from disturbance (Tuttle 1977; Tuttle 1979; Tuttle and Taylor 1998). All of the barriers in the Dales fall into the first category and most are likely to obstruct the passage of bats to varying degrees. If these barriers are to be replaced, this must be done with well-designed gates.

Despite good intentions, many of the early barriers put in place to protect bats from disturbance led to severe declines in the number of bats using sites (Tuttle 1977; MacGregor 1993). Once this problem was recognised, new designs were introduced and many older gates replaced (Tuttle and Taylor 1998). A badly designed gate can cause two problems: it will either restrict access to bats and/or alter the microclimate of an underground site (Tuttle and Stevenson 1977; Richter *et al.* 1993). Changes in microclimate occur through impaired air flow in and out of the cave. This may be due simply to a change in aperture area or to more subtle effects. For example, even a low barrier can alter the flow of cold air. Tuttle and Stevenson (1977) deal with these issues in some detail. The most common designs now in use incorporate a metal grille with horizontal spaces narrow enough to prevent entry by most humans, but large enough to allow bats unimpeded entry. The section and thickness of the bars are a compromise between the demands of strength and unrestricted air flow. In the United States, at least 1,620 'bat-friendly' cave gates had been erected by 2002 (Sherwin *et al.* 2002) and the use of cave gates is widely promoted (e.g. Tuttle and Taylor 1998). Bats can clearly fly through these gates, and gating can lead to substantial increases in the number of bats using a site. However, the effects of gates on bats have not been studied rigorously: there are very few studies of the effects of gates on bats relative to the large number of gated sites.

Virtually all published studies on cave and mine gating relate to medium or long-term effects on the numbers of bats occupying hibernacula or summer nursery sites, based on visual counts (e.g. MacGregor 1993; Ludlow and Gore 2000; Martin *et al.* 2003). Results vary greatly and depend on gate design and also any modifications made to the cave or mine entrance itself during gate construction. Recent designs have yielded encouraging results (Tuttle and Taylor 1998; Martin *et al.* 2003; Mitchell-Jones

1995), but the data are often difficult to interpret since they are complicated by other factors such as inadequate monitoring before gating, the removal of old gates or alterations to other parts of the cave or mine.

Figure 6.1 shows the total number of bats (*Myotis* and *Plecotus*) recorded by visual survey at a site in Essex (Mitchell-Jones 1995). Prior to 1962 the maximum number of bats recorded was "about 20". After gating there has been a steady increase in numbers.

Figure 6.1 The effect of gating a small hibernation site on hibernating bats. Data from Mitchell-Jones (1995). The maximum number of bats recorded prior to 1962 was 'about 20'. Human access was restricted in 1986.

Ludlow and Gore (2000) monitored an all-year *Myotis* cave roost with two entrances, for one year before and one year after the removal of the gate that covered one entrance. Prior to removal, less than 10% of the bats used the gated entrance; the rest used the ungated entrance. After removal of the gate 50% of the bats made use of each entrance. The gate that was removed was a circular steel bar grille, with 100 mm x 465 mm spaces. Martin *et al.* (2003) studied the impact of gates at six caves used by *M. grisescens*, over a 20 year period. Gates were made of angle-iron with 150 mm spacing. The number of bats decreased at one cave, increased at two and remained unchanged at three. Data were available for variable pre-gating periods, population sizes were estimated from the size of guano piles and the data set were incomplete.

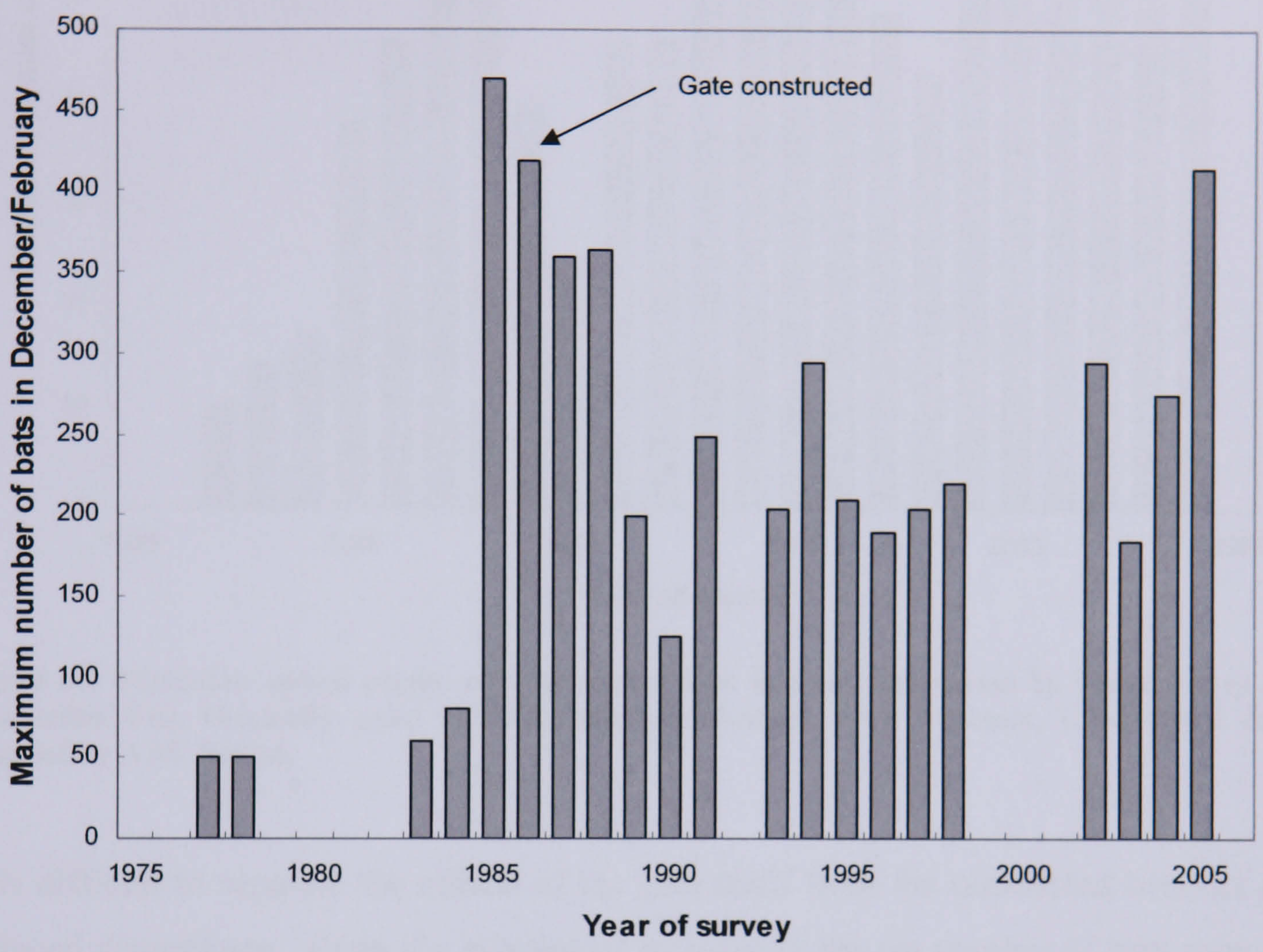

Figure 6.2 Maximum number of hibernating bats (predominantly *Myotis* species) in Greywell Tunnel, Hampshire, determined by visual survey (December to end February). Unpublished data supplied by A.J. Mitchell-Jones.

Unpublished data from Greywell Tunnel in Hampshire (Figure 6.2) appears to show a marked increase in the number of hibernating bats after gating, but note that the highest count actually preceded gating. A similar upward trend has been observed at Westerham Mines in Kent (Figure 6.3). This site was originally gated in 1972, but bat numbers did not begin to increase until a series of modifications were begun in 1981 to improve airflow (to lower temperature) and to increase the size of some of the entrances. Most of the modifications were carried out 1981-1987.

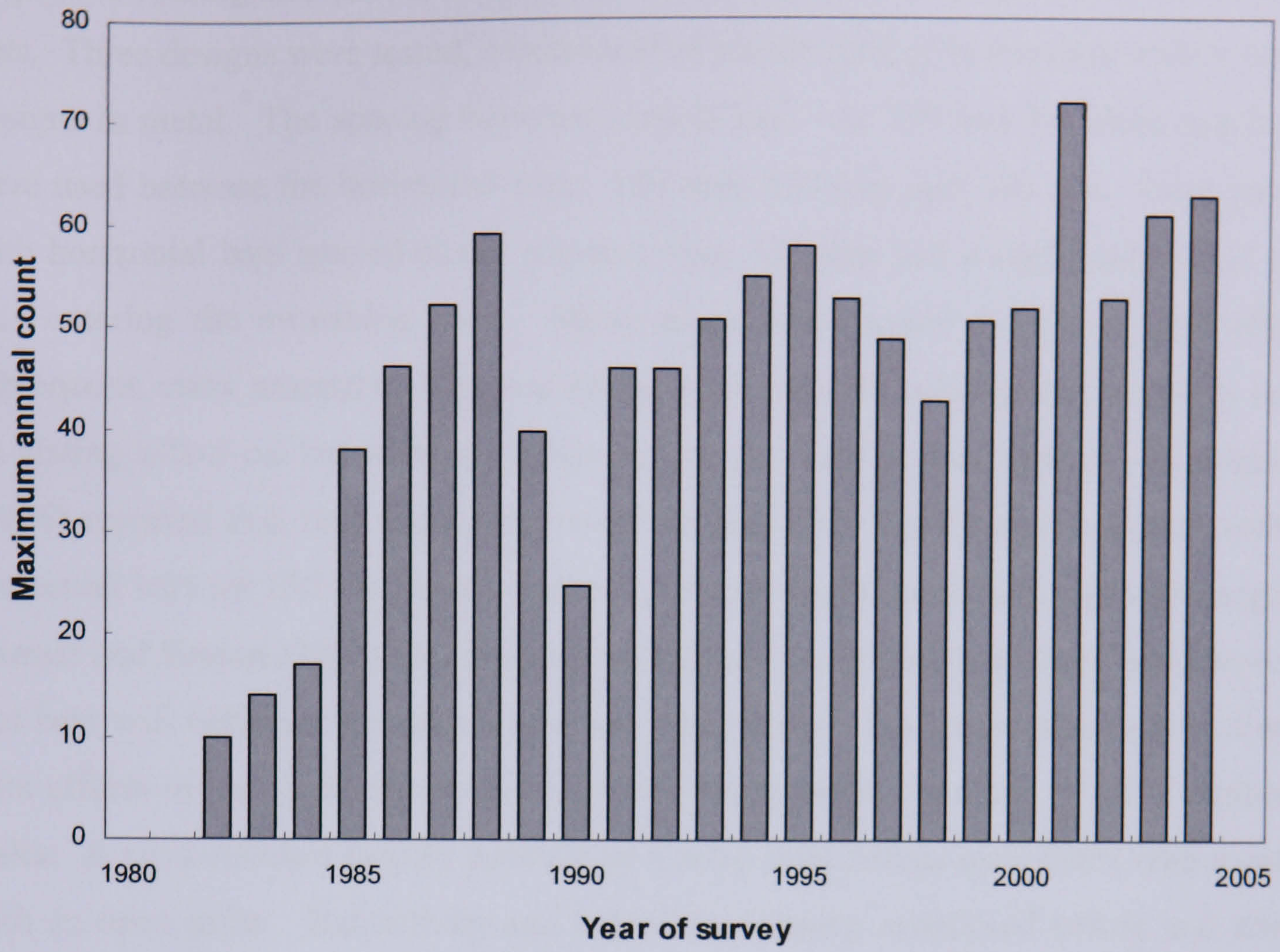

Figure 6.3 Maximum annual counts at Westerham Mines in Kent, determined by visual survey of hibernating bats. Originally gated in 1972, with modifications 1981 - present. Unpublished data supplied by A.M. Hutson.

It is difficult to separate the effects of the gate itself from the undoubted benefits of reduced disturbance. Even if a substantial increase in the net number of bats using a site is seen, this does not mean that the gate itself is not a deterrent to individual bats.

Few studies have looked at behaviour, in particular the response of flying bats to the gate itself. Martin *et al*. (2003) found no evidence to suggest that gates delayed the emergence of gray bats (*M. grisescens*). Rodrigues (1996) showed that the introduction of gates rapidly decreased the number of individuals of several species of rhinolophid and vesper bat visiting a cave. White and Seginak (1987) carried out perhaps the only published study to look at different gate designs: Virginia big-eared bats (*Corynorhinus townsendii virginianus*) and gray bats preferred to exit via a grille rather than a funnel, and a grille with horizontal, round iron bars (19 mm diameter) spaced 154 mm apart was preferred to a grille of angle iron (103 mm diameter) at the same spacing. However, all three gate types deterred bats to some degree.

Pugh and Altringham (2005) studied the immediate effects of grilles on swarming bats. Three designs were tested, constructed of plastic tubing, to simulate widely used designs in metal. The spacing between vertical bars was 750 mm but three spacings were used between the horizontal bars: 150 mm, 130 mm and 100 mm. Cave gates with horizontal bars spaced closer together than 150 mm had a significant effect on bats entering the swarming cave. Many more bats aborted their first, and often subsequent, entry attempts. Removal of the gates after 30 min showed that they had no lasting effect on bat numbers when *in situ* for such a short period. Rodrigues (1996) reported that bats spent more time circling after the insertion of gates (with horizontal bars set 150 mm apart) and reduced their flight speed and approach height. Spanjer and Fenton (2005) also reported an increase in circling behaviour and showed that bats will habituate to gates, but never completely. The immediate and medium term effects of gate replacement are currently being studied at a site in the Yorkshire Dales. A cave entrance largely covered by a solid steel lid has been fitted with a gate with an open grille. Bat activity and behaviour is being monitored before and after installation using automated loggers and infra-red video recording (see section 6.4).

It is important that the longer term effects of gates are investigated since published data are non-existent: only anecdotal evidence is available. The Vincent Wildlife Trust has installed grilles with a spacing of only 125 mm on a number of lesser horseshoe bat summer roosts. The bats do alter their behaviour and long after gating they continue to light sample behind the gate when previously they would have flown further from the roost (J. Messenger, pers. comm.). However, at several sites the number of lesser horseshoe bats increased dramatically after gating, suggesting that any adverse effects of gating were much less significant than the benefits of reduced disturbance. However, both gated and ungated lesser horseshoe bat roosts in the Usk valley have increased in size over the last 20 years, although no data are available for analysis (J. Messenger, pers. comm.).

In conclusion, bats are reluctant to pass through the narrow grilles (spacing ≤150 mm) used in many current gate designs. What impact the gates themselves have on hibernating and swarming behaviour is uncertain, since the limited data available are difficult to interpret. Although some early gates led to (often massive) declines in underground bat populations, many gated sites continue to be used and have indeed seen population increases. It is currently impossible to separate the potentially negative effects of the gate itself from the positive benefits of reduced disturbance or from underlying population trends. A gating programme, replacing inappropriate barriers to sites in the Yorkshire Dales would not aim to restrict human access, but simply exclude livestock, so gates could have broad grilles (>150 mm) that appear not to impede bats. There is no suggestion that unobstructed sites should be altered in any way. It should be emphasised that where possible, adequate monitoring of bat populations should be carried out before and after installation of a grille in order to determine the short- to long- term effects of such action. In addition, concurrent monitoring of unaltered sites close by could control for underlying population trends. The following section discusses current techniques available for survey and monitoring.

6.4 Methods for surveying and monitoring bat populations at underground sites

The value of monitoring is self-evident:

- For assessing the relative importance of a site
- For monitoring population fluctuations
- For assessing the effects of gating or other human intervention
- For scientific study

Visual winter surveys have been used for many years to estimate the number of bats occupying a site at a given time. This method can be time-consuming and it has severe limitations. Many underground sites, particularly natural sites, are large, complex and have many inaccessible hiding places for bats. Only horseshoe bats are easy to find and most other species are seriously underestimated (Altringham 2003). Repeated visits to sites over the winter also run the risk of causing excessive disturbance, increasing arousal frequency and causing the bats to use up vital energy reserves (Thomas 1995). Monitoring of the movements of bats as they go in and out of roosts is even more labour-intensive without automated systems, particularly at hibernacula, since activity is low and the monitoring periods long. Automated logging systems have the potential to collect long runs of quantitative or semi-quantitative data on roost use, with minimal effort. In theory they can be used to address all of the issues raised at the beginning of this section. The ideal automated logging system would count the number of bats going into and out of the site, identify each bat to species and log the time of each event. In reality, counting individual bats and identifying the species can be highly problematic.

6.4.1 Automated logging systems

A logging system attempts to count bats, but since bats frequently fly around the entrance to a cave or mine before entering or leaving, many loggers count 'bat passes'. In the case of logging systems based on ultrasound, a bat pass is the discrete train of echolocation pulses heard as a passing bat enters and leaves the detection range of a bat detector. Most logging systems therefore give an index of activity in the vicinity of the logging device, not a count of the number of individual bats. Activity can only be compared across sites if it is assumed that bat behaviour is similar at all sites. It must also be assumed that all bat passes are logged even when sites have entrances of very different dimensions.

Some loggers can determine the direction of a bat pass and are therefore capable of counting net flow into and out of a cave. However, individual bats may still be logged more than once if they make repeated flights in and out of a site, so once again these systems do not provide a population count.

Bats can be detected in several ways:

- They emit ultrasonic echolocation calls that can be detected and even recorded.
- They can be made to pass through barriers of infra-red beams that detect their passage.
- They can be photographed or recorded on video.

Each method has advantages and disadvantages, both in terms of the information that can be obtained, their practicality, versatility and cost. The generic principles, advantages and disadvantages of each method are described below. Further details can be found in Glover and Altringham (2005)

6.4.1.1 Ultrasound

Bats echolocate using short (<50ms), high intensity (up to 120 dB at 10 cm), high frequency (15-150 kHz) sound pulses. All sounds decrease in intensity as they travel away from a point source due to spherical spreading and attenuation. Attenuation increases with frequency, so bat echolocation calls are rapidly attenuated and are detectable only over short distances. Although dependent on bat species and detection method, a logging system is unlikely to record many bats more than 25 m away and may be unreliable at distances >15 m.

Echolocation calls vary from species to species and may be relatively invariant within a species, at least with respect to the call properties currently measured. There is therefore considerable potential for species identification from their calls. Some species are easily identified, with minimal analysis, others require more detailed analysis and separation of some species is still beyond current methodology (see Chapter 4).

Other sources of ultrasound may interfere with logging systems. The most common problem in underground sites is the noise of dripping or turbulent water, which can have a significant ultrasonic component. A significant advantage of all ultrasound systems is that they are portable, self contained units (low power, compact, robust) that are not site-specific.

Three techniques are currently in use to detect and/or analyse echolocation calls:

- Tuneable, narrowband heterodyne detectors
- Broadband frequency division detectors
- Broadband time-expansion detectors

6.4.1.1.1 Heterodyne

Echolocation calls are picked up by an ultrasonic microphone. The signal from the microphone is mixed with that from a tuneable oscillator in the detector, to bring the output down to a frequency we can hear. Most detectors have a bandwidth of about 10 kHz, (±5 kHz of the tuned frequency) and will produce an audible output, and one that will trigger a logger when a bat's call is within this range. Most detectors can be tuned to frequencies between 10 and 150 kHz and typically have a high signal to noise ratio. Each bat species has a particular frequency/frequency range at which it focuses most of the energy in its call.

Since the bandwidth of heterodyne detectors is typically 5-10 kHz they can, in principle, be tuned to detect specific species. However, this requires empirical testing and species recognition can be unreliable. For example, greater and lesser horseshoe bats, with calls of say 84 kHz and 110 kHz, have detectable first harmonics at around 42 kHz and 55 kHz and could be logged by detectors set to record other species at these frequencies. If the aim is to detect all species, a single narrowband detector will not record all species when both *Myotis* and *Rhinolophus* species are present.

The output from a heterodyne detector can be used in two ways:

- Each bat pass can be counted and totalled over a given time. The information is therefore limited and there is no way of knowing that the count is uncontaminated by artefacts (see below).
- If the bat passes are sent to a logger that adds a 'time stamp' then a plot of bat passes against time is obtained. This can provide valuable information and also help to eliminate counts due to triggering by non-bat sources, e.g. dripping water, since they will have a different 'activity pattern' to bats. This method has been successfully used to identify key underground sites in the Yorkshire Dales (Chapter 2 and 3).

6.4.1.1.2 Frequency division

This method uses a zero-crossing circuit to identify the fundamental frequency component of the echolocation call. This is then divided, typically ten-fold, to lower the frequency into the audible range. The major advantage is that these are broadband detectors, and are able to pick up species echolocating between 10 and 150 kHz or beyond, without constant tuning. When connected to a logger, all UK species will be detected, although the low intensity calls of long-eared bats may again go unlogged. Although all harmonic and intensity information is lost, frequency information is preserved, so basic spectrographic analysis is possible if the calls are recorded for later analysis. A major disadvantage is that all sounds, including background noise, are amplified. Signal to noise ratios are typically low and some frequency division detectors can be noisy.

The output from a frequency detector can be used in two ways:

- Count only, as for heterodyne
- Bat calls stored digitally for later analysis

6.4.1.1.3 Time expansion

These detectors are the most expensive, but offer significant advantages. The signal from the ultrasonic microphone is captured by a fast analogue to digital (A/D) converter at very high sampling rates: fast enough to preserve all the detail of even the highest frequency echolocation calls. This information is temporarily stored in a memory buffer. It can then be played back through a D/A converter at a slower rate (usually one tenth), and thus at a frequency range low enough to be recorded onto an ordinary audio recording system.

Time-expansion bat detectors are the best option if the aim is to record calls for later analysis and species identification. By preserving virtually all the frequency and intensity information contained in the calls, including that of any harmonics, they make detailed spectrographic analysis possible (see Chapter 4). This is the only reliable and verifiable way to identify some bat species.

6.4.1.2 Infra-red (IR) beam barriers

These use an array of light-emitting diodes (LEDs) that work in the infra-red (IR) region of the spectrum. Opposite each LED is a photodiode sensor. When one of the beams is interrupted by the passage of a bat, cutting off light to one of the photodiodes, a signal is sent to the logger. If two arrays are used in series across the entrance, then the direction in which a bat is flying can be determined and so the number of passes into and out of the site can be counted. Arrays are often custom-built for a particular site, to cover the entire passage but it is difficult to build systems which cover large areas. Such systems have been used successfully at underground sites e.g. Degn *et al.* (1995); Redell (2004).

An alternative approach is to use a passive IR monitor that detects body heat and movement. These have the advantage that they pick up animals in a large cone of air space. They are similar in principle and design to many burglar alarms, which are known to be triggered by bats, so there may be scope for development here.

6.4.1.2 Automated photography/video

Active and passive IR monitors can be used to trigger cameras or camcorders, although the slow response of camcorders would make them more suited to control by timers. Photography has the potential for species identification, but limited storage capabilities. This sort of system has been implemented successfully in the monitoring of a hibernaculum in Germany (Lubeczyk and Nagel 1995).

Automated logging systems based on heterodyne detectors are reliable and portable, and as such are the best option for identifying important underground sites. They are particularly useful when surveying large numbers of sites. Logging autumn swarming activity is currently without doubt the best method of identifying hibernation sites of crevice-dwelling species. For long term/year round monitoring of important sites, IR double arrays can provide information on the net movement of bats. Population estimates of hibernating bats can then be made that are more reliable than those obtained from visual surveys. It should be noted that the installation of monitoring systems such as IR arrays are likely to modify entrances by reducing the aperture through which the bats fly. Reliable species identification is currently limited by the overlap in call structure between many species and the limited data storage capacity of most systems.

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