Factors Affecting Distribution and Habitat Selection of Water Shrews *Neomys fodiens*

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"It is a ravening beast, feigning itself gentle and tame, but being touched it biteth deep, and poisoneth deadly. It beareth a cruel mind, desiring to hurt anything, neither is there any creature it loveth" (Topsell, 1607).

Abstract

The water shrew *Neomys fodiens* is one of Britain's least known mammals and its habitat requirements are poorly understood. The purpose of this study was to determine occurrence and associated habitat preferences of water shrews, a species of conservation concern, by comparing populations in central England freshwater habitats. Bait tube surveys were undertaken at 32 freshwater sites to establish water shrew presence, half of which were found to contain water shrews. Habitat surveys were undertaken and, in addition to water shrew presence/absence data, were used to develop habitat suitability index models by means of artificial neural networks. Management intensity (occasional or frequent bankside management) was identified as the most important predictor of water shrew presence and, when combined with dissolved oxygen (0-2.99mg l^{-1}) and water depth (<25cm), created the highest performing model. These models will allow sites to be rapidly assessed for water shrew presence without labour intensive and costly live-trapping techniques. Prey availability was investigated by undertaking invertebrate surveys at four water shrew-positive sites, as well as at an additional four sites with unknown water shrew presence with which to compare. Overall, there was no significant difference between the total numbers of terrestrial and aquatic invertebrates at sites with known/unknown water shrew presence although there were differences in composition of potential prey. POPAN abundance of water shrews was estimated, and its relationship with other small mammal species investigated, using live trapping at the four water shrew positive sites. Negative relationships were found between water shrews and the terrestrial shrew species although these were not significant. Individually identifying captured water shrews using traditional fur-clipping marking methods is difficult. Therefore, buccal swab samples were taken to identify individuals via genetic profiling. Determining numbers of water shrews via genetic profiling was found to be more accurate than through fur-clipping which overestimated populations. Furthermore, buccal swab sampling is a new, minimally invasive method of identifying individuals which can be used to give accurate information about water shrew population densities and dynamics across seasons. This is the first in-depth study of factors affecting the occurrence and habitat selection of water shrews in central England and has made some important contributions to the understanding of habitat analysis and species identification.

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Chapter 1
General Introduction

The principal aim of this thesis is to determine occurrence and associated habitat preferences of water shrews *Neomys fodiens* (Pennant, 1771), a species of conservation concern, at various sites within central England. Secondly, habitat suitability indices will be developed for prediction of water shrew occurrence and to provide guidelines for effective management and conservation of this much understudied species.

1.1 Factors affecting species distributions

Species distributions are limited by many abiotic and biotic factors such as temperature (which is affected by latitude and altitude), dissolved gases and salinity, competition, predation, parasitism and disease (Connell, 1961; Paine, 1966; Terborgh and Weske, 1975; Randall, 1982; Canterbury, 2002; Munguía *et al*., 2008). These abiotic and biotic factors do not necessarily act in isolation but interact with and affect each other. Globally, species diversity follows a latitudinal gradient with the highest levels found in the tropics, lowest at the poles and intermediate levels in the temperate regions (Rohde, 1992; Gaston and Spicer, 2004; Krebs 2009). These patterns of diversity are typically explained by climate and energy availability. The tropics have the most favourable climate and highest levels of energy availability which are the ideal conditions for increasing plant and animal diversity and distribution (Connell, 1978; Huston, 1979; Stevens, 1989; Clarke and Gaston, 2006).

The pattern of species distribution is determined fundamentally by the ability of individuals to reach a particular geographical area. Topographical features such as water, deserts and mountain ranges are the main physical barriers limiting species distributions (Jeffree and Jeffree, 1994; Gaston, 2003). For example, although shrews can swim several kilometres across water (Hanski, 1986) they were absent from Newfoundland, which is

separated from Labrador by only 25 km of water, until *Sorex cinereus* was introduced in 1958 (Hanski and Kaikusalo, 1989). Therefore, the conditions of a particular habitat may be ideal for a species but if it cannot physically access the area colonisation is not possible.

Temperature is one of the main determinants of species distribution affecting not just single species, but the behaviour of all organisms including predators, prey and parasites (Coope, 1977; Randall, 1982; Atkinson *et al.,* 1987; Davis and Shaw, 2001; Pearson and Dawson, 2003). For example, the limit of distribution of rabbits *Oryctolagus cuniculus* in Australia is marked by the 27°C isotherm (Cooke, 1977) and the southern limit of northern hemisphere seals is restricted to sea surface temperatures below 20°C (Lavigne *et al*., 1989). However, in most mobile species it is usually the effect of temperature on the frequency and quality of their food, and not on the species directly, which is the limiting factor (Jeffree and Jeffree, 1994). Therefore, an organism will struggle to survive in an area if it depends on another species for food and that species cannot tolerate the prevailing environmental conditions. Opportunistic feeders such as Soricine shrews which feed on a wide variety of invertebrate prey may not be particularly affected by the lack of availability of any one prey type which may explain their wide distribution (Churchfield, 2008). However, the general lack of food availability at very high latitudes and elevations may limit their distribution in such areas (Hanski and Kaikusalo, 1989).

In aquatic environments, temperature interacts with the concentration of dissolved gases and is therefore an important factor in the distribution of aquatic gill breathing species (Brett, 1956; Begon *et al*., 2006; Sato *et al*., 2009). For example, the downstream limit to the distribution of brown trout *Salmo trutta* to upstream waters is determined by its particular oxygen requirements which are indirectly affected by temperature (Vincent and Miller, 1969). Temperatures are lowest and oxygen concentration highest in upstream waters. However, an increase in temperature downstream creates an increase in oxygen requirements for the trout but the increased water temperature causes a decrease in dissolved oxygen concentration (Jonsson and Jonsson, 2011).

If the physical and environmental conditions in a given area are within the species' optimum tolerance range and the animal is physiologically and behaviourally adapted to the environment, whether or not a species can become successfully established is determined by biotic factors such as predation, and interspecific and intraspecific competition for resources such as food, shelter from weather, nesting sites and territories (Sinclair *et al*., 2006). The principle of competitive exclusion (Gause, 1932) states that no two species can occupy the same niche. Co-existing species must differ in certain aspects to enable them to exploit different resources. If species are too similar selection will either lead to extinction of all but one of the species trying to occupy the same niche or lead to character displacement to make them less similar, thereby reducing competition and avoiding competitive exclusion (Hardin, 1960). An example of competitive exclusion caused by interspecific competition and exacerbated by disease can be seen in the United Kingdom between the native red squirrel *Sciurus vulgaris* and the introduced American grey squirrel *Sciurus carolinensis* (Bryce *et al*., 2002; Tompkins *et al*., 2002; Tompkins *et al*., 2003; Bruemmer *et al*., 2010).

Finally, anthropogenic factors such as exploitation, human-induced climate change, habitat destruction and fragmentation are major limiting factors making species survival more difficult as populations become small and fragmented and therefore less stable (Dirzo and Raven, 2003; Pimm *et al*., 2006; Isaac, 2009).

1.2 Species abundance and rarity

Species able to exploit a wide range of resources (generalists) tend to be both widespread and locally abundant whereas those which are narrowly restricted (specialists) tend to only occur at low local abundances (Brown, 1984). This has been widely demonstrated at a variety of spatial scales by the positive relationship between local abundance of a species and the size of its distributional range (Gaston and Lawton, 1990; Kouki and Hayrinen 1991, Hanski, *et al*. 1993; Gaston, 1996; Gaston and Curnutt, 1998). For

example, a positive abundance/range relationship has been reported in insects (e.g. Gaston, 1988; Williams, 1988), birds (e.g. O'Connor, 1987; Ford, 1990) and mammals (e.g. Brown, 1984). However, this is not a universal rule. Some studies have found no relationship between local abundance and size of distributional range (e.g. insects, Thomas and Mallorie, 1985; birds, Wilson, 1974) and others a negative one (e.g. birds, Ford, 1990; Schoener, 1990).

Rarity of a species can be defined in terms of low abundance and/or small range (Gaston, 1994; Rodrigues and Gaston, 2002; Lennon *et al*., 2004). In addition to this rather simple definition, other factors which have been used to identify rare species include habitat specificity and taxonomic distinctness. The state of rarity is both temporally and spatially scale dependent, and may have one apparent cause at the large scale (e.g. global climate) and another at the smaller scale (e.g. soil type) (Kunin and Gaston, 1993). The spatial distribution of a species can be described on three scales: local, regional and biogeographic (Gaston, 1994; see Table 1.1).

Table 1.1 Description of the spatial distribution of a species at different scales (after Gaston, 1994).

Scale	**Range**	**Definition**
Local	Micro	Small area of homogenous habitat
Regional	Meso	An area large enough to embrace many habitats, but not so large as to encompass the entire geographic range
Biogeographic	Macro	An area large enough to encompass the entire geographic range

Furthermore, a species may be globally rare but locally abundant (Murray and Lepschi, 2004). For example, many species inhabiting the tropics are common within tropical regions but on a global scale are rare (Williams *et al*., 2009). Large scale rarity usually refers to endemics, those species which occur only in a specific area and nowhere else. These species typically have smaller ranges and population numbers than non-endemics

(Kruckeberg and Rabinowitz, 1985). However, endemism does not necessarily equate to rarity as some endemic species may be very abundant (Williams *et al*., 2009). Gaston (1994) proposes that the least-abundant 25% of species in an assemblage should be defined as rare.

There are various factors which contribute to species rarity such as being restricted to an uncommon type of habitat, limited to a small geographic range and/or occurring at only low population densities (Rabinowitz *et al*., 1986). An example of a species restricted to an uncommon habitat is the Devil's Hole pupfish *Cyprinodon diabolis*, which occurs only in a single freshwater spring in Death Valley, California with a surface area of less than $100m^2$ (Brown *et al*., 1996). The Devil's Hole pupfish was isolated from other populations up to 30,000 years ago and like other cave-dwelling organisms, are blind and lack pigmentation, which restricts them to very specific environmental conditions (Culver *et al*., 2000).

Some species may be rare because they are limited to a small range by geographical barriers such as islands surrounded by ocean or lakes surrounded by land. For example, approximately 700 species of cichlid fish are endemic to Lake Malawi in Africa (Turner *et al*., 2001). A small geographic range may also be caused by more subtle barriers, such as soil type or water temperature, for species with narrow tolerances (Brown, 1984). Furthermore, the colonisation ability of a species, which is determined by both its dispersal and establishment ability, are factors which contribute to small geographic ranges in rare species (Gaston, 1994). For example, shrews are relatively poor dispersers due to their high metabolic rate, small body reserves and consequent short starvation times (Vogel, 1976). Establishment ability is affected by various aspects of reproductive biology, such as relatively lower fecundity and smaller litter sizes, both of which have been associated with species rarity (Glazier, 1980).

Finally, a species may be rare because it occurs only at low population densities for which there are many causes (Gaston and Lawton, 1990). However, the two main factors contributing to species with low population densities are large body size, as they simply require more space and have

higher energetic requirements than smaller organisms, and/or scarce and dispersed resources (Gaston, 1994). Carnivores are a classic example of species that live at relatively low densities because they are commonly top level predators which require large areas to range to obtain food (Williams and Thomas, 2009).

All species are limited in their distribution and abundance by the same processes, but rare species are more severely constrained (Gaston, 1994). Therefore, conservationists are particularly concerned with rare species because they may be more likely to become extinct (O'Grady *et al*., 2004; Seoane *et al*., 2011). In addition, rarity is used as a way of classifying species on the basis of their supposed risk of extinction (Hamaide *et al*., 2006; Mace *et al*., 2008). A species which only inhabits a small geographic range could be pushed into extinction by an environmental event which may encompass the species' entire range. For example, a specific catastrophe, such as a volcanic eruption on an island (Diamond, 1974; 1975) or a gradual change such as the immigration of a competitive species (Sax and Gaines, 2008). Likewise, a species which is restricted to an uncommon habitat may be more vulnerable to environmental change than habitat generalists (Isaac, 2009). Furthermore, the ability to adapt to a changing environment may be reduced in species which, over long periods occur at low population densities, caused by decreased genetic diversity from genetic drift, inbreeding and bottlenecks (Rojas-Bracho *et al*., 2006).

The distinction between species which are 'naturally' rare and those whose rarity is as a consequence of human activities is an important one. Rarity is a natural state, in fact in most ecological communities, only a few species are common while most others are more or less rare (Loreau, 1992; de Lange and Norton, 1998; Hartley and Kunin, 2003; Magurran and Henderson, 2003). Naturally rare species may possess life history characteristics that enable them to persist in this state (Kunin and Gaston, 1993; Harrison *et al*., 2008). Therefore, rarity in itself does not necessarily mean a species is under threat of extinction (de Lange and Norton, 1998; Robbirt *et al*., 2006; Mace *et al*., 2008). Rabinowitz (1981) developed the 'seven forms of rarity' model to categorise rare species. The model focuses

on three characteristics of species: (i) the species distribution area, (ii) the variety of habitats occupied by a species and (iii) the local population density. Each of these three measures is a simple dichotomy yielding eight possible categories, seven of them indicating rarity (Pagel *et al*., 1991; Kryštufek *et al*., 2009).

The International Union for the Conservation of Nature (IUCN) Red List of Threatened Species is a system of measuring extinction risk using five independent criteria relating to aspects of population loss and decline of range size (Vie *et al*., 2008). Threatened species are categorised as Critically Endangered, Endangered, and Vulnerable depending on the following criteria: A) high decline rate, B) small range area and decline, C) small population size and decline, D) very small population size and E) unfavourable quantitative analysis. The state of natural rarity has been part of an ongoing debate regarding the categorisation of species by the IUCN (Mace *et al*, 1992; Mace and Kunin, 1994; Mace *et al*., 2008). For example, a small and stable population may be less susceptible to extinction than a large but declining population (Mace *et al*., 2008). Therefore, placing rare species into threatened categories simply on the basis of rarity would greatly increase the numbers of species listed and include many that are under no particular threat of extinction. However, placing rare species in the same category as widespread and more abundant species is also inappropriate. Rare species (very restricted in population size or very restricted in area) are now listed as Vulnerable under subcriterion (D2) of criterion D which allows species to qualify solely on the basis of a very restricted distribution. For example, the Isarog shrew mouse *Archboldomys luzonensis* comes under this classification not because its populations are declining, in fact they are a moderately common species with a stable population, but because they are restricted to Mount Isarog on Luzon Island in the Phillipines (Balete and Heaney, 2008). Similarly, the black-crowned dwarf marmoset *Callibella humilis* is also listed as Vulnerable under this subcriterion despite no evidence of any major threats at present (Mittermeier and Rylands, 2008). The species was previously listed as Least Concern but has been reclassified as Vulnerable on the basis that it is confined to a very small unprotected range and that its habitat is potentially

vulnerable to future destruction for agriculture (Van Roosmalen *et al,* 1998; Van Roosmalen and Van Roosmalen, 2003). Criterion D allows species to be listed as threatened without evidence of an actual or potential decline because theoretical models show that small populations can have relatively high extinction risks (Mace *et al*., 2008). This categorisation has been criticised for not recognising that rarity is a natural state and not automatically a sign of endangerment (de Lange and Norton 1998).

Species are assessed by the IUCN at the global level because this is the scale at which extinctions occur (Vié *et al*., 2008). However, the status of a species at local level is of concern because local declines, if not managed, can ultimately lead to global threat. The population status of a species may be deemed relatively stable within a country or region but still be at risk globally, whereas the status of another species may be deemed relatively secure globally but highly at risk in a particular area. While it is important and makes sense to assess a species risk of extinction at a global level effective conservation generally takes place nationally and locally (Mace *et al*., 2008).

In order to conserve a species effectively and implement the best conservation management its occurrence needs to be established (Mackenzie and Kendall, 2002; McCallum, 2005). Determining patterns in species occurrence to make inferences on habitat selection and to predict species distributions is increasingly being used in biodiversity conservation (Ruiz-Gutierrez and Zipkin, 2011). However, observed patterns of occurrence can be influenced by differences in detectability (the probability of observing a species or individual when present) during surveying, between species and in different habitat types (Kéry 2002; Tyre *et al*., 2003; Gu and Swihart, 2004; Mazerolle *et al*., 2005; O'Connell *et al*., 2006; Pellet, 2008; Gibson; 2011). These differences in detectability, if not taken into consideration, can lead to misrepresentation of habitat preferences (MacKenzie and Nichols, 2004; MacKenzie and Royle, 2005; Pagano and Arnold, 2009; Jeffress *et al*., 2011).

1.3 Habitat selection

The role of habitat selection in the structure or species communities is a key topic in ecology (Huey, 1991; Resetarits, 2005) as the study of habitat use is vital for understanding the conservation needs and management of wild species (Orrock *et al*., 2000; Freitas *et al*., 2008). Habitat selection by a species is affected by many factors such as morphological, physiological and behavioural adaptations (Morris, 1989), as well as predation (Vijayan *et al*., 2012), mate selection (Rosenzweig, 1979) and biogeographical constraints (Ruby, 1986). However, presence and abundance of competitors play a key role (Rosenzweig, 1981). Selection of the best habitat will depend on competition for key resources by other species (Vijayan *et al*., 2012) and it is this differential selection of habitats which allows species to coexist (Rosenzweig, 1981; 1991). At the small scale habitat selection is likely to be influenced by foraging areas within the home range, whereas at the larger scale dispersal and ability to relocate home range are likely to be the most important (Morris, 1992).

Habitat selection theory states that a species is able to coexist with its competitors by being selective with respect to habitat but may alter this strategy when the density of the competitors is experimentally reduced (Rosenzweig, 1981). Neet and Hausser (1990) found evidence that habitat selection in parapatric shrews *S. araneus* and *S. coronatus* is a response to the presence of a competitor and interspecific interactions, resulting from territoriality, maintain habitat selection in the contact zone.

Hanski and Kaikusalo (1989) found that habitat selection in boreal shrews was determined by a combination of food availability and interference competition. The larger *Sorex* species were more abundant in the most productive habitat whereas the smaller species were relatively and absolutely more frequent in the unproductive habitats. This was linked to large body size with the larger shrew species being competitively superior to the smaller species and the absence of a species from a given habitat was likely to be the result of interference competition with larger species.

Studies of a multispecies community of shrews in wetland habitats of the Białowieża Forest in eastern Poland found that the segregation of microhabitat of each species was determined by ground wetness and distance to a stream (Rychlik, 2000; 2001). The order of species from closest to furthest from the wetland areas was Neomys fodiens, *N. Anomalus*, *Sorex minutus*, *S. araneus.* A similar pattern of habitat segregation was found among shrews coexisting in Montesinho, central Portugal with *N. anomalus* occupying wet habitats directly at the water's edge, *Sorex granarius* occupying areas of intermediate wetness and distance from water and *Crocidura russula* occupying dry habitats up to 15 m from water (Rychlik and Ramalhinho, 2005).

Ultimately habitat selection is determined by multiple cost-benefit tradeoffs such as food availability, competition, reproductive success and risk of predation etc. (Bastille-Rousseau *et al*., 2010). The ideal habitat may need to encompass a mixture of patches in order to contain all of the resources required for the species leading to compromises to be made. For example, good foraging habitats may not necessarily provide the best cover from predators and vice versa (Orians and Wittenberger, 1991). Morris (1989) found evidence that habitat selection by white-footed mice was density dependent (litter sized declined with density). However habitat selection is determined, the evidence that this is occurring in a taxon is provided by the distribution patterns of species where there is presumed equal access to the habitats being examined.

1.4 Shrew distribution, classification and ecology

Shrews are distributed widely throughout the world; absent only from the polar regions and Australasia (Macdonald, 2001; Churchfield, 2008). A number of morphological features characterise shrews including a narrow pointed snout, small eyes, short, rounded ears, short legs, plantigrade feet (walk with soles and heels on the ground) with five digits, slender tail, short dense fur and scent glands on the flanks (Churchfield, 2008).

It was believed that possession of certain primitive features such as relatively small brains with few wrinkles to increase the surface area, intra-abdominal testes, a plantigrade gait and possession of a cloaca (Macdonald, 2001) place shrews (Soricidae) firmly in the Insectivora (Lipotyphla) (Wilson and Reeder, 2005; Churchfield, 2008) along with four other insectivorous small mammal families: Chrysochloridae (golden moles), Erinaceidae (hedgehogs and gymnures), Tenrecidae (tenrecs) and Solenodontidae (solenodons) (Stanhope *et al*., 1998; Macdonald, 2001; Carter and Churchfield, 2006b). However, there is still much debate regarding the phylogenetic relationships between the families in the light of anatomical and molecular studies (Emerson, 1999; Wilson and Reeder, 2005). For example, Stanhope *et al.* (1998) found molecular evidence that the mammals of Insectivora are not monophyletic (originating from a single common ancestor) as traditionally believed, but paraphyletic (containing some, but not all, of the descendants from a common ancestor) and therefore should be reclassified to reflect this by partitioning Insectivora and placing the African families Chrysochloridae (golden moles) and Tenrecidae (tenrecs) into a new order Afrosoricida. Furthermore, Douady *et al.* (2002) found strong molecular evidence that shrews and hedgehogs share a sister-group relationship to the exclusion of moles. Thus, currently, the order Insectivora has been abandoned although how many orders have replaced it is unclear. According to Wilson and Reeder (2005) and Churchfield (2008) Insectivora has been replaced by three separate orders Erinaceomorpha (hedgehogs, gymnures and moonrats), Afrosoricida (golden moles and tenrecs) and Soricomorpha (shrews, moles and solenodons). However, according to Macdonald (2009) Insectivora has been replaced by two orders Afrotheria (containing tenrecs and golden moles) and the Eulipotyphla (which is divided into two sub-orders; the Soricomorpha and the Erinaceomorpha).

There are currently 26 genera and 384 known species within the shrew family Soricidae which is currently divided into three sub-families; the white-toothed shrews (Crocidurinae), the African mouse shrews (Myosoricinae) and the red-toothed shrews (Soricinae) (Wilson and Reeder, 2005; Macdonald, 2009). The Myosoricinae is a recently assigned sub-

family of three genera previously belonging to the sub-family Crocidurinae (Macdonald, 2009). Red-toothed shrews possess a deposition of iron in the outer layer of the enamel on the tips of their teeth which may increase resistance to wear; white-toothed shrews do not have this feature (Macdonald, 2001; Carter and Churchfield, 2006b).

1.4.1 Water shrews

There are thirteen species of water shrew belonging to four genera within Soricinae; *Chimarrogale*, *Nectogale*, *Neomys* and *Sorex* (Churchfield, 1998) (see Table 1.2). Water shrews possess a number of anatomical adaptations which distinguish them from terrestrial shrews and equip them for their semi-aquatic existence. For example, *Neomys* and *Chimarrogale* have a fringe of stiff hairs on both lateral edges of each toe (Hutterer, 1985) probably to aid propulsion during swimming (Churchfield, 2008) and *Nectogale* has webbed feet. Some water shrew species have a wide geographic distribution although generally they are more restricted globally than the terrestrial species (Churchfield, 1998). For example, water shrews are absent from Africa despite many other genera and species of shrew being present (Wilson and Reeder, 2005) which may reflect the limited availability of suitable riparian habitats. There is also morphologically distinct subspecies of *Neomys fodiens*, *N. niethammeri* in Western Spain which has a restricted range and may be threatened although further taxonomic investigation and population monitoring is needed (Hutterer *et al*., 2008).

Although they can be widespread, water shrews generally occur at lower population densities than their terrestrial counterparts (Churchfield, 1998). For example, in multi-species communities of shrews, water shrews generally constitute only a small proportion of the population (Aulak, 1970; Yalden, 1973; Sheftel, 1989; Cantoni, 1993; Rychlik and Ramalhinho, 2005). Eurasian water shrews *Neomys fodiens* are one of three species belonging to the genus *Neomys* and are widely distributed across Europe and Asia (see Figure 1.1) (French *et al.*, 2001; Aloise *et al.*, 2005). As the

focus of this study is the Eurasian water shrew *Neomys fodiens*, from this point 'water shrew' will refer to this species.

Table 1.2 Species of water shrew (Churchfield, 1998).

American water shrews	**Asian web-footed water shrew**	**Eurasian water shrews**	**Oriental water shrews**
Sorex alaskanus	*Nectogale elegans*	*Neomys anomalus*	*Chimarrogale hantu*
S. bendirii		*N. fodiens*	*C. himalayica*
S. palustris		*N. teres*	*C. phaeura*
			C. platycephala
			C. styani
			C. sumatrana

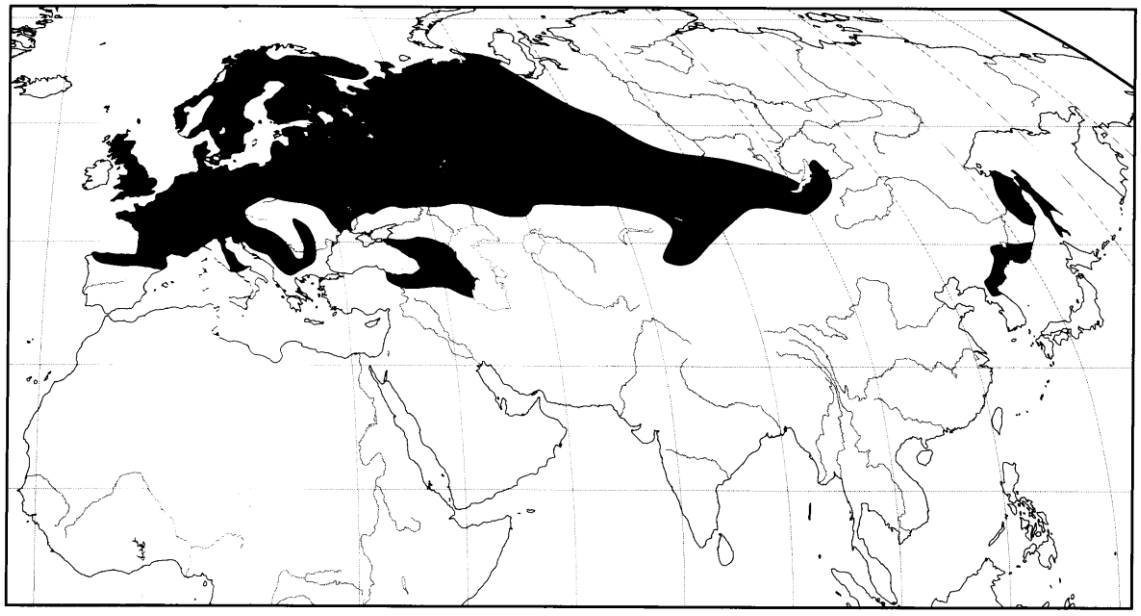


Figure 1.1 Worldwide range of the water shrew *Neomys fodiens* (Harris and Yalden, 2008).

1.4.1.1 Distribution

The water shrew is the largest of six species of shrew inhabiting the British Isles and one of the three shrew species inhabiting the UK mainland (see Table 1.3). Within the British Isles water shrews have a wide distribution and are present on many islands including Skye, Mull, Anglesey and the Isle of Wight (Churchfield *et al.*, 2000; Greenwood *et al.*, 2002; Carter and Churchfield 2006a; Churchfield, 2008). However, they are more localised in Scotland and absent from Ireland (Tew, 1995).

Table 1.3 The six species of shrew inhabiting the British Isles (Churchfield, 2008).

Sub-family	Species	Common name	Distribution
Soricinae (Red-toothed shrews)	*Neomys fodiens*	Water shrew	Mainland Britain, but absent from Ireland
	Sorex araneus	Common shrew	Mainland Britain, but absent from Ireland
	Sorex minutus	Pygmy shrew	Widespread in the British Isles
	Sorex coronatus	Millet's shrew	Jersey
Crocidurinae (White-toothed shrews)	*Crocidura russula*	Greater white-toothed shrew	Alderney, Guernsey and Herm Islands
	Crocidura suaveolens	Lesser white-toothed shrew	Sark and Jersey

In 2004-2005 the first nationwide survey of water shrews was undertaken in the UK (Carter and Churchfield, 2006a). Water shrews were detected at 387 (17.4%) of the 2159 sites surveyed and were distributed throughout mainland Britain, but with predominance in central and eastern England and a scarcity in northern Scotland (see Figure 1.2). The lower frequency of occurrence of water shrews in upland regions throughout Britain could be related to the colder and wetter climate, high altitude, steep topography and possibly low pH, due to geology and the presence of acid soils and peat, which may affect their aquatic invertebrate prey (Bell, 1971; Allard and Moreau, 1987).

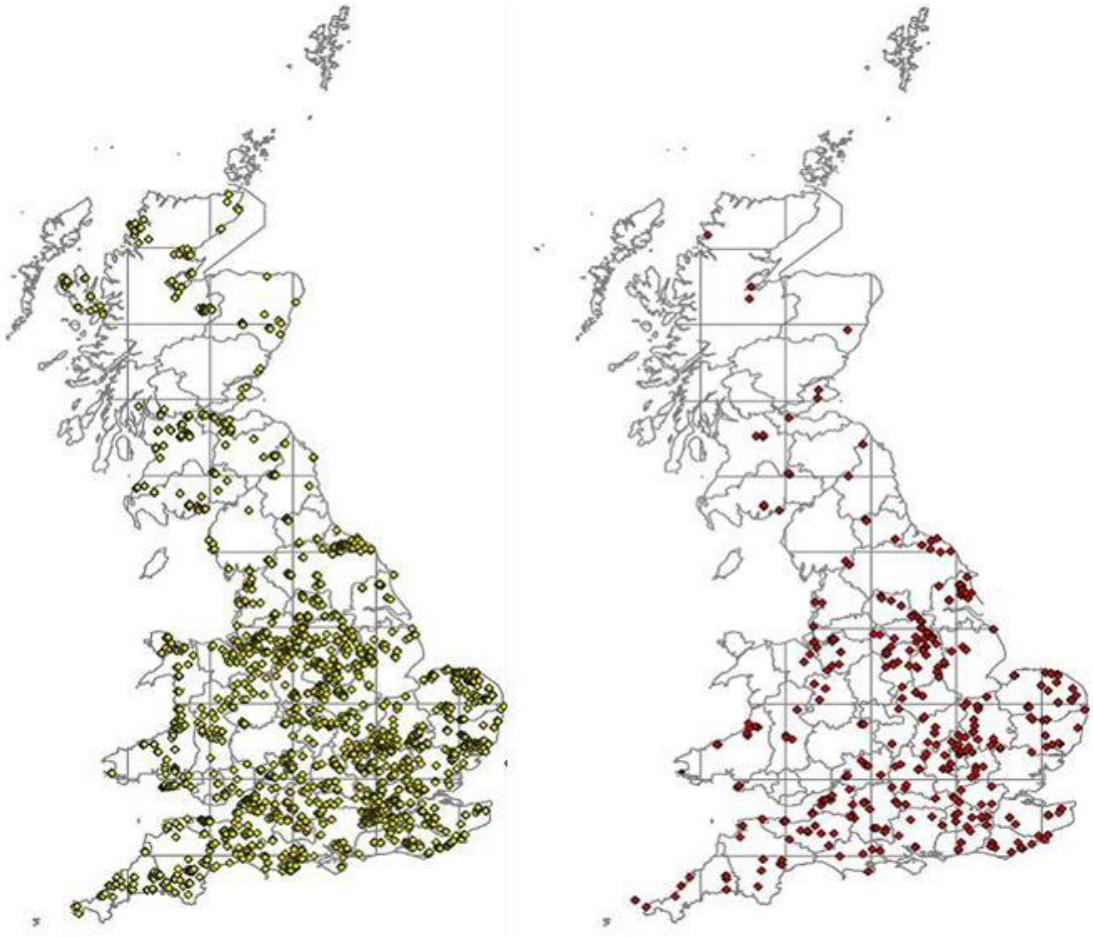


Figure 1.2 Maps showing all sites surveyed during the National Water Shrew Survey (left) and the sites which generated positive records of water shrew (right) (Carter and Churchfield, 2006a).

The National Water Shrew Survey has provided the first evidence that easting is a significant factor in the distribution of water shrews. The reasons for this might be related to the relatively warmer and drier climate (Lake *et al*., 2003), low altitude, low topography and type of habitats (especially lowland riparian) available in eastern England. However, there are some interesting exceptions to this pattern. For example, there seems to be a relative lack of water shrews in Lincolnshire and parts of East Anglia such as the Fens, west Norfolk, large parts of Suffolk and Essex. Much of these regions are subject to intensive arable agriculture (Robinson and Sutherland, 2002; Critchley *et al*., 2004) and it may be that the associated lack of suitable habitat and poorer water quality (for example high levels of nitrates) is responsible for this pattern in water shrew distribution.

However, despite the evidence for an association between water shrews and easting, Carter and Churchfield (2006a) advise caution when interpreting these findings as the predictive ability of the statistical models was poor suggesting other (as yet unidentified) more important factors in predicting occurrence. Furthermore, it must be acknowledged that the whole UK has not been surveyed and the distribution of water shrews largely reflects the distribution of surveyors.

Prior to the National Water Shrew Survey, the Mammal Society produced a distribution map of historical water shrew records in the British Isles, collected between 1993 and 2006, from a variety of sources including live-trapping, cat kills and owl pellet analysis (see Figure 1.3). When this map is compared with the records of the National Water Shrew Survey (see Figure 1.2) water shrew distribution appears to have declined in some regions. For example, it would appear that water shrew distribution in western Scotland has reduced since 1993. However, many of the records from this region are 'other method or unknown source', which suggest there may be issues with their reliability as records from 'live sightings' and 'live-trapping' do seem to follow a similar distribution to the National Water Shrew Survey. Unlike the Water Shrew Survey maps which show both the sites surveyed and the sites positive for water shrews there is no way of knowing whether water shrews are absent from the areas of the historic map without records or if they just have not been surveyed. Therefore, the map should be interpreted with caution.

1.4.1.2 Habitat

Over its worldwide range, the water shrew typically inhabits temperate deciduous forests and coniferous taiga where it is associated with wetland environments such as streams, rivers, marshes and bogs (Lardet, 1988; Churchfield, 1998; Macdonald, 2001).

Water shrews have been recorded extensively within the British Isles, in a wide variety of riparian habitats. These include ponds, drainage ditches, canals, reed beds, fens, marshes and bogs but particularly clear, fast-flowing, unpolluted rivers, streams, and watercress beds with which they

have been traditionally associated (Wolk, 1976; Churchfield, 1998; French *et al*., 2001; Greenwood *et al*., 2002; Aloise *et al*., 2005). However, in rare examples they have also been found in woodland, hedgerows and grassland up to 3 kilometres from water (Churchfield, 2008).

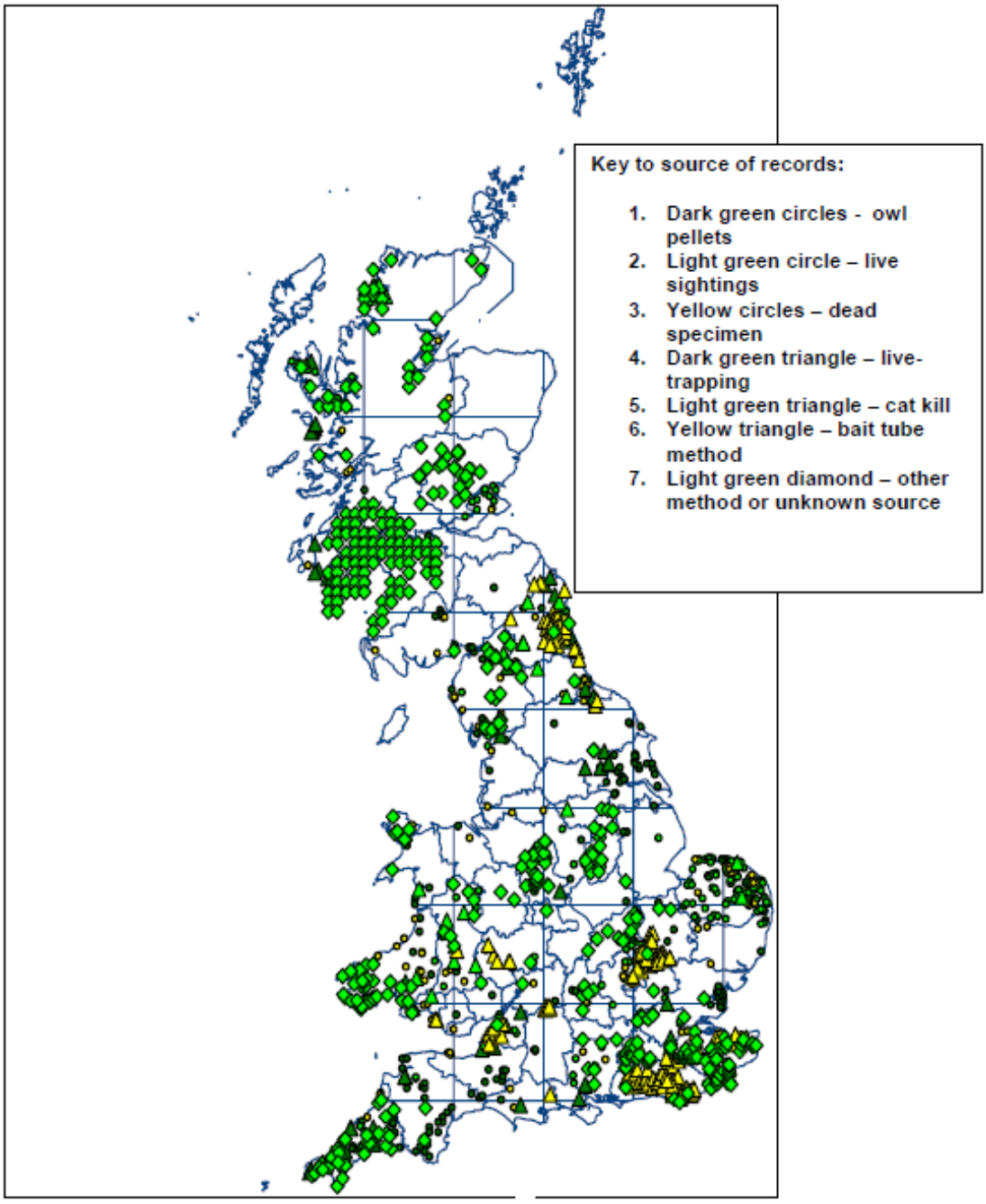


Figure 1.3 Historical records of water shrews from 1993-2006 (not including records from the National Water Shrew Survey) (Carter and Churchfield, 2006a).

Water shrews occupy extensive underground burrow systems in the banks of streams with the entrances above water level (Lardet, 1988). They may modify and inhabit the burrows of other small mammals. Their one or two rounded nests of moss, dried leaves and grass are usually below ground but above the highest level of the water (Churchfield, 2008). Nests are sometimes made in old tree stumps (Lardet, 1988).

Greenwood *et al.* (2002) surveyed 96 sites for the presence of water shrews using bait tubes at a variety of lentic and lotic freshwater habitats (including rivers, streams, canals and ditches) within the catchments of five rivers in the Weald. The majority of habitats investigated in the study were lotic, although no evidence was found for preference of either lentic or lotic habitats.

Sites surveyed during the National Water Shrew Survey (Carter and Churchfield, 2006a) included rivers, streams, canals, ponds/lakes, ditches, fens/marshes, reedbeds, bogs and cressbeds. Evidence of water shrews was found at all types of aquatic habitats surveyed except for the last two. This initially seems surprising as water shrews have been associated with cressbeds in the past (Churchfield 1984a; Churchfield, 1997a) however, this may be explained by the fact that only two cressbeds were actually surveyed. In addition, water shrews were found at a greater number of lentic sites such as canals and ponds than lotic ones such as rivers and streams with which they have been typically associated. This finding was in contrast to that of Greenwood *et al*. (2002).

1.4.1.2 Population dynamics

1.4.1.2.1 Density

Water shrew population density varies greatly according to habitat and season but is always much lower than that of the common and pygmy shrews (see Table 1.4). Harris *et al.*, (1995) estimated the spring population of water shrews in the British Isles at 1.9 million (England, 1.2 million; Scotland, 0.4 million; Wales 0.3 million) which is considerably less than the estimate for pygmy shrews (4.8 million) and common shrews (41.7 million). Their dependence on freshwater habitats means that there

is a much smaller optimal habitat for water shrews than other shrew species, which are terrestrial.

Studies have revealed that the percentage of shrew captures which constituted water shrews was similar for watercress beds in southern England (31%) (Churchfield, 1984a) and wetland habitat in Poland (>30%) (Aulak, 1970), but much lower in marshland in France (6-8.5%) (Yalden *et al*., 1973). The highest densities of water shrews to be recorded were at watercress beds in the south of England (3-5 per ha) (Churchfield, 1984a) and along a canal in Switzerland (<4.6 per 250m canal) (Cantoni, 1993).

Table 1.4 Estimates of population densities for three species of shrews.

Species	Density per ha	Habitat	Author
Water shrew	3	Watercress beds, England	Churchfield (1984a)
	<4.6 (per 250m canal)	Canal, Switzerland	Cantoni (1993)
Common shrew	2-69	Grassland, England	Churchfield (1995)
	12-18	Dune scrub, Netherlands	Michielson (1966)
	4-26	Spruce plantation, Germany	Kollars (1995)
Pygmy shrew	25-40	Dune scrub, Netherlands	Michielson (1966)
	25-40	Grassland, Ireland	Ellenbroek (1980)
	5-30	Grassland, England	Churchfield and Brown (1987)
	2-7	Spruce plantation, Germany	Kollars (1995)

1.4.1.2.2 Lifespan and breeding

Water shrews have a lifespan of 14-19 months (Price, 1953). Females produce between one and three litters (gestation 19-21 days) of, on average, 6 young between April and September with a peak in May-June (Price, 1953; Churchfield, 1984a). The young shrews overwinter to breed the following spring/summer and at the end of the breeding season most of the adults die off leaving the young to carry the population over to the following year (Carter and Churchfield, 2006a).

Water shrews therefore, follow a seasonal lifecycle, with a marked rise in numbers during the summer, a decrease in the autumn (as old shrews die

off) and low numbers throughout the winter. Following weaning, juvenile water shrews disperse from their natal area and during this time young shrews may be found hundreds of metres, even several kilometres, from water (Churchfield, 1990).

1.4.1.2.3 Predation factors

The main predators of water shrews are carnivorous birds such as owls, kestrels and buzzards. They are also occasionally eaten by mammals (e.g. weasels, stoats and foxes) and fish (e.g. pike) (Churchfield, 2008). However, few mammalian predators take shrews as a major component of their diet possibly due to their unpalatable odour (Eadie, 1938; Macdonald, 1977; Churchfield, 1990). For example, domestic cats will regularly kill shrews but rarely eat them. Both female and male shrews have a number of glands which, particularly the flank glands in males, exude a strong odour (Churchfield, 1990), although this odour acts more as a means of communication in shrew social organisation than as protection against predators. Predatory birds have a much poorer sense of smell compared with mammals (Smith and Reichman, 1984) however shrews still only constitute a small proportion of their diets compared with small rodents (Southern, 1955; Buckley and Goldsmith, 1975). For example, an analysis of tawny owl *Strix aluco* pellets revealed that shrews constituted 5.5% (common shrews 5%, pygmy shrews 0.3% and water shrews 0.2%) by weight of their total prey compared with 57% of rodents (bank voles *Clethrionomys glareolus* 24%, wood mice *Apodemus sylvaticus* 23% and field voles *Microtus agrestis* 10%) (Southern, 1955).

In a similar study on the diet of barn owls *Tyto alba* (Buckley and Goldsmith, 1975), shrews constituted 14% (common shrews 12%, pygmy shrews 1% and water shrews 1%) by weight of the total prey compared to 61% of rodents (field voles 52%, wood mice 6% and bank voles 3%). These comparatively low proportions of shrews probably reflect the difference in small mammal population densities in the habitats studied and therefore prey availability. For example, barn owls mainly hunt over open habitats and therefore take prey characteristic of these environments such as field voles and common shrews (Buckley and Goldsmith, 1975) whereas

tawny owls prefer woodland and therefore consume more bank voles and wood mice (Southern, 1955). Although barn owls take common shrews regularly throughout the year, they are caught more frequently in summer and autumn (Southern, 1955). During these times shrew population numbers are at their highest and more time is spent on the ground surface (Michielsen, 1966) as juveniles disperse and look for new territories which would explain the higher frequency of predation (Churchfield 1990). Water shrew population density is the lowest of the British shrews (Harris and Yalden, 2008) which together with its association with riparian habitats makes it an unlikely regular source of prey for many predators such as tawny and barn owls. It is unsurprising therefore that water shrews only counted for 1.2% of the combined diet of both species of owl (Southern, 1955; Buckley and Goldsmith, 1975).

1.4.1.2.4 Home range and territory size

The main purpose of territoriality is to defend resources, usually food (Ostfeld, 1990). Shrews are territorial animals and as such maintain clearly defined boundaries (Churchfield, 1990). Studies of common and pygmy shrews (e.g. Shillito, 1963; Michielsen, 1966; Cantoni, 1993) have revealed that they are solitary, territorial and display extreme aggression towards each other. As they mature, shrews establish territories and become socially dominant ousting strangers, old, and sometimes, socially inferior young shrews (Churchfield, 1990). Water shrews too are solitary and territorial (Krushinska and Rychlik; 1993; Lardet, 1988) but more tolerant of their own kind than common and pygmy shrews (Churchfield, 2008).

Several studies have examined the spatial behaviour and activity patterns of water shrews. Various techniques to measure home range size have been utilised such as visual assessment (Illing *et al*., 1981), capture-mark-recapture (Van Bemmel and Voesenek, 1984) and radioactive tracking (Lardet, 1988; Cantoni, 1993). The home ranges of shrews vary a great deal in their shape depending on factors such as proximity of neighbours, vegetation and topography (Churchfield, 1990). Home ranges of shrews living in hedgerows are linear whereas the home range in grassland may be more oval, triangular or square shaped. Water shrews usually occupy linear

home ranges based on the bank side of the water body (Van Bemmel and Voesenek, 1984; Churchfield, 1990; Cantoni, 1993). The home range sizes of the common and pygmy shrews are much larger than that of the water shrew which may be due to their considerably higher energy requirements (Genoud, 1985; Lardet, 1988; Rychlik and Jancewicz, 2002) (see Table 1.5). In addition, the prey availability of aquatic invertebrates may be more abundant and dependable than for terrestrial invertebrates (Churchfield, 1998). For example, Lardet (1988) investigated spatial behaviour and activity patterns of water shrews at a stream in Switzerland using radio-isotope tracking, and found the shrews had a home range size of 77-173m^2 (mean 106m^2) in winter and 101-373m^2 (mean 207m^2) in summer. This corresponds to the greater abundance of aquatic prey available during the winter compared with summer (Churchfield, 1998). Similar home range sizes have been recorded by Van Bemmel and Voesenek (1984), at peat bogs in the Netherlands (118-276m^2) and Illing *et al.* (1981), along a brook in Germany (only 20-30m^2 on land but 60-80m^2 when the water surface was included). Cantoni (1993) undertook a similar study to Lardet (1988) and found that water shrew home range sizes were larger in the winter (441-468m^2) than in summer (260-297m^2). Home range size was also similar for males and females and there was an increase at the beginning of winter and decrease following the breeding season. In addition, the water shrews were found to be territorial throughout the year with relatively low home range overlap. However, an increase in home range overlap was recorded between males and females during the breeding season. Females were territorial all year round with very little home range overlap (particularly apparent during the spring). Conversely, males did not defend territories during the breeding season as they wandered, often long distances (~500m), in search of females, suggesting an overlap promiscuous mating system (Cantoni, 1993).

Table 1.5 Examples of estimates of home range size (m^2) for three species of shrew.

Species	Winter	Summer	Habitat	Author
Water shrew	77-173	101-373	Stream	Lardet (1988)
	441-468	260-297	Canal	Cantoni (1993)
		118-276	Peat bog	Van Bemmel and Voesenek (1984)
Common shrew	500-600	400-450	Dune scrub	Michielson (1966)
	2,800		Woodland	Buckner (1969)
	800-1,700		Woodland	Ivanter *et al.* (1994)
Pygmy shrew	900-1,850	530-800	Dune scrub	Michielson (1966)
	1,400-1,700		Grassland	Pernetta (1977)

During the breeding season there is an apparent system of shifting home ranges, particularly amongst juveniles, with water shrews often leaving the water's edge, (where space is limited), and travelling through the countryside until other suitable habitats are reached (Chuchfield, 1990). These wandering tendencies explain the sudden appearance of water shrews in habitats such as hedgerows, woods or grasslands far from water (Churchfield, 1990). Such nomadic behaviour is supported by the low recapture rate of water shrews compared to other shrew species during population studies (Churchfield, 1990). For example, Shillito (1963) found that a water shrew population moved progressively through a woodland until eventually they left it completely. Of the fourteen animals caught during June and July, eight were not caught after August and then one shrew per month was lost until they had all gone. Shillito concluded that although a few may have died, they had probably left the wood in search of better resources. However, Lardet (1988) found that the home ranges of the water shrews he studied did not change more than a few metres over several weeks with the shrews still foraging within the same area. Another British riparian mammal species which appears to display similar nomadic tendencies is the otter *Lutra lutra* (Chanin, 1985). However, it is now known that although adult otters travel large distances (up to 48km) it is usually within their well defined (linear) home range (Kruuk *et al*., 1993).

The recorded daily movements of water shrews range from 10-200m although 10-60m is typical (Churchfield, 2008). During field observations of water shrews made through live-trapping in woodland, Shillito (1963) measured the distance they moved during daily activity. This varied between 28 and 162m compared with the maximum daily movement of 144m for common shrews and 60m for pygmy shrews. Other studies which have measured the distance water shrews travelled within their range recorded mean distances of 26m in watercress beds (Churchfield, 1984a) and 49m along a stream (Lardet, 1988).

Differences in food availability and habitat shape probably explain the variation in the mean distances travelled by water shrews within their home ranges. Watercress beds have an abundance of aquatic invertebrate prey such as *Asellus* throughout the year (Churchfield, 1984b) and are therefore a favoured habitat of water shrews having the highest recorded population densities (Churchfield, 1984a). The availability of prey and non-linear (rectangular) shape of watercress beds probably explains the shorter distances travelled by water shrews inhabiting such environments to those occupying linear habitats such as streams where they may need to travel further to forage.

1.4.1.2.5 Feeding ecology

Like all shrews, water shrews must eat every two to three hours and consume 50% of their body weight daily in order to sustain their high metabolic rate and avoid starvation (Crowcroft, 1957; Hawkins and Jewell, 1962; Rychlik and Jancewicz, 2002).

Water shrews exploit both terrestrial and aquatic environments in search of food, unlike the other purely terrestrial shrew species (Churchfield, 1984b). Foraging in water for prey may have an advantage over searching on land for terrestrial invertebrates as competition is limited mainly to insectivorous fish and birds, and food is in abundance (Churchfield, 1998).

For example, Churchfield (1984b) measured the abundance of aquatic invertebrates from streams supplying watercress beds and compared it with

the abundance of terrestrial invertebrates from a grassland/scrub area (Churchfield, 1982). Aquatic prey was found to have a significantly greater abundance (3358 per m^2) than terrestrial prey (1043 per m^2) (t = 14.12; p < 0.001) (Churchfield, 1984b).

The water shrew diet consists mainly of terrestrial and aquatic invertebrates plus frogs, newts and small fish (Dupasquier and Cantoni, 1992; Churchfield, 2008) although Haberl (2002), found evidence of water shrews feeding from carcasses of dead mice.

Water shrews secrete a narcotizing toxin in their saliva, an extremely rare phenomenon amongst mammals, which may enable larger prey, to be taken (Wolk, 1976; Churchfield, 1998). Commonly taken terrestrial prey typically include adult Coleoptera (beetles), Hemiptera (bugs), Myriapoda (centipedes and millipedes), Isopoda (woodlice), Araneae and Opiliones (spiders and harvestmen), Gastropoda (slugs and snails) and Lumbicidae (earthworms) (Churchfield, 1984b). Interestingly, of all the terrestrial invertebrates that water shrews commonly consume, millipedes are the only prey items not to be regularly eaten by the terrestrial common and pygmy shrews (Carter and Churchfield, 2006b).

Aquatic invertebrate prey include Trichoptera (caddis) larvae, Crustacea (*Asellus* and *Gammarus spp*.), aquatic snails, Diptera larvae and other insect nymphs and larvae (Churchfield, 1984b). The proportion of aquatic and terrestrial prey eaten varies, with an average component of 50% aquatic invertebrates being eaten by water shrews in freshwater environments in southern Britain (Churchfield, 1985). In contrast, in the Pyrenees the proportion of aquatic invertebrate prey was found to be up to 80% (Castien, 1995). Nevertheless, water shrews are capable of surviving on a diet solely containing terrestrial prey when they are away from water (Carter and Churchfield, 2006b).

1.4.1.4 Conservation status

Currently, global rates of species' extinctions are up to 1,000 times higher than the natural background rate (IUCN, 2011). This accelerated loss of biodiversity has been attributed to a number of factors the majority human-induced (Dirzo and Raven, 2003; Pimm *et al*., 2006; Isaac, 2009). Threats include habitat loss and degradation (which affect at least 86% of all threatened birds, mammals and amphibians), invasive species, over-exploitation, pollution and anthropogenic climate change (IUCN, 2011). According to the IUCN Red List (2011), of the 5,494 mammalian species worldwide for which there are data, 1,212 are in danger of extinction. Within Europe, 24% of mammal species are either classified as, or close to qualifying for, threatened status (Temple and Terry, 2007). Only 8% of species populations are known to be increasing, many of which (e.g. otters; Crawford, 2010) are due to successful species-specific conservation action (Temple and Terry, 2007). The commitment made by member states in 2001 to halt biodiversity loss and degradation of ecosystem services within the European Union by 2010, was not met. However, a new strategy has been adopted with the aim of meeting the commitment by 2020, as well as global commitments made by world leaders in 2010 to address global biodiversity loss (European Commission Environment, 2011).

The water shrew is listed on Appendix III of the Bern Convention which gives special protection through 'appropriate and necessary legislative and administrative measures', of the listed wild fauna species (Joint Nature Conservation Committee, 2012). Despite the apparent decline of local populations due to the loss and degradation of wetland habitats (Carter and Churchfield, 2006a) water shrews are classified as Least Concern by the IUCN as there is not deemed to be a serious threat to the global population at present (Hutterer *et al*., 2008). Justification for this classification include that the species is generally abundant, which appears contrary to other studies that have found water shrews to occur only at very low densities (e.g. Churchfield, 1984a; Churchfield, 1990; Cantoni, 1993; Greenwood *et al*., 2002; Carter and Churchfield, 2006a). However, in the UK water shrews are protected under Schedule 6 of the Wildlife and Countryside Act (1981) and are also on Natural England's (previously known as English Nature)

'conservation action priority' list (Wynne *et al*., 1995; Greenwood *et al*., 2002). In 1997, following an Environment Agency Research and Development project to assess the population status and methods of surveying water shrews in Hampshire (Churchfield, 1997a), the water shrew was identified as a 'species of concern' under the UK Biodiversity Action Plan and a Species Action Plan written. The water shrew was placed in this category due to its dependence on freshwater habitats and the threat to its population and habitats through the destruction of suitable bankside habitat via mechanized maintenance work, over-management of bankside vegetation and overgrazing by livestock (Churchfield, 1997b; Carter and Churchfield, 2006a).

Under the recommendation of the Species Action Plan to establish the nationwide status of the water shrew (Churchfield, 1997b), in 2004 the Mammal Society undertook a volunteer-based National Water Shrew Survey to investigate its distribution and habitat occurrence. Bait tubes were used by volunteers to detect the presence of water shrews in riparian habitats via faecal analysis (see Chapter 2, Section 2.1.3.1). Findings revealed water shrews to be widely distributed throughout Britain (Carter and Churchfield, 2006a) although, with the population size still undetermined it was not possible to fully assess their conservation needs. Although the survey provided good quality baseline data, several areas were elucidated as requiring further investigation including the impact of water quality and prey availability on water shrews (Carter and Churchfield, 2006a).

Prey availability was not investigated during the survey probably because of the limitations of using volunteers who would have needed some level of training to survey and identify aquatic invertebrates. Therefore, such a potentially crucial factor in water shrew distribution requires assessment. In addition, water quality (e.g. Biochemical Oxygen Demand, nitrates and pH) was found to have an effect on water shrew distribution. However, the water quality data used in the survey was provided by the Environment Agency National Data Unit's monitoring sites which, although close to (within 10km), did not always match the Water Shrew Survey sites and therefore more accurate water quality work is needed.

In order to conserve the species effectively and implement the best conservation management, habitat preferences need to be established. As with other riparian mammals such as otters, water shrews are vulnerable to pollutants and pesticides, which affect them both indirectly via prey and directly through grooming (Carter and Churchfield, 2006a). Accordingly, water shrews are largely absent in areas of low water quality (Greenwood *et al.*, 2002). Since water shrews occupy extensive underground burrow systems in the banks of streams (Lardet, 1988), their populations may also be affected by agricultural intensification including the disturbance and modification of waterside banks and vegetation (Macdonald and Tattersall, 2001). However, the exact relationship between environmental factors and occurrence remains unknown.

1.5 Research questions, aims and objectives

Previous work by the author in a preliminary study funded by the Mammals Trust UK, established presence of water shrews at a number of sites in the East Midlands an observation that coincided with the high density of water shrew records discovered in the National Water Shrew Survey. This study aims to determine occurrence and associated habitat preferences of water shrews, a species of conservation concern, at various sites in central England. These aims will be addressed by seeking to answer the following research questions:

1. What are the most important habitat features for predicting water shrew presence?

Establish evidence for water shrew occurrence at a range of freshwater sites (Chapter 2)

- Define the study area
- Select 32 freshwater sites
- Determine the occurrence of water shrews using bait tube sampling

Produce and test habitat suitability indices to establish the habitat preferences of water shrews (Chapter 3)

- Undertake habitat surveys at the 32 freshwater sites
- Develop habitat suitability indices using the data from the bait tube sampling and habitat survey data
- Test the habitat suitability indices on a subset of 'unseen' sites

2. Is water shrew presence associated with numbers and diversity of prey?

Investigate the effect of prey availability on water shrew presence at a subset of sites where water shrews were detected and a subset where they were not detected (Chapter 4)

- Select eight sites (four with and four without evidence of water shrew presence)
- Undertake aquatic and terrestrial invertebrate surveys at the eight sites
- Investigate the difference in invertebrate numbers and diversity at sites with and without evidence of water shrews

3. Is there an association between the relative abundance of water shrews and other small mammal species?

Investigate estimated numbers of water shrews and other small mammals at a subset of sites with known water shrew presence using live-trapping methods (Chapter 5)

- Select four sites where evidence of water shrew presence was found
- Undertake live-trapping over a number of seasons
- Estimate relative abundance of water shrews and other small mammal species
- Investigate the relationships between abundance of water shrews and other small mammal species

4. Can buccal swabs be used as a minimally-invasive method of genetic identification of water shrews?

Estimate abundance of live-trapped water shrews using DNA sampling to identify individuals (Chapter 6)

- Collect DNA samples from live-trapped water shrews using buccal swabs
- Evaluate buccal swab sampling as a method for obtaining DNA from water shrews
- Identify individual water shrews using genetic profiling
- Estimate water shrew abundance using genetic profiling

1.6 Projected outcome

The projected outcome of this work is production of a HSI as a system to assess rapidly sites for suitability of water shrews. This would enable:

- rapid evaluation of the suitability of a site for water shrews
- information to be obtained about key habitat features of importance to water shrews
- information to be obtained about habitat features which could be managed in such a way as to encourage water shrews
- predictions to be made about a particular site's suitability for water shrews and/or likelihood of having them

This HSI will help to inform conservation bodies and wildlife managers of how best to maintain and encourage water shrew populations and those of other riparian mammals.

Chapter 2
Determining Water Shrew Occurrence

The aim of this chapter is to establish water shrew occurrence at a range of freshwater sites. The process of defining the study area and site selection will be outlined and suitable methods of surveying for water shrews will be discussed.

2.1 Introduction

2.1.1 Determining species' occurrence

In order to conserve a species effectively and implement the best conservation management, information on its occurrence and habitat requirements needs to be determined (Mackenzie and Kendall, 2002; McCallum, 2005). A fundamental step in acquiring such information is to establish occurrence, which can be achieved through sampling an area for presence and absence (Kéry, 2002; Mackenzie, 2005a; Mackenzie, 2005b). Presence and absence surveys are useful for monitoring populations at large spatial scales, identifying habitats that are of high value to specific species and for assessing species' range, or distribution, including any changes over time (Orrock *et al*., 2000; Harvey, 2005; MacKenzie *et al*, 2006). In addition, presence and absence data can be used as a proxy for population size or abundance particularly when surveying at large scales, for elusive, low density and/or territorial species (MacKenzie, 2005b; Durso, 2011). Data collected from such surveys can be related to habitat characteristics of a given site and enable important features to be identified and appropriate management and/or protection to be undertaken. In addition, presence and absence data have been used for metapopulation models and incidence functions to investigate variation in species occupancy in different habitats (Hanski, 1999) allowing important habitats or patches to be prioritised for management or protection.

In order to assess the factors affecting occurrence and habitat selection of water shrews, their location and presence must first be determined. The water shrew is an elusive species which occurs in low densities and is patchily distributed (Aybes and Sargent, 1997; Churchfield *et al.*, 2000; Carter and Churchfield, 2006a). For example, of the 49 shrew records submitted to the Norfolk county mammal recorder in 1995, only 9 (18.4%) were actually confirmed as water shrews (Aybes and Sargent, 1997). Consequently, the water shrew is one of Britain's least known mammals and its habitat requirements are poorly understood (Churchfield, 1990; Greenwood *et al.*, 2002; Carter and Churchfield, 2006a). Water shrews have traditionally been associated with clear, fast-flowing, unpolluted rivers and streams, and watercress beds (e.g. Wolk, 1976; Churchfield, 1998; French *et al*., 2001; Aloise *et al*., 2005). However, later studies have recorded water shrews at both lotic and lentic sites (e.g. Greenwood *et al*., 2002; Carter and Churchfield, 2006a).

2.1.2 Site selection

Before surveys can be undertaken, potential sampling units (i.e. sites) need to be defined. A site may be a unit naturally occurring (e.g. a pond) or defined arbitrarily (e.g. one square kilometre of grassland) and could be a lake, a rock in a stream, or a plant depending on the size of the study organism (Southwood and Henderson, 2000).

Patterns of animal density in heterogeneous landscapes are likely to be affected by habitat selection on two scales (Morris, 1992). At the smaller scale habitat selection is affected by the variation of use of foraging locations within the home range (Williams *et al*., 2011) and at the larger scale by dispersal and the ability to transfer home range (Mladenoff *et al*., 1995). Therefore, consideration must be given to spatial scale and deciding whether determining the presence of a species within a given area (e.g. a woodland) is sufficient, or if a finer level of resolution is required (e.g. fraction of woodland occupied) (Johnson, 1980; Potvin *et al*., 2001). Such decisions depend on the purpose of the study and how the information is to be used (e.g. to inform habitat management or determine large-scale

distribution). Measures of occupancy are affected by scale (Jeffress *et al*., 2011) particularly for arbitrarily defined sites in contiguous habitats (Morris, 1992). For example, larger sites are more likely to have a higher probability of occupancy i.e. contain at least one individual of the target species than smaller sites (MacKenzie, 2006). Selecting sites using probabilistic sampling (e.g. simple random sampling and stratified random sampling) allows generalisation of the results and extrapolation to the wider population (Southwood and Henderson, 2000; O'Connell *et al*., 2010).

2.1.3 Potential surveying methods

There are a number of direct and indirect methods for detecting species (Harris and Yalden, 2004). Direct methods include drive counts (e.g. Roche *et al*., 2011), line transect counts (e.g. Petrovan *et al.*, 2011), point counts (e.g. Drapeau *et al*., 1999), aerial counts (e.g. Jachmann, 2002) and imaging techniques such as photography (e.g. De Bondi *et al*., 2010) and thermal imaging (e.g. Boonstra *et al*., 1994). Indirect methods include road kills (e.g. George *et al*., 2011), nest sites (e.g. Witherington, 2009), faecal pellet counts (e.g. Murray *et al*., 2002), feeding signs (e.g. Haberl, 2002), tracks (e.g. Beier and Cunningham, 1996), hair tubes (e.g. Pocock and Jennings, 2006), bait tubes (e.g. Churchfield *et al*., 2000), calls (McClintock *et al*., 2010) and owl pellet analysis (Buckley and Goldsmith, 1975). However, obtaining precise data on species presence or absence within a given area is practically impossible as a species which is present at a site will not always be detected and may go undetected even after lengthy searching (Kéry, 2002; Hirzel *et al*., 2002; MacKenzie *et al*., 2004; Durso, 2011). One of the main problems of determining presence and absence is that observations are usually contaminated by false zeros, which come from errors in detection of the species (Dorazio *et al*., 2011). This can lead to incorrect inferences of species distribution patterns and therefore make determining the influence of habitat variables on species presence difficult.

2.1.3.1 Surveying water shrews

Shrews leave few signs indicating their presence (Churchfield, 1990; Churchfield, 1997a) and are rarely sighted; therefore studying them

indirectly can be difficult. Until recently, live-trapping was the standard direct method of surveying shrews but it is a technique which is expensive, labour intensive and time consuming. However, a relatively new method, specifically for discriminating water shrews from terrestrial shrews by looking for the presence of aquatic prey remains in scats collected from baited tubes, has been developed (Churchfield *et al.*, 2000). This method has been used successfully in determining water shrew presence and distribution on both a small scale (e.g. French *et al*., 2001; Greenwood *et al*., 2002) and on a large scale with the National Water Shrew Survey (Carter and Churchfield, 2006a).

Bait tubes are short lengths of plastic piping (20cm x 5cm diameter) which are baited with blowfly pupae (Churchfield *et al.*, 2000). A piece of muslin is attached to one end of the tube with an elastic band to stop the bait from falling out. The tubes are placed within three metres of the edge of a water body and left in place for two weeks (see Figure 2.1). Observations of shrews (including water shrews) have found them to be curious of novel objects, willingly investigating and often defecating on, and inside, such items (Churchfield, 2000). Faecal scats deposited in the bait tubes (whilst the mammals feed on the bait) are then collected and examined. Faecal scats from shrews can be distinguished from rodent species, such as voles and mice, by their granular, uneven texture due to the content of invertebrates which, under light pressure, crumble easily (Carter and Churchfield, 2006b). Conversely, rodent faecal scats are smooth, fibrous, very hard when dry and do not crumble easily under pressure (Carter and Churchfield, 2006b). Water shrew scats are distinguished from terrestrial shrew (common and pygmy) scats by their size, shape, consistency and colour (see Figure 2.2) (Churchfield *et al.*, 2000; Carter and Churchfield, 2006b). Scats from the terrestrial shrews are approximately 2-5mm in length and black/grey in colour when are wet or dry whereas the scats of water shrews are approximately 3-10mm in length and are black when wet but become a pale grey/silver when dry due to shards of grey-white chitin from aquatic crustaceans and/or terrestrial millipedes (Carter and Churchfield, 2006b).

Whole water shrew scats can often be quite easily identified and distinguished from other small mammal scats by eye. Water shrew faecal scats which are difficult to distinguish because they are crushed or wet can be confirmed by examination under a low magnification (10x) microscope for the presence of aquatic prey remains (see Figure 2.3) (Churchfield *et al.,* 2000). Of the three shrew species inhabiting mainland Britain, the water shrew exploits both terrestrial and aquatic environments in search of food, unlike the other purely terrestrial shrew species. The main components of the water shrew's aquatic diet include water slaters *Asellus spp*., fresh water shrimps *Gammarus spp*. and caddis larvae Trichoptera *spp*. (Churchfield, 1985). Millipede remains also confirm water shrew presence as these terrestrial invertebrates are not eaten by pygmy or common shrews (Carter and Churchfield, 2006b).

Compared with live-trapping, the bait tube method is considerably cheaper and less labour intensive as the method does not require frequent checking of equipment, unlike live-traps, nor a shrew licence. Furthermore, Churchfield *et al*. (2000) looked at the relative success rates of bait tubes and live-trapping in determining habitat occurrence and found water shrews to be recorded in more habitats in a bait tube survey. Although live-trapping is a very good method of surveying, when only presence/absence information is needed, bait tube sampling is far more efficient as it allows water shrews to be inexpensively and relatively quickly surveyed over a large area. For example, bait tubes can be used to survey a large number of sites simultaneously, unlike live-trapping where sites would have to be surveyed individually. However, the method does rely on the correct identification of small invertebrate parts within faecal samples which can be time-consuming. In addition, terrestrial shrew species may sometimes consume aquatic invertebrate prey when inhabiting freshwater environments, although this is thought to be very rare (Churchfield *et al.*, 2000). For example, of 242 common shrew and 35 pygmy shrew scats, Churchfield *et al*. (2000) found that just six and one, respectively, contained minute traces of aquatic invertebrate prey. Because the method has been developed to identify water shrew presence by the remains of aquatic invertebrates, the method can only be used in freshwater habitats

where water shrews have access to such prey. In addition, the method cannot be used reliably to discriminate between the scats of the terrestrial pygmy and common shrews as the type of terrestrial invertebrates they both eat overlap considerably (Churchfield, 1990). Despite this, the method allows water shrews to be inexpensively surveyed over a large area.

Figure 2.1 Bait tube (Carter and Churchfield, 2006a).

Figure 2.2 The difference in appearance of common shrew scats (left) and water shrew scats (Carter and Churchfield, 2006b). Not to scale.

2.1.4 Aim and objectives

The aim of this part of the study is to establish evidence for water shrew occurrence at a range of freshwater sites. Objectives were to:

- Define the study area
- Select 32 freshwater sites
- Determine the occurrence of water shrews using bait tube sampling

2.2 Methods

2.2.1 Selection of study area

The study area (see Figure 2.1) encompasses a 40km x 40km square (SK400200 south west to SK800600 north east) centred on Nottingham which is within the River Trent drainage basin (Environment Agency, 2006). The study area was selected for a number of reasons:

- the countryside consists of lowland habitat representative of much of England, allowing generalisation of the habitat suitability indices to similar areas
- the size of the study area was considered sufficient to assess water shrew distribution and habitat selection at the regional scale (defined as an area large enough to embrace many habitats but not so large as to encompass the entire geographic range; Gaston, 1994)
- a concentration of positive records of water shrews was found in the central England area during the National Water Shrew Survey
- a population of water shrews were discovered in the area during an earlier study funded by the Mammals Trust UK
- accessibility
- a feasible size for study within the time and resource constraints of a PhD study

The study area is crossed by a major river, the Trent, and many minor rivers including the Soar, the Greet and the Dover Beck (Centre for Ecology

and Hydrology, 2010). It also has a diversity of artificial and natural ponds of both historic and recent origin. The topography is generally low lying with altitude varying between 30-120 metres (Ordnance Survey, 2002a, 2002b). Land use in the area is approximately 20% urban and 5% wooded with the remaining area predominantly agricultural.

2.2.2 Selection of sites

The records from the National Water Shrew Survey are infrequent and sporadic and historical water shrew records were scarce and often dated (pre 1960s). Therefore, it was decided not to base site selection upon previous records but to attempt to detect the presence of water shrews across a range of sites in the study area. Due to the labour intensive nature of surveying, it was decided to survey eight of the sixteen 10km squares (see Figure 2.3).These were chosen by systematic selection of alternate squares. Surveying eight of the sixteen 10km squares allowed coverage of a much larger area than if the study area had been defined as just eight 10km squares. As it is not possible to survey every single water body, a decision was made to select four sites within each of the eight 10km squares (two lentic and two lotic), giving a total of 32 sites (see Table 2.1).

Ideally a system of stratified sampling would have been undertaken but due to constraints of site availability and access, sites were selected by identifying potential sites on Ordnance Survey maps of the area and approaching the owners for permission. Potential sites were identified as a lentic or lotic waterbody within the selected grid square. Identifying potential sites and obtaining ownership was sometimes problematic and some grid squares had very few waterbodies to choose from. A range of water body types from a variety of habitats (such as woodland, grassland and arable) and in a variety of sizes were selected from small streams to large lakes. In common with Scott *et al*. (2012) each sampling unit was a 100m strip, parallel to the waterbody. Therefore, a minimum requirement of 100m circumference for ponds was chosen in order to accommodate the sampling unit.

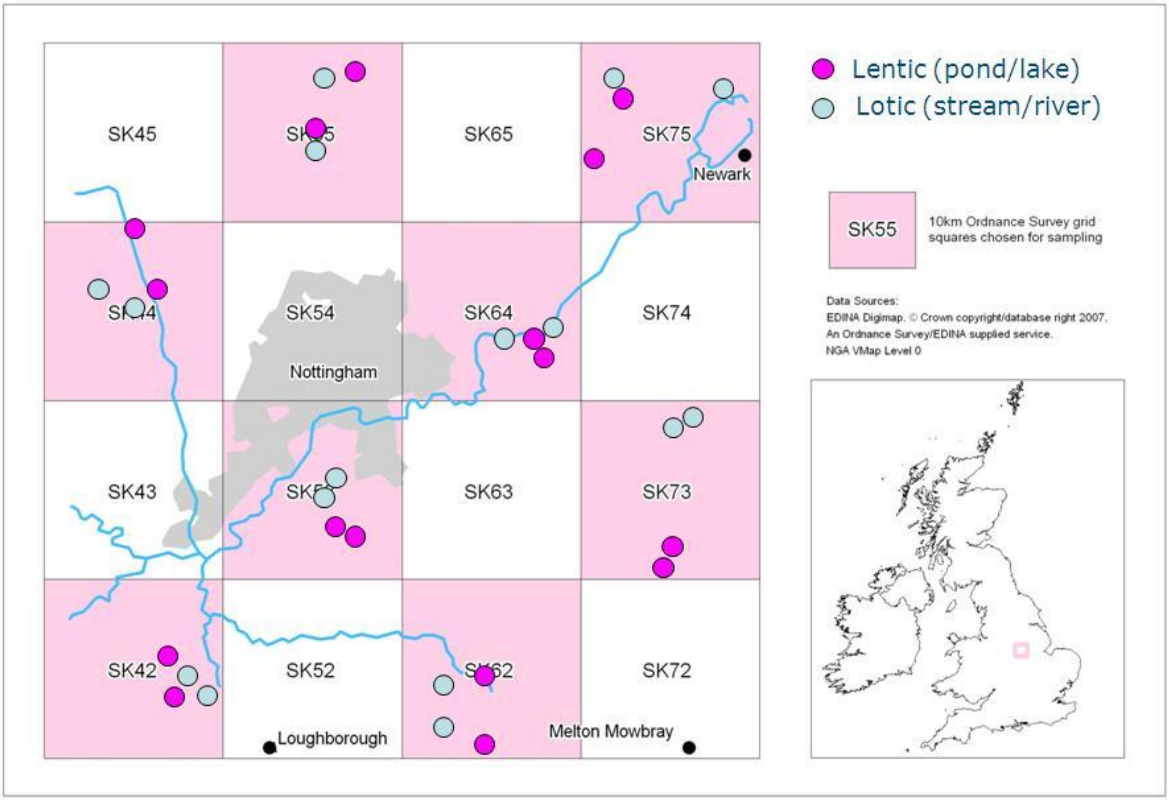


Figure 2.3 Map of study area showing the 32 sites selected for the bait tube survey.

Sites were separated spatially by at least one kilometre to minimise risk of detecting the same individual at two locations (Carter and Churchfield, 2006a). Publicly owned sites (e.g. Country Parks and Wildlife Trust sites) were chosen where possible, for ease of discovering ownership and obtaining permission. For areas without publicly owned sites, farms containing ponds or streams were selected by use of maps, and the owners identified and contacted. The nature of the methodology allows repeatability of the survey in other areas of the country.

2.2.3 Preliminary live-trapping

In order to confirm that bait tubes are as effective as live traps at determining water shrew presence, a preliminary survey was carried out. Standard live-trapping methodology (as described in Chapter 5, Section 5.2) and a bait tube survey was undertaken at two freshwater sites.

Table 2.1 The thirty-two sites surveyed during the bait tube survey, lentic (pond/lake) and lotic (stream/river). See Appendix 1 for images of all the sites.

National Grid Square	Grid reference	Site	Habitat type
SK42	SK470225 – SK470225	Drypot Lane Pond (DLP)	Lentic
	SK476240 – SK476240	Ash Spinney Pond (AS)	Lentic
	SK492232 – SK492233	Whatton Brook (Mill House) (WBMH)	Lotic
	SK484235 – SK485235	Whatton Brook (Mill Lane Bridge) (WBMLB)	Lotic
SK44	SK446501 – SK446501	Brinsley Flash (BF)	Lentic
	SK457438– SK452438	American Adventure Pond (AAP)	Lentic
	SK429441 – SK429440	Shipley Country Park Stream (SCPS)	Lotic
	SK448433 – SK448432	American Adventure Stream (AAS)	Lotic
SK53	SK561338 – SK561337	Fairham Brook (Road End) (FBR)	Lotic
	SK558333 – SK558334	Fairham Brook (School End) (FBS)	Lotic
	SK573321 – SK573320	Rushcliffe Country Park Lake (RCPL)	Lentic
	SK570324 – SK570324	Rushcliffe Country Park Pond (RCPP)	Lentic
SK55	SK562566 – SK562566	Harlow Wood Pond (HWP)	Lentic
	SK555564 – SK554564	Harlow Wood Stream (HWS)	Lotic
	SK543534 – SK543534	Newstead Park Pond (NPP)	Lentic
	SK542530 – SK542531	River Leen, Newstead Park (NPR)	Lotic
SK62	SK620222 – SK620221	Clock Farm Stream (CFS)	Lotic
	SK611231 – SK611231	Wymeswold Meadows (WM)	Lotic
	SK639211 – SK639211	Twenty-Acre Piece (TAP)	Lentic
	SK636235 – SK636235	Ella's Pond (EP)	Lentic
SK64	SK670435 – SK671435	Shelford Manor (River Pond) (SMRP)	Lentic
	SK664431 – SK665432	River Trent (Shelford End) (RTSE)	Lotic
	SK675427 – SK675427	Shelford Manor (Wood Pond) (SMWP)	Lentic
	SK679436 – SK680436	River Trent (Gunthorpe Bridge) (RTGB)	Lotic
SK73	SK750382 – SK749381	Whatton Manor (Mink End) (WMME)	Lotic
	SK742372 – SK743373	Whatton Manor (Road End) (WMRE)	Lotic
	SK761320 – SK761321	Washdyke Farm (Railway Pond) (WFRP)	Lentic
	SK757308 – SK758309	Washdyke Farm (Secret Pond) (WFSP)	Lentic
SK75	SK774556 – SK775556	Kelham Hall (KH)	Lotic
	SK718560 – SK718560	Hockerton Pond (HP)	Lentic
	SK716562 – SK716561	Hockerton Stream (HS)	Lotic
	SK758552 – SK758552	Sheepwalks Pond (SWP)	Lentic

Fifty live traps were set for one week at each site. Within 2 weeks of live-trapping at the two sites bait tube surveys were carried out using twenty bait tubes. Both methods detected water shrew presence at one of the two sites.

2.2.4 Bait tube survey

Following confirmation that bait tubes were as effective as live-trapping, a bait tube survey was undertaken at all 32 sites to establish water shrew presence or absence (see Figure 2.4). During the National Water Shrew Survey between four and eight tubes were used at each site, set at 10 metre intervals. Carter and Churchfield (2006a) found that the more tubes used per site, the greater the proportion of sites with water shrew presence. This was especially apparent when more than eight tubes were used per site. Therefore, in order to increase both the area covered by the tubes and the chance of the tubes being used by water shrews, the methodology was adapted slightly from the National Water Shrew Survey and twenty bait tubes were placed at five metre intervals at each of the 32 sites for a period of two weeks. Water shrews can travel up to 200 metres in a day (Carter and Churchfield, 2006b); therefore scats found in tubes at any one site are likely to belong to the same individual. Each tube was labelled with an individual number and site grid reference. The tubes were placed singly at ground level and under vegetation within two metres of the edge of the water body. At lotic sites, bait tubes were placed along one side of the water course except on some very narrow streams where access was blocked in parts and it was necessary to place tubes on both sides.

Unfortunately, during the two weeks (18^{th}–25^{th} June 2007) that the tubes were *in situ* there was heavy rainfall and severe flooding which impacted on the bait tube survey. According to the Environment Agency (2007), England and Wales suffered the wettest May to July period in the last 250 years with 414mm of rain. The majority of tubes were either lost to the floods or impossible to retrieve. The few tubes retrieved had any contents washed out by the rain. Therefore, during 20^{th}-28^{th} August 2007 the bait tube survey was repeated at all 32 sites.

On collection, each bait tube was placed in a small plastic bag, to ensure the contents did not get lost in transit, and all tubes from one site kept in a separate box. The tubes were then left in their boxes to allow the contents to dry before analysis. The scats were assessed by their size, shape, consistency and colour and divided into those belonging to either rodents or shrews. The shrew scats were further analysed using a microscope (10 x magnification) to distinguish between water and terrestrial shrews. Whole water shrew scats were easily distinguished (see Section 2.1.3.1) but scats which were crushed were examined under the microscope (10 x magnification) for the presence of aquatic prey remains. The identification of all water shrew scats was verified by Dr Sara Churchfield. Sites where no evidence of presence was found were recorded as 'not detected' as opposed to 'absent', as without repeated surveys it is difficult to know whether they were truly absent or just not detected.

2.2.5 Data analysis

Program PRESENCE version 4.1 (MacKenzie *et al*., 2002) was used to estimate the proportion of sites occupied and probability of detection of water shrews. The difference between presence and absence of water shrews at lentic and lotic sites was tested using a 1 x n chi-squared test and a power analysis was carried out to determine the validity of the result using Statistica 9.0.

2.3 Results

Water shrews were detected at 17 of the 32 sites (see Table 2.2 and Figure 2.4) therefore the naive estimate of occupancy (proportion of sites at which the species is detected) was 17/32 = 0.53.

Of the 17 sites with water shrew presence, eight were lentic and nine were lotic. Of the 15 sites where water shrew presence was undetected eight were lentic and seven were lotic. There was no significant difference in presence or absence of water shrews between lentic and lotic sites (χ^2= 0.25; d.f. =3; p=0.90). However, a power analysis of this result, conducted using a chi-square power model, found in order to achieve a power of 0.8 with this level of difference based upon preliminary results, would require in excess of 528 sites to be surveyed if they demonstrate this proportionality of difference. In fact, the difference in numbers of water shrew presence at lentic and lotic sites required to achieve significance is a ratio of between 2-3 lentic and 14-15 lotic (see Table 2.3).

Because the bait tube survey was only undertaken once detection probabilities from this data were unable to be estimated. Therefore, each survey within a 10 km square was treated as an independent survey of a population, with the assumption that water shrews have uniform presence over that scale. Program PRESENCE (MacKenzie *et al*., 2002) was then used to estimate the proportion of sites occupied and probability of detection. A single-season model was selected and the model represented probability of occupancy and probability of detection as constant across all surveys. As water shrews were detected at six of the eight 10 km grid squares (see Figure 2.4) the naive estimate of occupancy (proportion of grid squares at which the species is detected) was 6/8 = 0.75. PRESENCE indicated a detection probability of 0.7 which gives a corrected occupancy estimate of 0.76 ± 0.15.

Table 2.2 The thirty-two sites surveyed during the bait tube survey showing presence or non-detection of water shrews.

National Grid Square	Grid reference	Site	Water shrews
SK42	SK470225 – SK470225	Drypot Lane Pond (DLP)*	Present
	SK476240 – SK476240	Ash Spinney Pond (AS)*	Present
	SK492232 – SK492233	Whatton Brook (Mill House) (WBMH)	Present
	SK484235 – SK485235	Whatton Brook (Mill Lane Bridge) (WBMLB)	Present
SK44	SK446501 – SK446501	Brinsley Flash (BF)*	Present
	SK457438– SK452438	American Adventure Pond (AAP)*	Not detected
	SK429441 – SK429440	Shipley Country Park Stream (SCPS)	Present
	SK448433 – SK448432	American Adventure Stream (AAS)	Not detected
SK53	SK561338 – SK561337	Fairham Brook (Road End) (FBR)	Present
	SK558333 – SK558334	Fairham Brook (School End) (FBS)	Present
	SK573321 – SK573320	Rushcliffe Country Park Lake (RCPL)*	Present
	SK570324 – SK570324	Rushcliffe Country Park Pond (RCPP)*	Not detected
SK55	SK562566 – SK562566	Harlow Wood Pond (HWP)*	Not detected
	SK555564 – SK554564	Harlow Wood Stream (HWS)	Not detected
	SK543534 – SK543534	Newstead Park Pond (NPP)*	Not detected
	SK542530 – SK542531	River Leen, Newstead Park (NPR)	Not detected
SK62	SK620222 – SK620221	Clock Farm Stream (CFS)	Present
	SK611231 – SK611231	Wymeswold Meadows (WM)	Present
	SK639211 – SK639211	Twenty-Acre Piece (TAP)*	Present
	SK636235 – SK636235	Ella's Pond (EP)*	Not detected
SK64	SK670435 – SK671435	Shelford Manor (River Pond) (SMRP)*	Not detected
	SK664431 – SK665432	River Trent (Shelford End) (RTSE)	Not detected
	SK675427 – SK675427	Shelford Manor (Wood Pond) (SMWP)*	Present
	SK679436 – SK680436	River Trent (Gunthorpe Bridge) (RTGB)	Not detected
SK73	SK750382 – SK749381	Whatton Manor (Mink End) (WMME)	Not detected
	SK742372 – SK743373	Whatton Manor (Road End) (WMRE)	Not detected
	SK761320 – SK761321	Washdyke Farm (Railway Pond) (WFRP)*	Not detected
	SK757308 – SK758309	Washdyke Farm (Secret Pond) (WFSP)*	Not detected
SK75	SK774556 – SK775556	Kelham Hall (KH)	Present
	SK718560 – SK718560	Hockerton Pond (HP)*	Present
	SK716562 – SK716561	Hockerton Stream (HS)	Present
	SK758552 – SK758552	Sheepwalks Pond (SWP)*	Present

*lentic site

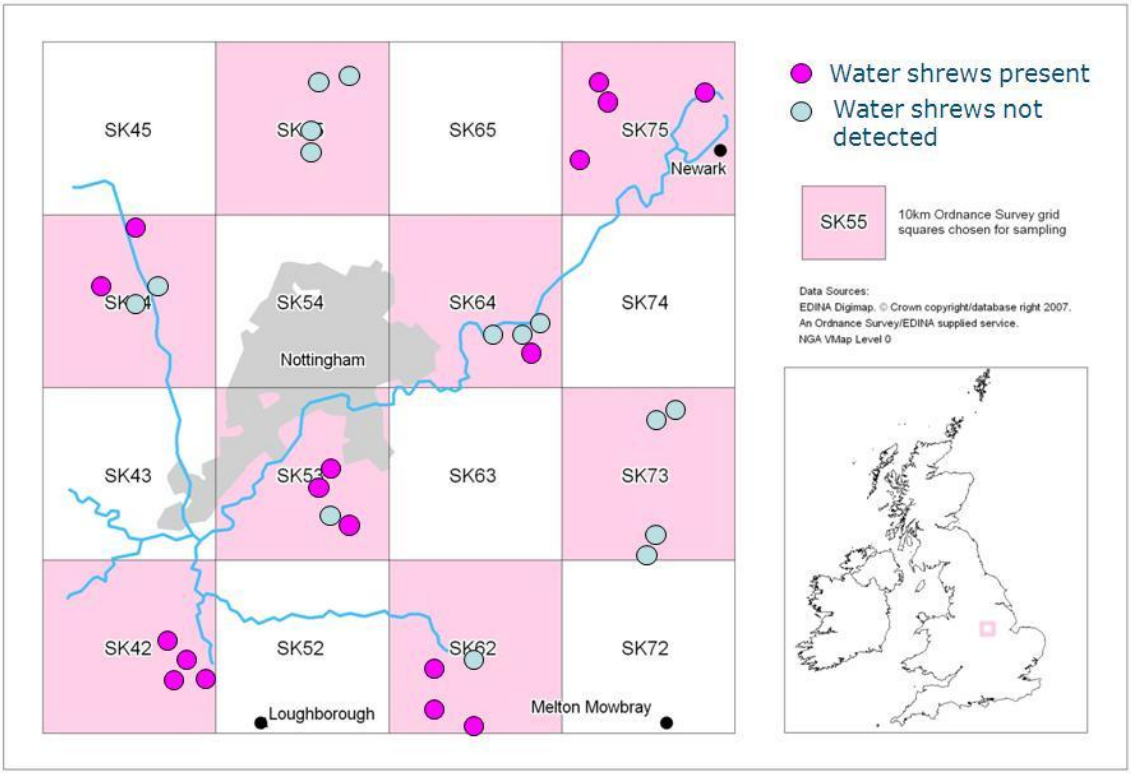


Figure 2.4 Distribution of water shrews following the bait tube survey.

Table 2.3 The difference in numbers of water shrew presence at lentic and lotic sites required in order to achieve significance.

Lentic	**Lotic**	**Ratio**	***p***
8	9	0.88	0.97
7	10	0.7	0.86
6	11	0.55	0.625
5	12	0.42	0.35
4	13	0.3	0.15
3	14	0.21	0.051
2	15	0.13	0.013

2.4 Discussion

Although water shrews were detected at over half of the sites surveyed, because the issue of imperfect detection was not addressed it is likely that there were a number of sites where water shrews were not detected but were in fact present. These false absences may result in biased estimates of occupancy and consequently misrepresentation of habitat preferences (MacKenzie and Nichols, 2004; MacKenzie and Royle, 2005; Pagano and Arnold, 2009; Jeffress *et al*., 2011). There are methods now available to account for this problem by estimating the probability of detecting a species during a given survey (MacKenzie *et al*., 2006). Such methods incorporate detection probability through multiple visits in time or space to a survey site (MacKenzie *et al*., 2002). Obtaining occupancy rates corrected for detection probability improves the reliability of inferences made about species and habitat associations (Jeffress *et al*., 2011), but repetition of the bait tube survey was not possible in the current study due to resource limitations.

It must be acknowledged that the assumption of even distribution of water shrews within a 10 km square (as used for estimation of detection probabilities) has not been tested. If the estimated detection probabilities are representative of water shrews at a smaller spatial scale, the fact that the naive occupancy estimate for the 10 km squares (0.75) was so similar to the estimate corrected for detectability (0.76), suggests that bait tube surveys are a relatively accurate method of detecting water shrews. The detection probability of water shrews was relatively high compared with other mammal species. For example, in America, detection probabilities for seven species of small mammals ranged from 0.25-1.00 (Gu and Swihart, 2004) and for 10 species of meso-mammals 0.07-0.48 (O'Connell *et al*., 2006). Furthermore, Gibson (2011) found detection probabilities of six species of Australian small mammal to be considerably lower than one with naive estimates of occupancy underestimating occupancy rates corrected for detection probability by up to 45%.

Determining the number of repeated surveys necessary in occupancy

studies is an important aspect of their design. One way is to determine the number of surveys required to have 95% confidence of detecting the species at a site if it is present (Stauffer *et al*., 2002). Another is to modify the number of surveys depending on the detection probabilities of the target species. For example, when detection probabilities are high it is better to survey more sites, rather than increasing the number of repeated surveys, whereas when detection probabilities are low more surveys per site should be undertaken (Tyre *et al*., 2003). MacKenzie *et al*. (2002) suggest the number of surveys at a given site required to provide a 'reasonable' estimate of occupancy to be a minimum of two if occupancy is greater than 0.7 and detection probabilities, in a single survey, are greater than 0.3. Therefore, based on the 0.70 detection probability and 0.76 estimate of occupancy, the bait tube survey undertaken in the current study should have been repeated at least once. Therefore, the results of this survey and subsequent inferences regarding habitat selection must be interpreted with caution.

Bait tube surveys are a cheap and easy technique to confirm presence of water shrews in a given area although they are unable to provide information on population density (Churchfield *et al*., 2000). It is acknowledged that the 100m sampling unit used in this survey would fall within a single home range of a water shrew. However, since the aim of the survey was simply to establish water shrew occurrence at a range of sites the bait tube method was regarded as satisfactory to achieve this aim. Furthermore, the length of the sampling unit is in fact greater than most other bait tube surveys of water shrews (e.g. 30m by French *et al*., 2001; 50m by Greenwood *et al*., 2002; and 40-80m by Carter and Churchfield, 2006a).

A number of different riparian habitats were assessed during the bait tube survey including grassland, woodland and arable. For each habitat type surveyed, water shrews occurred at approximately half (eight of the fifteen grassland sites, six of the eleven woodland sites and three of the five arable sites) suggesting no preference between any of these habitat types. Carter and Churchfield (2006a) found water shrews to occur most commonly in

freshwater habitats adjacent to arable (25.3%) followed by woodland (19.6%) and grassland (17.3%).

Water shrew presence was detected at sites which were very close to human habitation, as well those more isolated. For example, Fairham Brook which is on the edge of a large housing estate; Kelham Hall stream which runs through a busy council offices car park; and Hockerton pond which is within metres of 'eco' houses. Conversely, they also occurred at more remote sites such as Brinsley Flash which is wetland habitat approximately a mile from the nearest village which suggests no preference for sites either close or far from human habitation. Water shrews appear not to mind close proximity to humans with records of their presence in urban habitats and gardens and even a scrapyard (Carter and Churchfield, 2006a).

As demonstrated by the power analysis, there are insufficient data to indicate a preference for still or flowing water since many more sites would have to be sampled. However, the findings are similar to previous studies. For example, Greenwood *et al.* (2002) found no evidence that water shrews have a particular habitat preference as they were present at a variety of the 96 lentic and lotic habitats (rivers, streams, canals, ditches and a pond) surveyed in the Weald. Similarly, the findings of the National Water Shrew Survey also found water shrews to occur at a wide range of lentic and lotic habitats (Carter and Churchfield, 2006a). These recent findings are interesting as water shrews have previously typically been associated with fast flowing streams and rivers (Churchfield, 1990; Macdonald and Tattersall, 2001; French *et al.*, 2001) with records from lentic habitats such as ponds and lakes scarce (Carter and Churchfield, 2006a).

The pattern of water shrew presence revealed by the bait tube survey does not give any obvious indications as to habitat requirements. Therefore, a much more detailed survey of specific habitat features was required to elucidate the pattern of occurrence.

Chapter 3
Developing and Testing Habitat Suitability Indices

The aim of this chapter is to produce and test habitat suitability indices to establish the habitat preferences of water shrews by relating water shrew occurrence to habitat characteristics of each site.

3.1 Introduction

3.1.1 Freshwater species and habitats

Freshwater environments are important habitats for a wide range of mammal, bird and invertebrate species. Besides the water shrew, there are two other riparian mammal species native to Britain, the otter *Lutra lutra* and the water vole *Arvicola amphibious*, as well as several species of bat such as Natterer's *Myotis nattereri*, Daubenton's *Myotis daubentonii* and Pipistrelle *Pipistrellus nathusius*, *P*. *Pipistrellus and P. pygmaeus* which are particularly associated with freshwater habitats (Furniss and Lane, 1992). Many riparian species have suffered declines due to habitat loss and degradation, therefore methods of determining the environmental attributes, which constitute suitable and unsuitable habitats, are crucial in assisting the recovery and conservation of vulnerable species. For example, both otters and water voles have undergone dramatic declines in population numbers in the last 50 years (Woodroffe, 2000; Strachan and Moorhouse, 2006). During the 1950s-70s, the otter declined to near extinction largely as a result of poisoning by organochlorine pesticides including dieldrin, Dichloro-Diphenyl-Trichloroethane (DDT), Polychlorinated biphenyls (PCBS) and heavy metals (Chanin and Jefferies, 1978). However, in the 1960s the use of organochlorines was banned and the release of particularly harmful substances into watercourses was controlled by the EC Dangerous Substances Directive (Macdonald and Tattersall, 2001). As a result, otter population numbers have been slowly recovering and the number of sites

with evidence of otter has increased from 5.8% in 1977-79 to 58.8% in 2009/10 (Crawford, 2010).

Over the last hundred years, water voles have suffered the most dramatic decline in numbers of any British wild mammal in the twentieth century with an estimated loss of 94% (Jefferies *et al*., 1989). This decline appears to have accelerated over the last thirty years leaving the water vole as one of our most threatened species (Woodroffe, 2000). One of the main contributing factors to the dramatic loss of water voles is the destruction of good quality habitat such as densely vegetated banks. However, the most serious threat that water vole currently face is predation by the non-native American mink *Neovison vison* (Strachan and Moorhouse, 2006). American mink are a semi-aquatic species, much smaller than the otter (Dunstone, 1993) that were originally brought to fur farms in Britain from 1929 onwards, but subsequently escaped or were released (Woodroffe, 2000). Mink are now widely distributed throughout mainland Britain and Ireland (Dunstone and Macdonald, 2008). Unfortunately, the anti-predator strategies (e.g. diving into the water and kicking up a cloud of mud, escaping to its burrow or hiding in bankside vegetation) employed by the water vole against native predators such as weasels, stoats and cats are ineffective against mink (Strachan and Moorhouse, 2006). This predation on water voles has left the already vulnerable population extinct in many of its former core sites (Woodroffe, 2000).

Water quality in the freshwater habitats where riparian mammals are found is directly affected by pollution from agricultural, domestic and industrial wastes (Macdonald and Tattersall, 2001) which can affect wildlife both directly and indirectly. Direct effects of pollution such as damage to the nervous system, kidneys and reproductive system (Sànchez-Chardi and Nadal, 2007), can occur through ingestion of polluted water, for example during grooming (Carter and Churchfield, 2006a). Factors such as Biochemical Oxygen Demand (BOD), nitrates, phosphates and pH, as well as pollutants such as insecticides and molluscicides, may have indirect effects on riparian mammals such as water shrews through impacts on their invertebrate prey (Carter and Churchfield, 2006a). For example, chemicals

such as insecticides and molluscicides are readily accumulated by the invertebrates and fish, on which riparian mammals may feed, and the chemical residues and heavy metals in these toxins can accumulate in the mammal species (Shore *et al*., 1997; Walker *et al*., 2007). This process, known as bioaccumulation, was partly responsible for the large decline in otter numbers (Macdonald and Tattersall, 2001). In addition, aquatic invertebrates are sensitive to acidification with low pH levels associated with poorer invertebrate populations (Mason, 2002). Similarly, nutrients in the water such as nitrates and phosphates provide ideal substrates for bacterial growth which can lead to eutrophication and de-oxygenation, resulting in lack of vegetation and associated invertebrate life (Jeffries and Mills, 1990).

The exact relationship between water shrew survival and environmental factors is unknown; however, their dependence on freshwater habitats makes it likely that they too are affected by changes in habitat and water quality. The development of a successful HSI for water shrews, by identifying the variables of the greatest importance in determining water shrew occurrence, would allow rapid assessment of sites for likely presence, without labour intensive and costly techniques. As there is not currently a reliable method of assessing habitat suitability for water shrews, a successful HSI would be a useful tool in water shrew conservation and could contribute towards a national species management plan by informing best practice.

3.1.2 Habitat suitability indices

HSI models predict species occurrence by measuring the suitability of a habitat based on an assessment of habitat attributes such as diversity of vegetation, water quality and adjacent land use. The identification of features, which predict well for species occurrence, allows rapid assessment of new sites for habitat suitability, without the need for intensive species trapping. The ability to predict species occurrences is a vital tool in the field of applied ecology and has many uses for conservation. A number of conservation applications, as identified by Manel *et al*. (2001), include the use of species prediction to:

- identify sites expected to have important species using environmental data
- identify sites suitable for reintroductions
- inform site management by manipulating features known to favour species presence
- identify gaps in species distribution and diagnose their cause
- identify locations at risk of species extinction
- identify major influences on species distribution
- discriminate effects of habitat and pollution on species distribution to identify which is responsible for absence
- predict distributional change in response to climate change and land use

HSI models have been applied to a range of individual species. For example, minnows *Phoxinus phoxinus* (Mastrorillo *et al*., 1997), great-crested newts *Triturus cristatus* (Oldham, 2000), wolves *Canis lupus* (Glenz *et al*., 2001), dormice *Muscardinus avellanarius* (Greaves *et al*., 2006), red-winged blackbirds *Agelaius phoeniceus* (Ozesmi *et al*., 2006), lynx *Lynx lynx* (Doswald *et al*., 2007), badgers *Meles meles* (Newton-Cross *et al*., 2007), otters *Lutra lutra* (Ottaviani *et al*., 2009; Gallant *et al*., 2009) and Arctic ground squirrels *Spermophilus parryi* (Barker *et al*., 2010), as well as groups of species, including butterflies (Fleishman *et al*., 2003), aquatic invertebrates (Dedecker *et al.*, 2004, 2005; Goethals *et al*., 2007), insects (Hein *et al*., 2007), ungulates (Habib *et al*., 2010) and African mammals (Boitani *et al*., 2008).

In general, these HSIs were capable of making predictions about the target species. For example, a dormouse HSI produced satisfactory predictions using an information-theoretic model (Greaves *et al*., 2006). Doswald *et al*. (2007) developed two successful HSI models for lynx in the Swiss Alps, a local expert model (using data from game wardens) and a scientific expert model (using data from lynx researchers experienced in monitoring and radio-tracking). Both models performed well although the local expert model performed better ($r_s = 0.964$, $p < 0.001$) than the scientific expert

model (rs = 0.833, $p < 0.001$) when evaluated with data taken from the study area. However, when the models were evaluated in the Jura mountains, as expected, the local expert model performed less well (rs = 0.939, $p < 0.001$) than the scientific expert model (rs = 0.967, $p < 0.001$). Newton-Cross *et al*., (2007) successfully modelled the distribution of badgers using field-based and remotely derived habitat data and found all four training models had classification accuracies in excess of 69%. Ecological consultants have reported on the effectiveness of HSIs for assessing site suitability for great-crested newts. For example, Maben (2011) analysed the data of great-crested newt surveys at 92 waterbodies and found a positive correlation between HSI score and population size.

However, a mink HSI developed by the United States Fish and Wildlife Service's to determine suitable mink habitat was not so successful. The model was tested on randomly selected sections of streams in the Lake Michigan and Lake Superior basins of Wisconsin (Loukmas and Halbrook, 2001). The model performed poorly in predicting mink habitat suitability in those areas with correlation analyses determining no association between HSI values and mink activity (r = -0.09, $p = 0.729$). The model was flawed because not enough value had been assigned to habitats that potentially support prey populations.

Previous attempts to model habitat characteristics and water shrew presence have been attempted with varying success (e.g. French *et al*., 2001; Greenwood *et al*., 2002 and Carter and Churchfield, 2006a). French *et al.* (2001) developed a model incorporating short grass and freshwater crustaceans, which had 90% sensitivity (ability to predict true positives i.e. water shrew presence), 71% specificity (ability to predict true negatives i.e. water shrew absence) and an overall predictive ability of 80%. The presence of short grass had a negative effect on water shrew presence whereas presence of freshwater crustaceans had a positive effect. In addition, Greenwood *et al*. (2002) constructed a model using the variables current speed, water depth, bank incline and bankside vegetation and found their model had 83% predictive ability. Water shrews were more likely to occur at sites with fast-flowing, shallow water with dense bankside

vegetation and steep bank inclines. Furthermore, the National Water Shrew Survey (Carter and Churchfield, 2006a) identified a number of variables which showed significant interactions with water shrew presence which were used to construct a model. Easting (east-west component of grid reference) and presence of herbaceous vegetation had a positive effect on water shrew presence, whereas the presence of trees and shrubs together at a site had a negative effect. However, the model performed poorly, correctly predicting only 2% of sites with known water shrew presence. Although French *et al.* (2001) and Greenwood *et al.* (2002) produced models that gave good predictive performance for determining water shrew presence for the training data, none of the discussed studies tested their models independently, using unseen data, therefore generalisation ability of the models is unknown.

3.1.3 Methods of surveying habitats

There are numerous methods of surveying habitats both generally and specifically such as the Phase 1 Habitat Survey, Phase 2 Vegetation Survey, River Habitat Survey and Pond Monitoring Network Survey. The Phase 1 Habitat Survey and Phase 2 Vegetation Survey are standardized systems for surveying, classifying and mapping wildlife habitats, and classifying terrestrial and freshwater vegetation (Joint Nature Conservation Committee, 2007). The River Habitat Survey is a standard methodology developed by the Environment Agency to record physical and habitat features of sections of river (Raven *et al*., 1998). The Pond Monitoring Network survey is a methodology developed by the Environment Agency and Pond Conservation to assess a wide range of chemical and physical characteristics of a pond and identification of plant and invertebrate species (Biggs *et al*., 1998).

The quality of fresh water can be defined and measured by its physical, chemical and biological characteristics. Many of these characteristics relate to the geographical location and local geology of the water body and are responsible for the natural variation in water quality throughout the country (Dodds, 2002). For example, lowland rivers and streams tend to be slow-flowing with a sandy or clay substrate resulting in poorly oxygenated water,

and uplands have a natural presence of organic acids and therefore tend to be more acidic than lowland streams (Rundle and Ramsay, 1997).

Water quality can be assessed using biological and/or chemical analyses. Biological assessment of water quality uses macro-invertebrate communities as an indicator of water quality and to assess organic pollution (Hawkes, 1998). Samples of macro-invertebrates (e.g. snails, worms, leeches, mayflies, dragonflies, beetles etc.) collected from a water body are assigned a set of simple numerical values according to their tolerance to pollution. Commonly assessed chemical characteristics of water bodies such as pH, dissolved oxygen, conductivity, and nutrients (nitrates and phosphates), have a large influence on the type and composition of aquatic flora and fauna (Dodds, 2002). For example, a lack of vegetation and low aquatic invertebrate diversity is associated with nutrient rich water (Jeffries and Mills, 1990).

3.1.4 Methods of analysing HSIs

Analysis of complex multiple ecological variables requires a multivariate approach (Hirzel *et al*., 2002). Commonly used methods of multivariate analyses for HSI models include logistic regressions (Glenz e*t al*., 2001; Dettki *et al*., 2003; Hein *et al*., 2007; Newton-Cross *et al*., 2007; Gallant *et al.*, 2009), stepwise logistic regressions (French *et al*., 2001; Greenwood *et al*., 2002; Greaves *et al*., 2006; Carter and Churchfield, 2006a), generalized linear models (Ozesmi *et al*., 2006) and discriminant analyses (Buckton and Ormerod, 1997; Manel *et al.*, 1999). However, an alternative method of analysis, which has become increasingly popular in ecological modelling, is artificial neural networks (e.g. Balls *et al*., 1996; Mastrorillo *et al*., 1997; Lek and Guégan, 1999; Dedecker *et al*., 2004, 2005; Goethals *et al*., 2007; Tirelli *et al*., 2009).

3.1.5 Artificial neural networks

Artificial neural networks (ANNs) are a form of machine-learning algorithms, commonly described as computational modelling systems (Lemetre, 2010). They were developed in the 1940s by neuroscientists to simulate the function of biological neural networks (McCulloch and Pits, 1943), hence the name, and further developed during the late 1950s (Rosenblatt, 1958). However, it was not until the late 1980s that interest in the technique resurfaced and the use of ANNs in a wide range of applications became increasingly popular (Lek and Guégan, 1999).

ANNs can learn from patterns and are able to make predictions from non-linear, highly dimensional and noisy data (e.g. data containing measurement errors, human mistakes or missing data values), which they do in a similar way to learning in the human mind (Lancashire *et al*., 2008); the more a particular pattern is represented the stronger the recognition of it by the ANN (Balls *et al*., 1996).

Models produced by ANNs have the ability to predict accurately for unseen data and therefore possess highly reliable generalisation ability (Lemetre, 2010). Consequently, they represent one of the most robust and reliable methods of analysing complex data and are used widely in biomedical research such as determining breast cancer biomarkers (Lancashire *et al*., 2008; Lemetre, 2010) and in disease diagnosis and survival prediction (e.g. Song *et al.,* 2005). Although ANNs were originally developed to simulate biological neurons and the process of memory, they are used today in a broad range of applications, including stock market predictions and speech and image recognition (e.g. Egmont-Petersen *et al*., 2002; Vanstone and Finnie, 2009; Dede and Sazli, 2010).

ANNs have been used in the field of zoology to classify the echolocation calls of bat species with a high degree of accuracy (Parsons, 2001), performing 75% better than humans in their ability to classify recordings of bat calls (Jennings *et al*., 2008). Similarly, they have been used successfully

to classify primate vocalisations, performing better than both Discriminate Function Analysis and Cluster Analysis (Pozzi *et al*., 2010).

ANNs have been increasingly used in ecological studies to make predictions of species distributions. For example, predicting nest occurrence and breeding success of red-winged blackbirds, where they performed better than Generalised Linear Models (GLM), (Ozesmi *et al*., 2006), predicting minnow abundance with 92% predictive performance (Mastrorillo *et al*., 1997), and for predicting presence of aquatic invertebrates in streams and rivers (Dedecker *et al*., 2004, 2005; Goethals *et al*., 2007).

Manel *et al*. (1999) looked at alternative methods of predicting species distribution using Himalayan river birds as an illustration. They found that when using calibration data ANNs performed better (89-100%) than logistic regression (75-92%) and discriminant analysis (81-95%) at predicting presence or absence of birds. When applied to unseen test data, prediction success of all methods averaged 71-80%, with logistic regression marginally outperforming ANNs and discriminant analysis. Nevertheless, all methods predicted true absences (83-92% success) better than true presences (31-44%).

In a similar study, Tirelli *et al*. (2009) assessed alternative methods of predicting the presence of an endangered salmonid *Salmo marmoratus*, by using discriminant function analysis, logistic regression, decision trees and ANNs, and comparing the performances of the different models. They found that the ANN models were more effective than all the other classification techniques at predicting salmonid presence at a site. Moreover, the ANNs were very effective when applied in models to make decisions with respect to river and conservation management.

The use of machine-learning techniques such as ANNs in environmental and ecological sciences has become increasingly popular (Lek and Guégan, 1999) as the ability of ANNs to make predictions from complex and often non-linear data sets, with a multitude of variables, makes them an ideal choice for ecological studies and modelling (Williams and Poff, 2006). Using

ANNs for ecological applications has a number of advantages over traditional ecological models such as multiple regression and logistic regression, which are limited by assumptions of normality, linearity and zero values, consistently outperforming such techniques when analysing non-linear data sets (Ozesmi and Ozesmi, 1999; Biancon *et al*., 2010). Furthermore, ANNs do not depend on any prior type of function such as binomial or poisson, but rather develop a function that best suits the problem being addressed. Therefore, on this basis, ANNs were selected as the method of analysis for the current study.

3.1.6 Aims and objectives

The aims of this part of the study were to produce and test habitat suitability indices to establish the habitat preferences of water shrews. The objectives were to:

- Undertake habitat surveys at the 32 freshwater sites
- Develop habitat suitability indices using the data from the bait tube sampling and habitat survey data
- Test the habitat suitability indices on a subset of 'unseen' sites

3.2 Methods

3.2.1 Experimental design

A preliminary survey using baited tubes was undertaken at 32 sites in the East Midlands to determine water shrew presence via faecal pellet analysis (see Chapter 2 for site selection and experimental design). Habitat surveys of 31 sites from the bait tube survey were undertaken during winter 2007/8 and summer 2008 (the River Leen had to be excluded due to access issues). The habitat surveys were undertaken in both winter and summer in case there were any seasonal differences between the important habitat variables.

3.2.2 Designing a habitat survey for water shrews

In order to a design a habitat survey specifically for water shrews a method which included assessment of both aquatic and terrestrial habitat was required as the species utilises both. The design of the habitat survey was based upon the National Water Shrew Survey field form (to allow results from this study to be compared) but additionally encompassed relevant aspects of Phase 1 Habitat Survey, Phase 2 Vegetation Survey, River Habitat Survey and Pond Monitoring Network Survey in order to collect as diverse a range of data as possible. The habitat survey included physical attributes of the water body, percentages of aquatic and terrestrial vegetation and measurements of water quality.

3.2.3 Variables measured and their importance for water shrews

Thirty-two variables were measured including habitat, physical and chemical characteristics, vegetation and environmental impacts (see Table 3.1). All of the variables measured were considered to have a potential positive or negative effect on a site's suitability for water shrews. For example, proximity to other water bodies could affect dispersal and distribution of the water shrews, adjacent land use e.g. woodland or grassland could affect the type and abundance of invertebrate prey, as could water quality, and presence and density of terrestrial and aquatic vegetation.

Table 3.1 The thirty-two variables measured/recorded during the habitat survey.

Habitat Characteristics	Physical characteristics	Chemical characteristics	Terrestrial vegetation	Aquatic vegetation	Environmental impacts
Habitat type	Substrate complexity	Conductivity	Vegetation cover	Submerged vegetation	Adjacent land use
Ponds and lakes up to 500m	Sediment	pH	Overhanging vegetation	Emergent vegetation	Human activity
Streams and ditches up to 500m	Permanence	Dissolved oxygen	Bankside trees	Floating vegetation	Management intensity
	Water depth	Phosphates	Bankside shrubs	No. of aquatic plants species	Pollution sources
	Width	Nitrates	Bankside herbs		Livestock use
	Current		Bankside grasses		
	Bank incline		No. of bankside plant species		
	Bank height				

Environmental impacts to a site such as intensity of management, level of human activity and potential sources of pollution such as road runoff could also impact negatively on water shrew presence. Over-management may lead to loss of habitat, human activity could cause disturbance and potentially habitat loss and pollution could affect water quality and therefore food supply.

Habitat survey field forms were produced in order for variables to be recorded (see Table 3.2). All measurements were taken within the 100m transects used in the bait tube survey. Habitat type, adjacent land use, potential sources of pollution (e.g. road runoff, livestock, wildfowl), substrate complexity and sediment type were assessed through observation at each site. Permanence of the water body (and depth if difficult to assess), livestock use and management practice were ascertained through speaking with the landowner.

The number of waterbodies within 500m of the site was recorded and their width was measured using Ordnance Survey Explorer maps and Google Earth. A metre ruler was used to measure water depth (measured from the water's edge) and bank height (in cms). Three measurements were taken of each and then averaged. Surface water velocity (current) was measured in ms^{-1} using a floating object timed for five seconds.

Vegetation was measured in a number of ways. Firstly, the percentage of the waterbody within the 100m transect shaded by overhanging vegetation and percentage of the surface covered by vegetation were recorded. Secondly, terrestrial (bankside) vegetation (e.g. trees, shrubs, herbs and grasses) and aquatic vegetation (e.g. submerged, emergent and floating) were recorded as present, absent or dense. Finally, aquatic and terrestrial vegetation were recorded using the DAFOR scale, which works on percentage cover of species (Dominant = >81%, Abundant = 61–80%, Frequent = 41–60%, Occasional = 21–40%, Rare = 1–20 %), and the numbers of plant species calculated.

Table 3.2 Example of a completed habitat survey field form.

Habitat type	Lake	Pond ✓	River	Stream
Adjacent land use	Grassland ✓	Scrub	Arable	Woodland
Human activity	Minimal (private site) ✓	Sporadic		Frequent (public site)
Management	None	Occasional	Frequent ✓	Description: Mowing
Permanence	Stays same size ✓	Shrinks up to half size	Shrinks more than half size/dries completely	

Water depth	<0.25m	0.25-0.5m ✓	0.5-1m	1-2m	>2m
Width	<1m	1-2m	2-5m	5-10m	>10m ✓
Current	Static (0ml/s) ✓	Slow (0.1-0.5m/s)		Fast (>0.5m/s)	
Substrate complexity	Clay/silt ✓	Clay/silt/sand	Clay/silt/gravel	Clay/silt/rocks/stones	
Sediment	None	Plant material		Leaves/twigs ✓	
Bank type	Rocks	Earth ✓	Concrete	Wood	
Bank incline	<45° ✓		>45°		
Bank height	<1m ✓	1-2m		>2m	
No. of waterbodies <500m	Ponds and lakes 10		Streams and ditches 3		

Water quality	Conductivity (µs cm^{-1}) 839	pH 7.58	DO_2 (mg^{-1}) 11.75	Temp (°c) 6.9
	Nitrates (mg/l NO_3- -N) Date: 23/01/08 De-ionised water: 0.16 Sample: 3.3		Phosphates (mg/l PO_4^{3-}) Date: 23/01/08 De-ionised water: 0.12 Sample: 0.62	

Pollution sources	None ✓	Farming	Road runoff	Wildfowl	Livestock / Other:
Use by livestock	None ✓		Light (e.g. few rare breed cattle)		Heavy (e.g. dairy cows)

Vegetation cover (surface)	None ✓	<1/4	1/4-1/2	1/2-3/4	>3/4
Overhung (any height)	None ✓	<1/4	1/4-1/2	1/2-3/4	>3/4

Aquatic vegetation*	**Submerged**	**Emergent**	**Floating**
Absent			✓
Present	✓	✓	
Dense			

Aquatic vegetation DAFOR	**Dominant (81-100%)**	**Abundant (61-80%)**	**Frequent (41-60%)**	**Occasional (21-40%)**	**Rare (1-20%)**
			Phragmites	Lesser reed Mace	
				Soft rush	

Number of aquatic plant species: 3

Bankside vegetation	**Trees**	**Shrubs**	**Herbs**	**Grasses**
Absent	✓	✓		
Present			✓	✓
Dense				

Bankside vegetation DAFOR	**Dominant (81-100%)**	**Abundant (61-80%)**	**Frequent (41-60%)**	**Occasional (21-40%)**	**Rare (1-20%)**
	Grass				Rosebay Willowherb

Number of bankside plant species: 2

Water quality was assessed by measuring pH, conductivity and the presence of nitrates and phosphates. A further indication of water quality was achieved by measuring dissolved oxygen concentration. Although this has limitations it was carried out in preference to using BOD due to the difficulty in obtaining the necessary stable conditions. Water quality assessment was undertaken in the field except for nitrates and phosphates, which were assessed in the laboratory. pH, conductivity and dissolved oxygen were assessed in the field at the water's edge using portable meters (Jenway

350, Jenway 470 and Hach HQ30d, respectively). Readings were taken when the meters had stabilised, which usually took about one minute. The pH meter was calibrated each day it was used. In order to test for nitrates and phosphates, two 500ml samples of water were collected from each site in new polyethylene bottles to test for nitrates and phosphates and care was taken to minimise contamination during handling by rinsing the sample bottles with de-ionised water and several times with the sample before collection. Samples were then taken back to the lab and stored at 5°c until they were analysed (within 3 days). Samples were filtered prior to analysis and then tested using a Hach DR 2800 spectrophotometer using PhosVer 3 (molybdenum) for phosphates and NitraVer 5 (cadmium reduction) for nitrates.

3.2.4 Data analysis

Data collected during the habitat surveys were converted using a categorical scoring system in order to allow inclusion of variables which had a wide range of difference in variation, or in some cases no numerical value at all (see Table 3.3 for variable scoring categories). Although it is more desirable to use continuous data, complex survey information, which includes qualitative or category information such as habitat type or adjacent land use, is frequently scaled.

3.2.4.1 ANN analysis

Each data set (winter 2008 and summer 2008) were analysed with ANNs using a stepwise approach (see Figure 3.1), similar to stepwise regression, which involves the sequential selection and addition of input variables to the ANN in order to identify those variables (or combination of variables) with optimum predictive performance (Lancashire *et al*., 2008). Dissolved oxygen had to be excluded from the winter 2008 data analysis as the meter was faulty and therefore the results were spurious.

Table 3.3 Definition of variable categories used in the habitat surveys.

	Category					
Variable	**0**	**1**	**2**	**3**	**4**	**5**
Habitat characteristics						
Habitat type		Lake (>10,000m^2)	Pond (<10,000m^2)	River	Stream	
Ponds and lakes within 500m		None	1-5	6-10	>10	
Streams and ditches within 500m		None	1-5	6-10	>10	
Adjacent land use		Grassland	Scrub	Arable	Woodland	
Human activity		Minimal	Sporadic	Frequent		
Management intensity		None	Occasional	Frequent		
Pollution sources (e.g. road runoff, farming)		None	Minimal	Several		
Livestock use		None	Light	Heavy		
Physical characteristics						
Substrate complexity		Clay/silt	Sand	Gravel	Stones/rocks	
Sediment		None	Plant material	Leaves and twigs		
Permanence (water body)		Stays same size	Shrinks up to ½ size	Shrinks > ½ size/dries		
Water depth		<25cm	25-49cm	50-99cm	1-2m	>2m
Width		>1m	1-1.99m	2-4.99m	5-9.99m	>10m
Current		Static	Slow	Fast		
Bank incline		<30°	30-59°	>60°		
Bank height		<1m	1-2m	>2m		
Chemical characteristics						
Conductivity (µs cm^{-1})	No water	<249	250-499	500-999	1,000-1,499	>1,500
pH	No water	<7	7-8	>8		
Dissolved oxygen (mg l^{-1})	No water	0-2.99	3-5.99	6-8.99	9-11.99	>12
Phosphates (mg/l)	No water	0-0.99	1-1.99	2-2.99	3-3.99	>4
Nitrates (mg/l)	No water	0-1.99	2-4.99	5-7.99	8-10.99	>11
Vegetation						
Vegetation cover (surface of water body)		None	<1/4	1/4 - 1/2	1/2 – 3/4	>3/4
Overhanging vegetation		None	<1/4	1/4 - 1/2	1/2 – 3/4	>3/4
Bankside trees		Absent	Present	Dense		
Bankside shrubs		Absent	Present	Dense		
Bankside herbs		Absent	Present	Dense		
Bankside grasses		Absent	Present	Dense		
Bankside plant species		None	1-9	10-19	>20	
Submerged vegetation		Absent	Present	Dense		
Emergent vegetation		Absent	Present	Dense		
Floating vegetation		Absent	Present	Dense		
Aquatic plant species		None	1-9	10-19	>20	

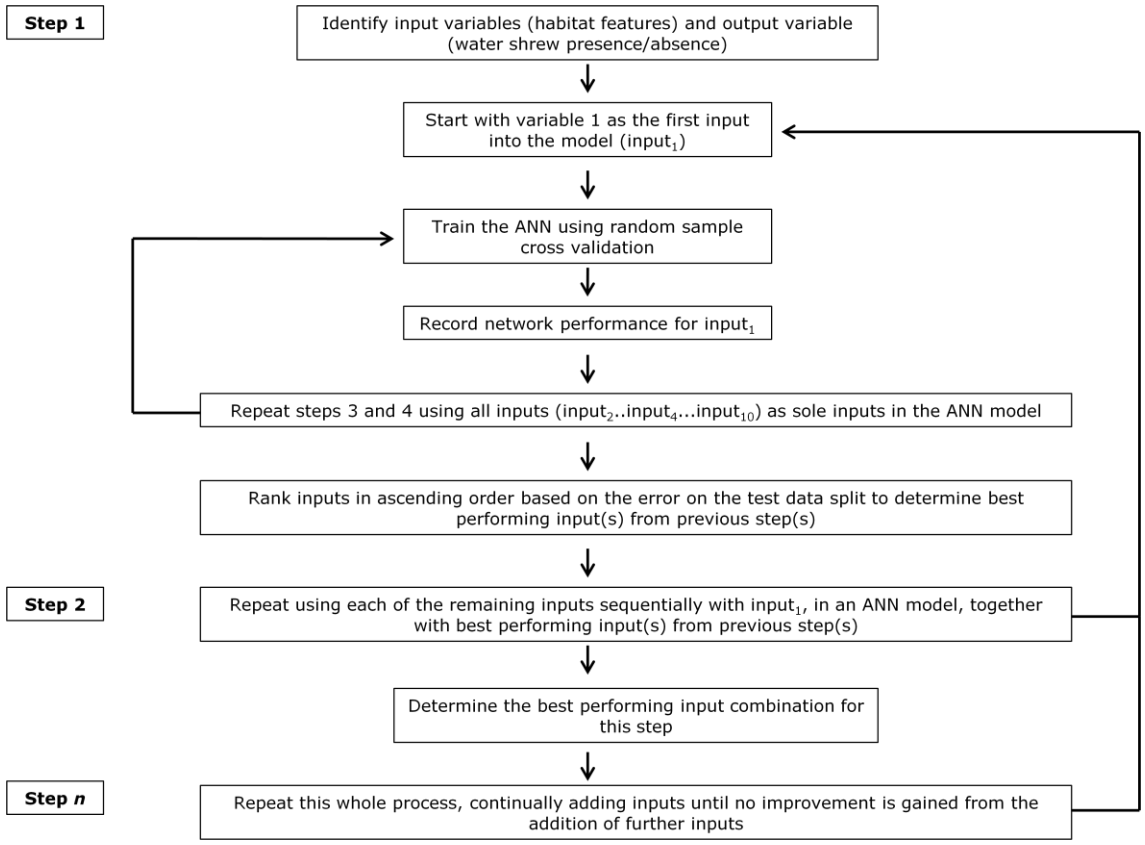


Figure 3.1 Summary of the stepwise ANN algorithm modelling process (after Lancashire *et al*., 2008).

The learning algorithm used within the ANN was a backpropogation (BP) algorithm. This algorithm is a very powerful method capable of modelling complex relationships between variables allowing prediction of an output vector (e.g. water shrew presence/absence) for a given input vector (e.g. habitat variable) (Lek and Guégan, 1999; Ball *et al.*, 2002). The BP algorithm builds a model based upon examples of data with known outputs (training data). The model is constructed entirely from the examples presented in the training data which is assumed to be fully representative of the whole set of possible data (Balls *et al*., 1996).

A problem associated with training neural networks can be the overtraining of a data set resulting in the networks memorising the training data and associated noise (Balls *et al*., 1996). This can lead to the neural networks over-fitting the training data, which reduces their ability to make

generalisations and therefore accurate predictions on previously unseen data (Tirelli *et al.*, 2009). In order to prevent overtraining, a cross-validation method is used whereby a proportion of the data set is not used for training the neural network but instead to repeatedly test the progression of the network's training and indicate its ability to generalise. This indication comes in the form of the error value of the test data which is when it has reached convergence i.e. cannot improve on the prediction at that point. When the error value no longer decreases, the training process is stopped and over-training is prevented (Balls *et al*., 1996).

Step 1 of the ANN tested all of the variables individually for their predictive performance for the presence of water shrews. Each variable was then ranked according to its predictive ability. The best predictor from step 1 of the analysis was then selected for each subsequent step, of which there were ten and all other variables were tested in combination with it, thus trying to identify the optimum two-variable model at step 2, the best three-variable model at step 3 and so on. Following the stepwise analysis the variables were ranked in order of predictive performance (based on test error) both as individual variables and overall as variables which in combination with other variables ranked highly for predictive performance. The stepwise analysis gives a probability of water shrew presence of *between* 0 and 1 whereas the presence or absence of water shrews is *either* 0 or 1. Therefore, a typically used threshold of 0.5 was applied to the model output to determine whether a prediction was correct (Fielding and Bell, 1997).

3.2.4.2 Model creation and interrogation

The highest-ranking predictors, both individually and in combination with other variables, were selected to create models for each data set (winter 2008 and summer 2008). Each model was trained over 50 randomly selected subsets and the average probability values of the 50 models calculated. The models created were then applied to the same training data to illustrate how effective those particular variables were in determining water shrew presence for that data set. In addition, in order to measure the performance of each model (the ability to correctly classify sites with and

without water shrew presence) the area under the relative operator characteristic (ROC) curve was calculated. A ROC curve plots the sensitivity (true positives) and specificity (true negatives) of a model at incremental threshold probabilities between zero and one (Greaves *et al.*, 2006) as opposed to just once at the 0.5 threshold. The area under the curve, expressed as a proportion of the area given by a model with perfect accuracy, gives a measure of the model's discrimination ability (Pearce and Ferrier, 2000). An area of 0.5 (resulting in a line of 45°) indicates that the predicted probabilities were the same as if obtained by chance (Greaves *et al.*, 2006). As discrimination ability improves, the area under the curve becomes closer to the maximum value of one, and the model more accurately describes the data. Area under the ROC curve values of 0.5-0.7 are taken to indicate low accuracy, 0.7-0.9 indicate useful applications, and values of >0.9 indicate high accuracy (Swets, 1988; Manel *et al*., 2001). The area under the ROC curve is an important index because its measure of accuracy is not dependent on a particular threshold (Deleo, 1993). Response curves were produced to show the general trend of the effect of the variables on the probability of water shrew presence using an average of all 50 models. They are a snapshot of the different variables contributions to the model and a way of looking at a four-dimensional model in two dimensions. ANN analysis was undertaken using a dedicated standalone programme (Lancashire *et al*., 2008) created and made available to the author by the bioinformatics group at Nottingham Trent University. Models were created and interrogated using STATISTICA 7.0.

3.3 HSI model development

3.3.1 Winter 2008 ANN analysis

The best individual predictor for water shrew presence was management intensity (see Table 3.4.). The predictive ability of the ANN model decreased (and the test error increased), after the fourth step (see Table 3.5) therefore a four-variable model of management intensity, bank height, floating vegetation and phosphates was created. However, water depth was

identified as one of the top individual variables for predicting water shrew presence (see Table 3.4) and is known to be an important factor for foraging (e.g. Illing *et al*, 1981; Lardet 1988; Churchfield, 1998). Therefore, a second, three-variable, model was created using the top two overall predictors, management intensity and bank height, and water depth.

Table 3.4 Step 1 summary of highest-ranking individual variables (see Appendix for complete list) for winter 2008.

Input ID	Average Test Error
Management intensity	0.203275
Water depth	0.214449
Substrate complexity	0.235448
Bank height	0.241037
Conductivity	0.241343
Width	0.242770
Overhanging vegetation	0.244705
Submerged vegetation	0.245684
Ponds and lakes up to 500m	0.252384
Bankside shrubs	0.252571

Table 3.5 Overall summary of each step showing the highest-ranking combined variables for winter 2008.

Step	Input ID	Average Test Error
1	Management intensity	0.203275
2	Bank height	0.160208
3	Floating vegetation	0.150160
4	Phosphates	0.138483
5	Water depth	0.142192
6	Overhanging vegetation	0.143075
7	Bankside herbs	0.142598
8	Current	0.134660
9	Substrate complexity	0.134754
10	Emergent vegetation	0.133221

3.3.1.1 Effect of the variables on the winter 2008 four-variable model

The probability of water shrew presence was greatest when the banksides were 'occasionally' managed, bank height was '1-2m' or above, floating vegetation was either 'absent' or 'present' and phosphates were between '0-0.99mg/l' (see Figure 3.2).

3.3.1.2 Effect of the variables on the winter 2008 three-variable model

The probability of water shrew presence was greatest when the bank sides were 'occasionally' managed, bank height was '1-2m' or above, as per the four-variable model, and water depth was '<25cm' (see Figure 3.3).

3.3.2 Winter 2008 four-variable model

Overall, the four-variable model of management intensity, bank height, floating vegetation and phosphates had a predictive performance of 84% i.e. correctly classifying the presence/absence of water shrews at 26 out of the 31 sites (see Figure 3.4). The model showed 100% sensitivity (ability to predict water shrew presence) and 67% specificity (ability to predict water shrew absence). Only five sites were misclassified (AAP, NPP, WFRP, WMME and WMRE) and all of those were false positives, with the model predicting water shrew presence at sites where the bait tube survey had not detected any. The model's discrimination ability, as determined by the area under the ROC curve, was 0.82.

3.3.3 Winter 2008 three-variable model

Overall, the three-variable model of management intensity, bank height and water depth had a lower predictive performance (77%) than the four variable model (see Figure 3.5 and Table 3.6), correctly classifying the presence/absence of water shrews at 23 out 31 sites. The model also showed less sensitivity (88%), although specificity (67%) was the same. However, the model's discrimination ability, as determined by the area under the ROC curve, was 0.86, which was higher than the four-variable model. The sites AAP, NPP, WFRP, WMME and WMRE remained misclassified as false positives, and DLP, which was correctly classified as having water shrews by the four-variable model, was incorrectly classified by the three-variable model. In addition, RCPL and TAP both sites with known presence and correctly classified by the four-variable model, had a predictive probability of 0.5 so were not classified for either presence or absence.

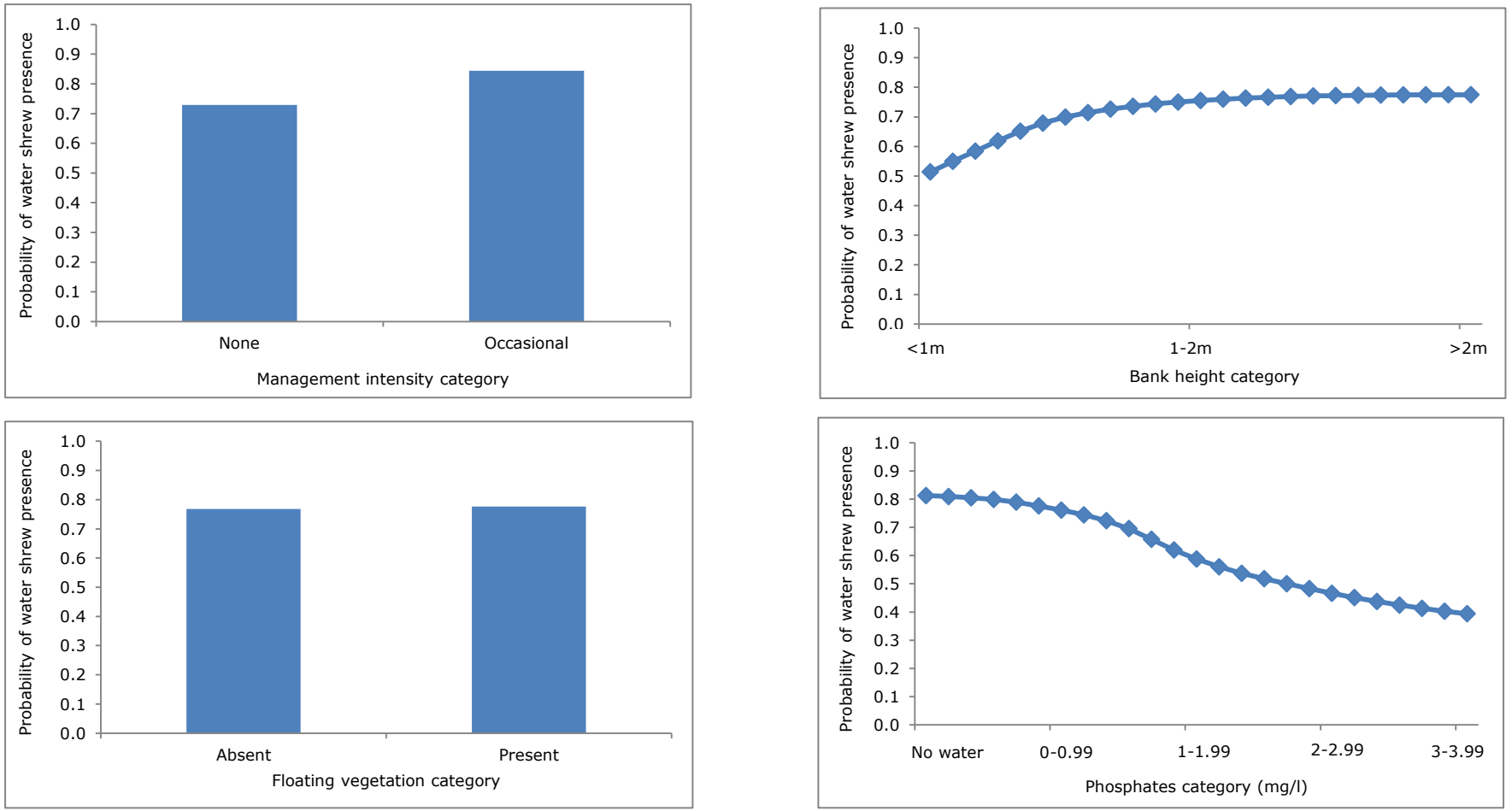
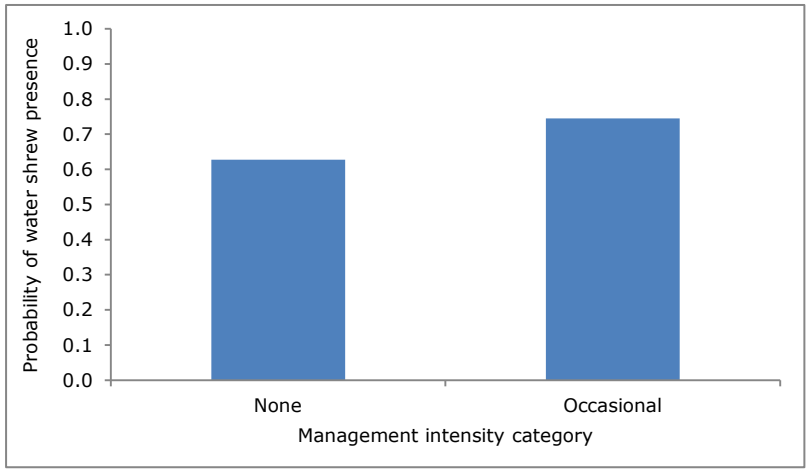
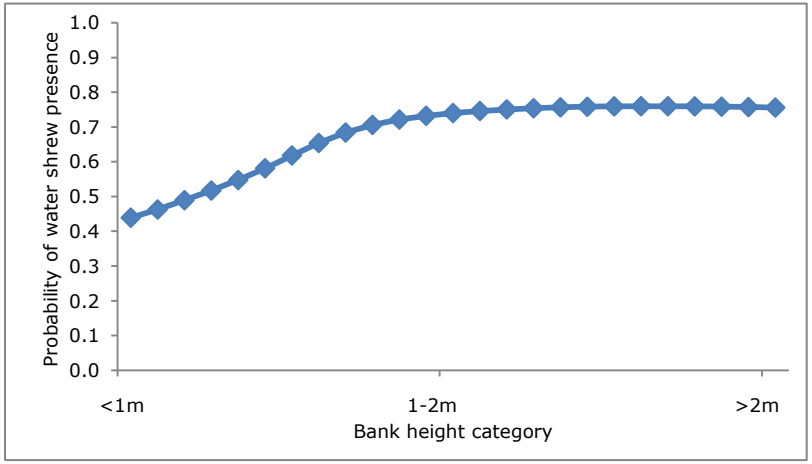


Figure 3.2 The effects of management intensity, bank height, floating vegetation and phosphates on probability of water shrew presence for the winter 2008 four-variable model.





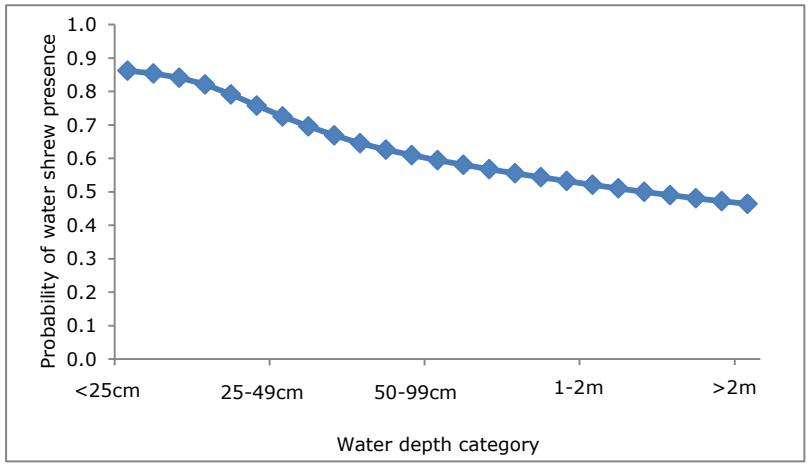


Figure 3.3 The effects of management intensity, bank height and water depth on probability of water shrew presence for the winter 2008 three-variable model.

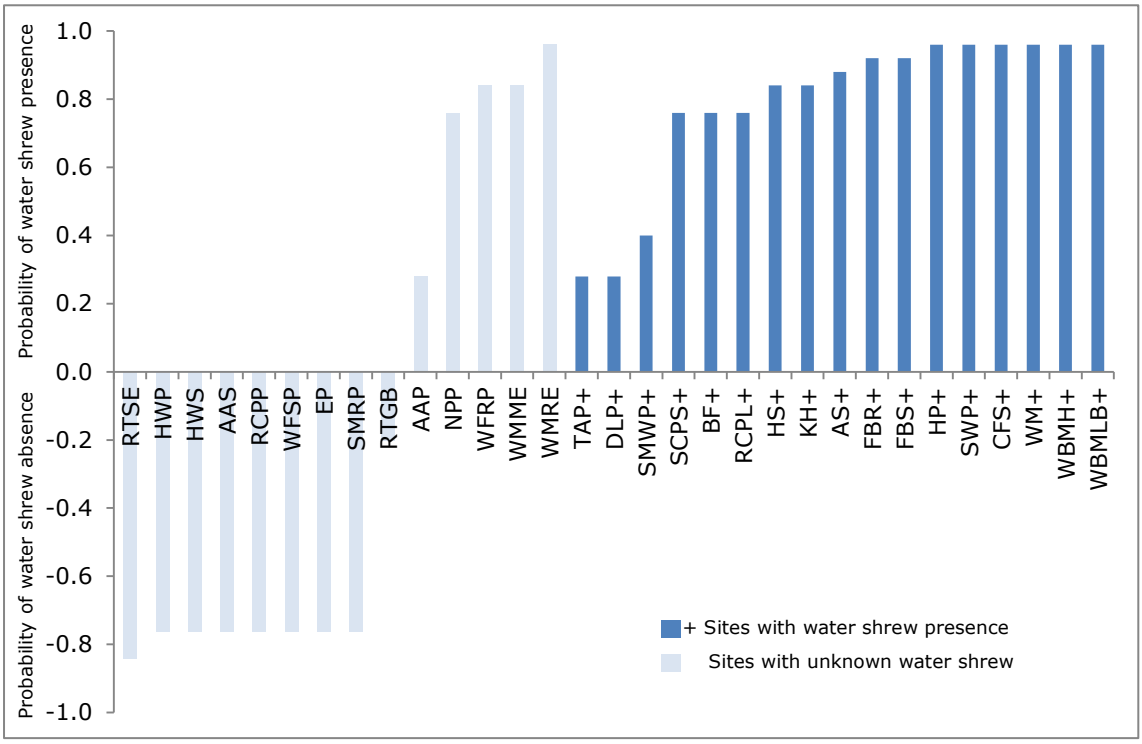


Figure 3.4 Winter 2008 four-variable model predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

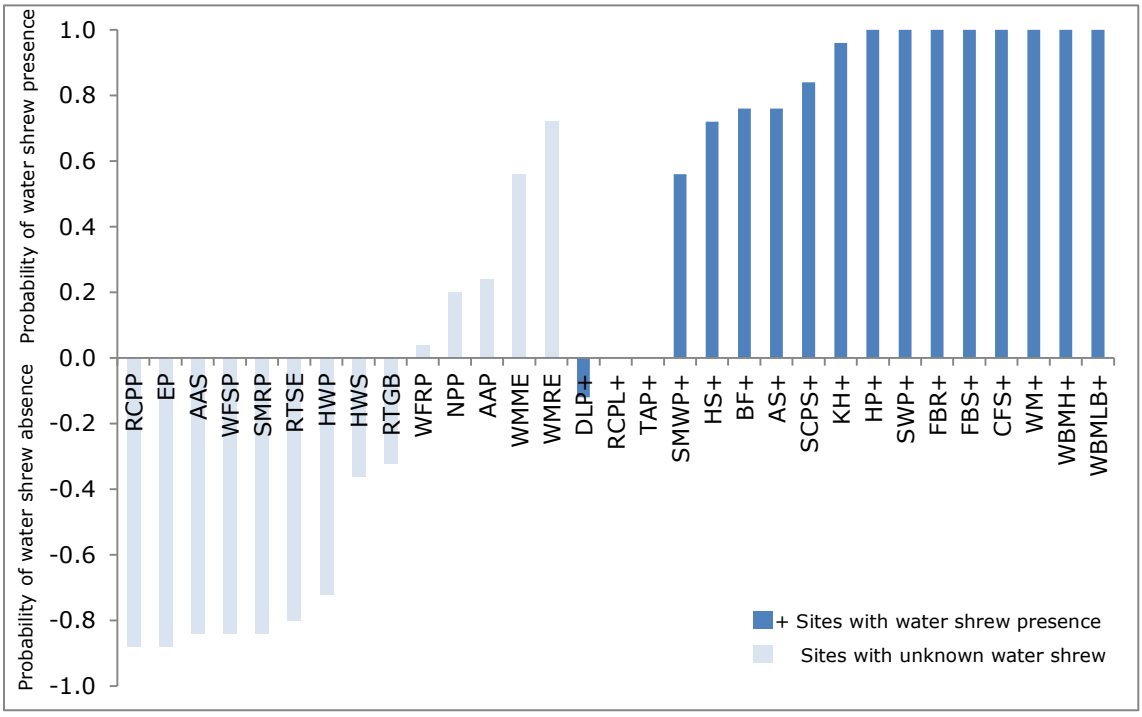


Figure 3.5 Winter 2008 three-variable model predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

Table 3.6 Summary of results for the winter 2008 models.

	Four-variable model	**Three-variable model**
Variables	Management intensity Bank height Floating vegetation Phosphates	Management intensity Bank height Water depth
Predictive performance	84%	77%
Sensitivity	100%	88%
Specificity	67%	67%
Area under ROC curve	0.82	0.86

3.3.4 Summer 2008 ANN analysis

The best individual predictor for water shrew presence, was again, management intensity (see Table 3.7.). The predictive ability of the ANN model decreased (and the test error increased), after the third step (see Table 3.8). Therefore, a three-variable model (A) of management intensity, dissolved oxygen and overhanging vegetation was created. Water depth was again identified as one of the top individual variables for predicting water shrew presence (see Table 3.4), therefore a second, three-variable, model (B) was created using the top two overall predictors, management intensity and dissolved oxygen, and water depth.

Table 3.7 Step 1 summary of highest-ranking individual variables (see Appendix for complete list) for summer 2008.

Input ID	**Average Test Error**
Management intensity	0.209859
Water depth	0.212653
Substrate complexity	0.233409
Bank height	0.234940
Overhanging vegetation	0.243771
pH	0.244675
Bank incline	0.244953
Aquatic plant species	0.246388
Human activity	0.248424
Floating vegetation	0.249641

Table 3.8 Overall summary of each step showing the highest-ranking combined variables for summer 2008.

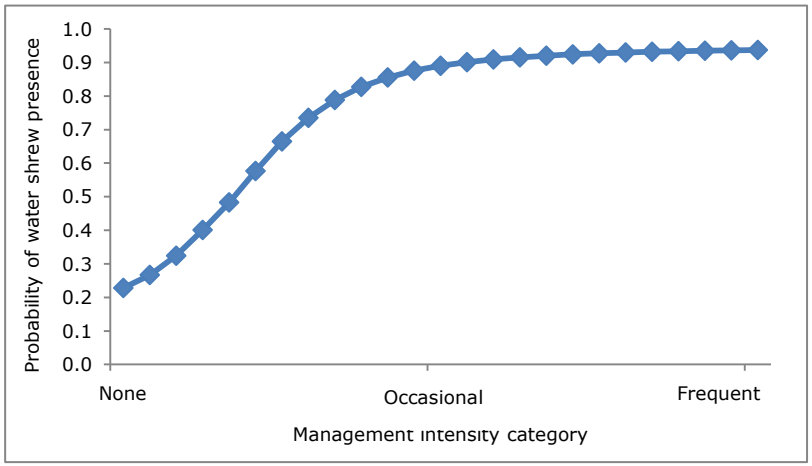
Step	Input ID	Average Test Error
1	Management intensity	0.209859
2	Dissolved oxygen	0.160084
3	Overhanging vegetation	0.133735
4	Water depth	0.136412
5	Floating vegetation	0.119890
6	Substrate complexity	0.111771
7	Human activity	0.114597
8	Conductivity	0.122696
9	Nitrates	0.137468
10	Sediment	0.147042

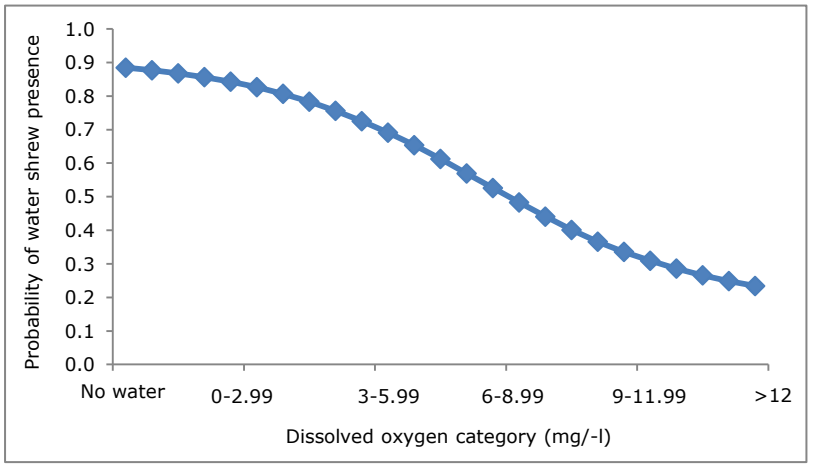
3.3.4.1 Effect of the variables on the summer 2008 three-variable model A

The probability of water shrew presence was greatest when the bank sides were 'occasionally' or 'frequently' managed, levels of dissolved oxygen were '0-2.99mg l^{-1}' and overhanging vegetation covered '>3/4' of the waterbody (see Figure 3.6).

3.3.4.2 Effect of the variables on the summer 2008 three-variable model B

The probability of water shrew presence was greatest when the bank sides were 'occasionally' managed, dissolved oxygen levels were '0-2.99mg $l^{-1'}$ and water depth '<25cm' (see Figure 3.7).





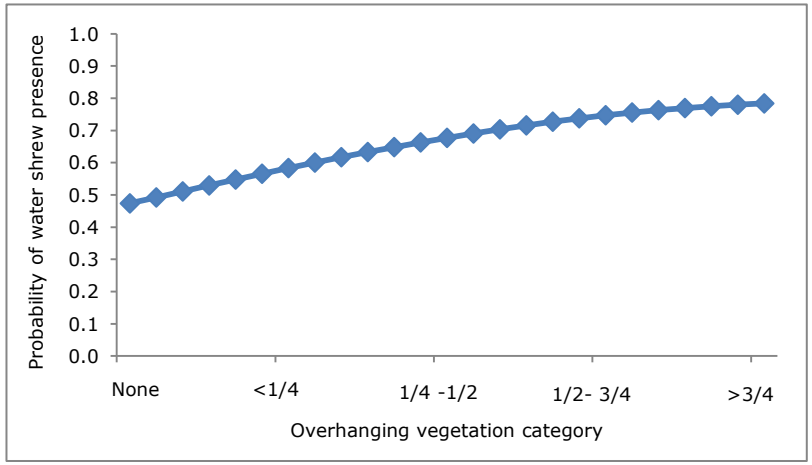
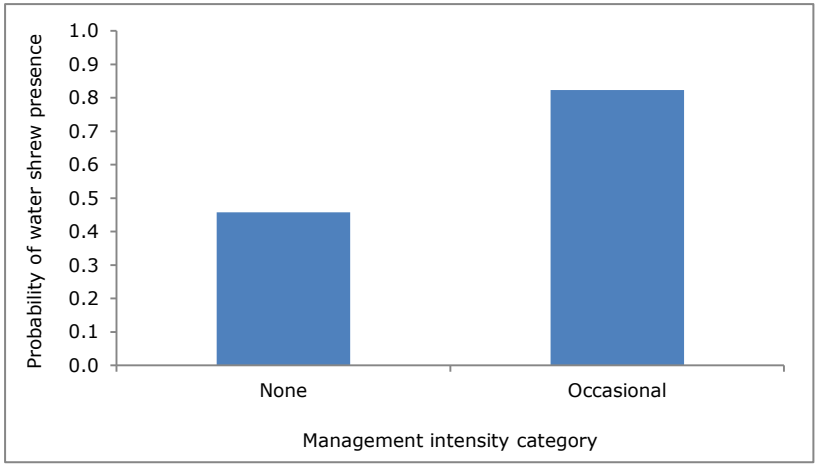
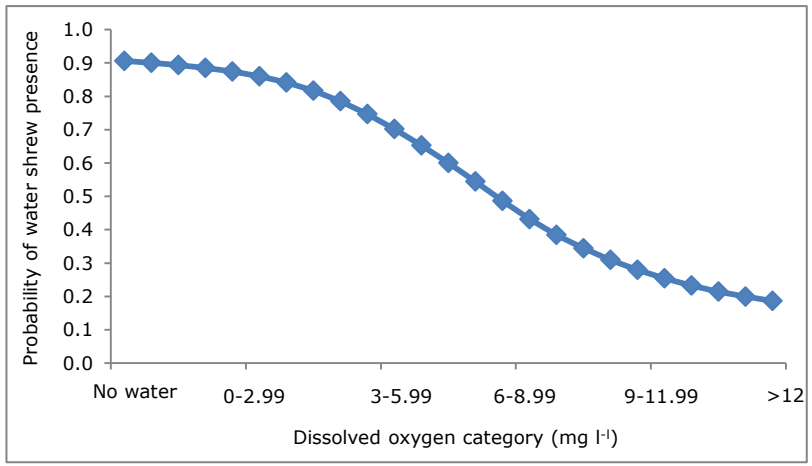


Figure 3.6 The effects of management intensity, dissolved oxygen and overhanging vegetation on probability of water shrew presence for the summer 2008 three-variable model A.





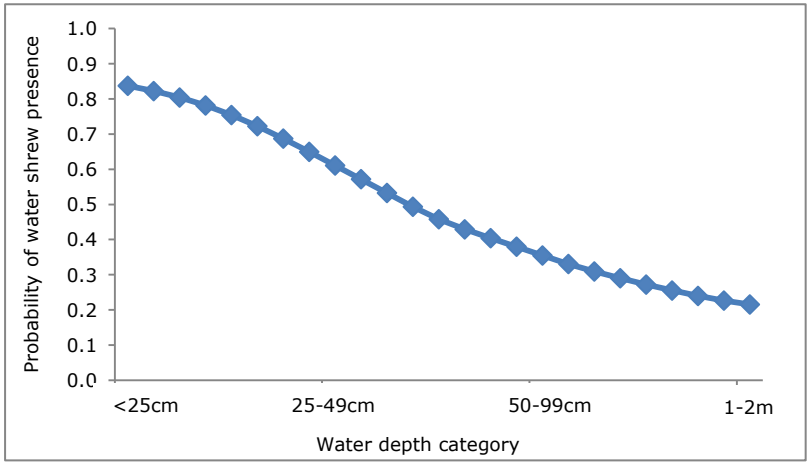


Figure 3.7 The effects of management intensity, dissolved oxygen and water depth on probability of water shrew presence for the summer 2008 three-variable model B.

3.3.5 Summer 2008 three-variable model A

Overall, the summer 2008 three-variable model A, of management intensity, dissolved oxygen and overhanging vegetation, had a predictive performance of 84%, i.e. correctly classifying the presence/absence of water shrews at 26 out of the 31 sites (see Figure 3.8). The model showed 88% sensitivity and 80% specificity. Of the five misclassified sites, three were false positives (EP, SMRP and WMRE) and two false negatives (DLP and SWP, with the model wrongly predicting water shrew absence at sites where the bait tube survey had detected their presence. The model's discrimination ability, as determined by the area under the ROC curve, was 0.87, which was higher than both winter models.

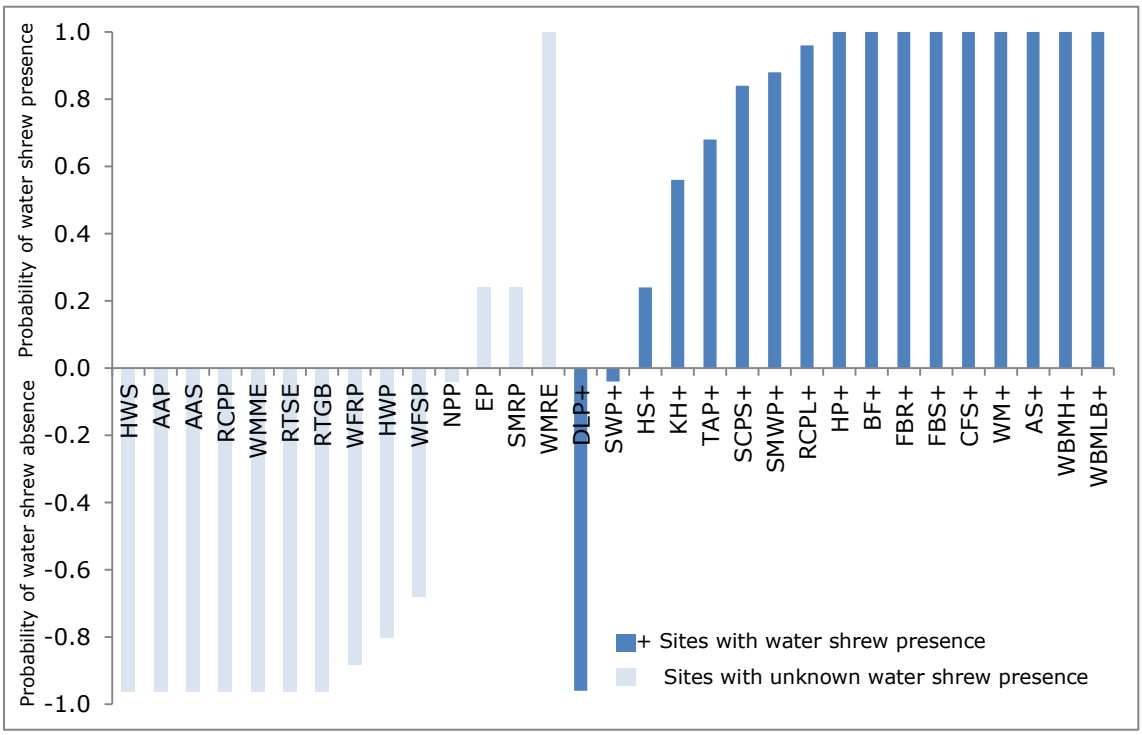


Figure 3.8 Summer 2008 three-variable model A predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

3.3.6 Summer 2008 three-variable model B

Overall, the summer 2008 three-variable model B had a higher predictive performance (90%) than the summer 2008 three-variable model A, correctly classifying the presence/absence of water shrews at 28 out 31 sites (see Figure 3.9 and Table 3.9). The model showed the same sensitivity (88%) as the summer 2008 three-variable model A but higher specificity (93%). EP and SMRP, sites misclassified by the summer 2008 three-variable model A, were correctly classified by the summer 2008 three-variable model B. However, WMRE and DLP remained misclassified and TAP, a site correctly classified by the summer 2008 three-variable model A as having water shrews, was misclassified. The model's discrimination ability, as determined by the area under the ROC curve, was 0.88, which was slightly higher than the summer 2008 three-variable model A, and again, higher than both winter models.

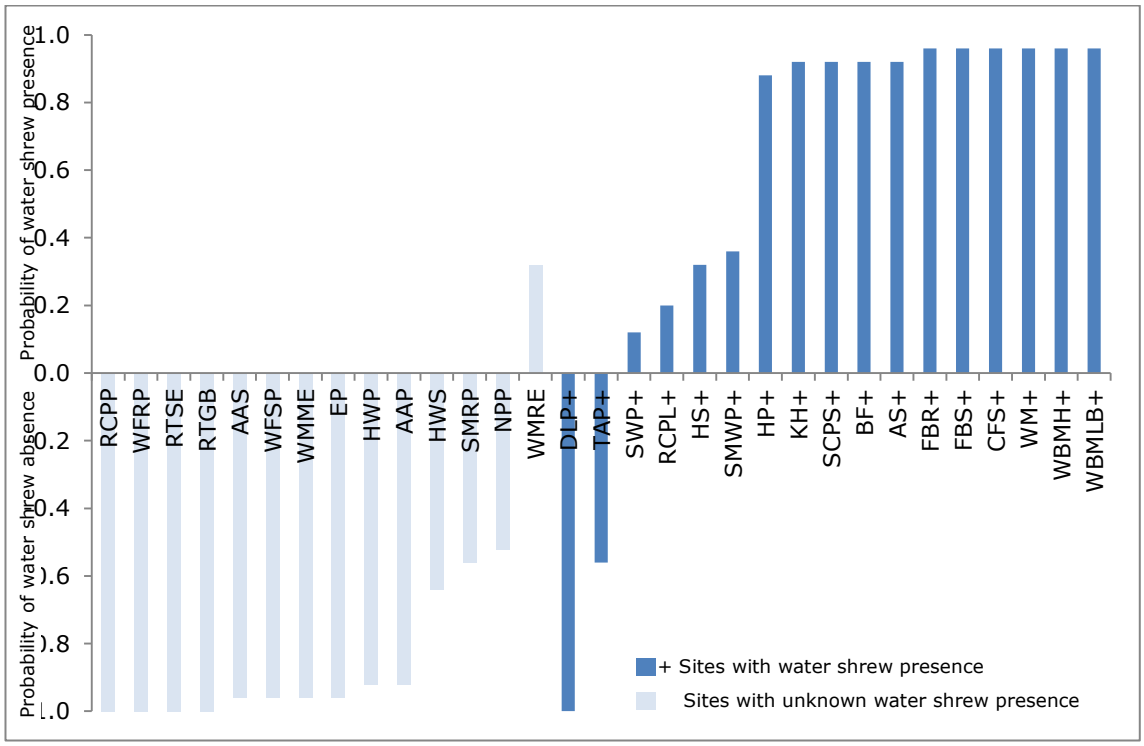


Figure 3.9 Summer 2008 three-variable model B predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

Table 3.9 Summary of results for the summer 2008 models.

	Three-variable model A	**Three-variable model B**
Variables	Management intensity Dissolved oxygen Overhanging vegetation	Management intensity Dissolved oxygen Water depth
Predictive performance	84%	90%
Sensitivity	88%	88%
Specificity	80%	93%
Area under ROC curve	0.87	0.88

3.3.7 Summary of the winter 2008 and summer 2008 models

Overall, the summer 2008 three-variable model B had the highest predictive performance (see Table 3.10) and the winter 2008 three-variable model the lowest. The best predictor for water shrew presence in all four models was management intensity, with the probability of water shrew presence more likely when bank sides were 'occasionally' or 'frequently' managed. Bank height featured as an important variable in the winter models, with water shrew presence more likely at sites with bank heights of '1-2m' or above. However, dissolved oxygen was an important feature in the summer models with water shrew presence more likely at sites with dissolved oxygen levels of '0-2.99mg l^{-1}'.

Table 3.10 Summary of results for the winter 2008 and summer 2008 models.

	Winter 2008		**Summer 2008**	
	Four-variable model	**Three-variable model**	**Three-variable model A**	**Three-variable model B**
Variables	Management intensity Bank height Floating vegetation Phosphates	Management intensity Bank height Water depth	Management intensity Dissolved oxygen Overhanging vegetation	Management intensity Dissolved oxygen Water depth
Predictive performance	84%	77%	84%	90%
Sensitivity	100%	88%	88%	88%
Specificity	67%	67%	80%	93%
Area under ROC curve	0.82	0.86	0.87	0.88
False positives	AAP NPP WFRP WMME WMRE	AAP NPP WFRP WMME WMRE	EP SMRP WMRE	WMRE
False negatives		DLP	DLP SWP	DLP TAP
Unclassified		RCPL TAP		

3.4 HSI model validation

In order to assess the generalisation ability of a model it is necessary to apply the model to an independent 'unseen' data set. Therefore, ten new validation sites (six lentic and four lotic) were selected from the alternate grid squares not used during the main bait tube survey (see Figure 3.10) using the same site selection process described previously (see Chapter 2, Section 2.2.2). Ideally, five lentic and five lotic sites would have been selected but this was not possible due to site accessibility.

3.4.1 Bait tube survey

Bait tube surveys were undertaken during summer 2009 at the ten new sites to establish water shrew presence, again, using methods described previously (see Chapter 2, Section 2.2.4).

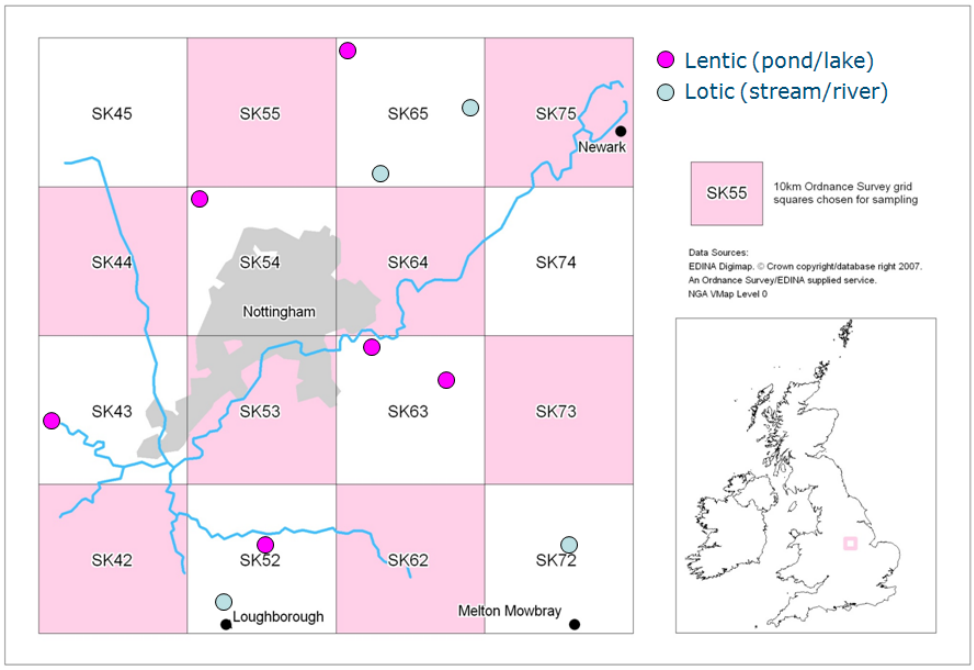


Figure 3.10 Map of study area showing the ten new HSI validation sites.

3.4.2 Results of bait tube survey

Following analysis of the contents of the bait tubes, four of the ten sites were found to be positive for water shrew presence (see Figure 3.11 and Table 3.11). Of these four positive sites, two were lentic and two were lotic. As previously, sites where no evidence of water shrews were found were recorded as 'not detected' as opposed to 'absent'.

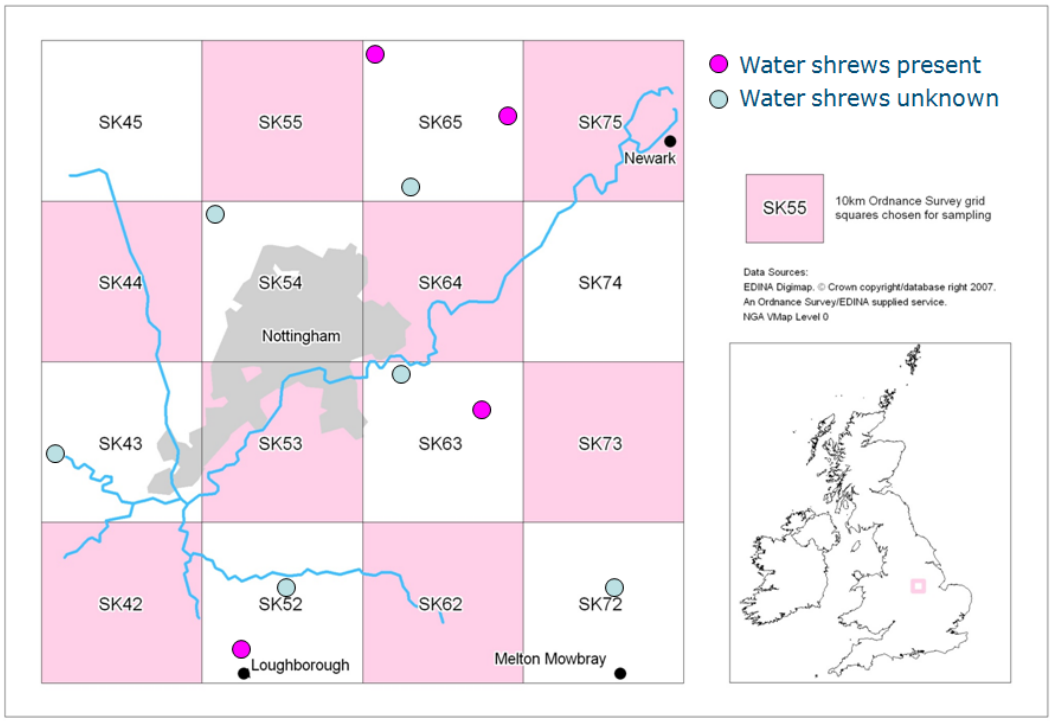


Figure 3.11 Distribution of water shrews following the HSI validation bait tube survey.

Table 3.11 The ten HSI validation sites surveyed during the bait tube survey showing presence or non-detection of water shrews.

National Grid Square	Grid reference	Site	Habitat type	Water shrews
SK43	SK406333-SK405333	Elvaston Castle	Lentic	Not detected
SK52	SK558254-SK558254	Manor Farm	Lentic	Not detected
	SK537214-SK537215	Loughborough Big Meadow	Lotic	Present
SK54	SK549480-SK549481	Mill Lakes	Lentic	Not detected
SK63	SK675367-SK676367	Spike's Island	Lentic	Present
	SK619391-SK619390	Skylarks Nature Reserve	Lentic	Not detected
SK65	SK622603-SK622603	Rainworth Water	Lentic	Present
	SK697557-SK697556	River Greet	Lotic	Present
	SK625502-SK624502	Dover Beck	Lotic	Not detected
SK72	SK754240-SK753239	Grange Farm	Lotic	Not detected

3.4.3 Discussion of bait tube survey

As with the previous bait tube survey, water shrews were found at similar numbers of lentic and lotic sites which further supports evidence of no preference for still or flowing water (Carter and Churchfield, 2006a), contrary to their traditional association with fast flowing streams and rivers (Churchfield, 1990; French *et al.*, 2001). Again, similar to the previous bait tube survey, a variety of riparian habitats were assessed including five grassland, one scrub, one arable and four woodland sites. However, unlike the previous survey which found no preference for adjacent habitat, no water shrews were found at any of the woodland sites but were present mainly at grassland sites as well as the scrub and arable sites. These findings are also contrary to other studies which have found water shrews least likely to occur at freshwater habitats adjacent to grassland (French *et al.*, 2001; Carter and Churchfield, 2006a). Water shrew presence was not detected at sites which were scored as being 'frequently' used by people, only occurring at sites with 'minimal' or 'sporadic' human use. This is, again, contrary to the previous survey and to other studies (Greenwood *et al*., 2002; Carter and Churchfield, 2006a) which found water shrew presence to

be unaffected by human disturbance. Apart from water shrews showing no particular preference for lentic or lotic sites, preliminary findings appear to contradict those from the previous survey.

3.4.4 Habitat surveys

Habitat surveys were also undertaken at the ten new sites during summer 2009 using methods previously described (see Section 3.2.3).

3.4.5 Data analysis

Data collected from the habitat surveys were converted to the scoring system used previously (see Table 3.3 for variable scoring categories). The models created with the summer 2008 data were applied to the new summer 2009 validation data set in order to assess the predictive performance of each model on unseen sites and therefore its generalisation ability. Due to the nature of seasonality and its effect on habitat variables such as vegetation cover and water depth, only the summer 2008 models were applied to the summer 2009 validation data.

3.4.6 Summer 2008 model validation

3.4.6.1 Summer 2008 three-variable model A validation

Overall, the summer 2008 three-variable model A had an extremely poor predictive performance of only 50%, correctly classifying the presence/absence of water shrews at only half of the ten 'unseen' sites (see Figure 3.12). The model showed only 50% sensitivity and 50% specificity. Of the five misclassified sites, three were false positives (EC, MF and ML) and two false negatives (LBM and RW). The area under the ROC curve was only 0.42 which meant that the model's predictive probability was less than if predictions were made by random.

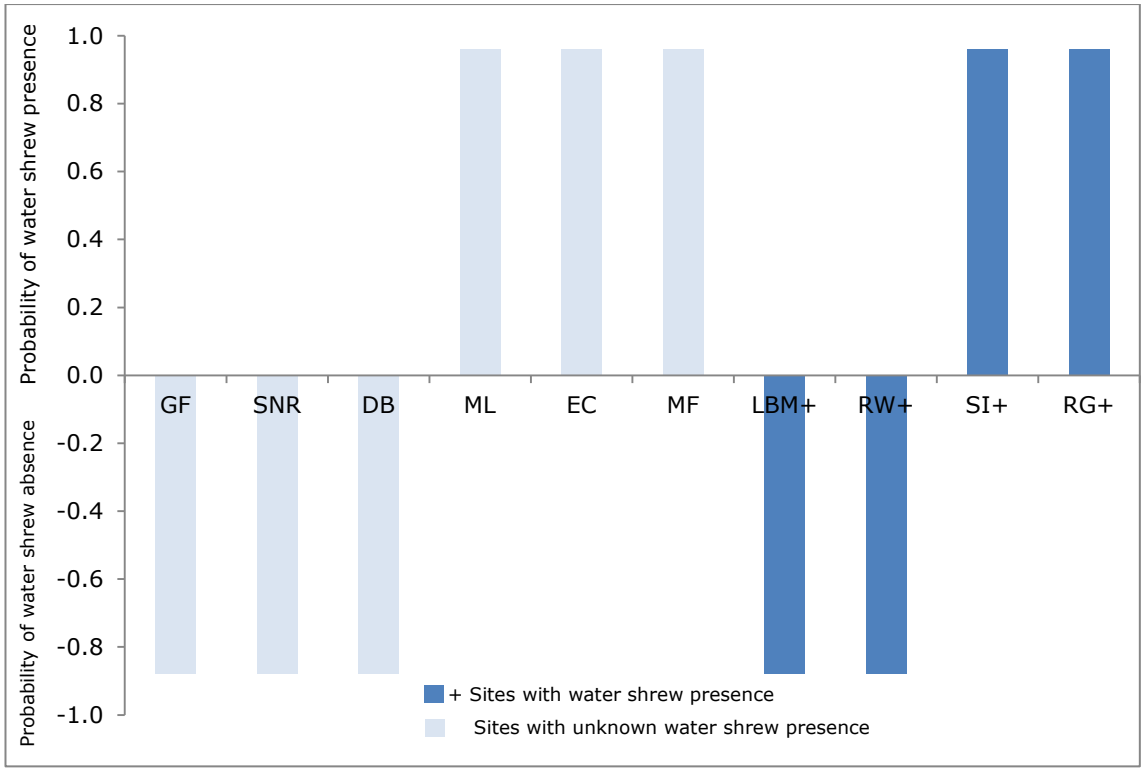


Figure 3.12 Summer 2008 three-variable model A validation predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

3.4.6.2 Summer 2008 three-variable model B validation

Overall, the summer 2008 three-variable model B had an even poorer predictive performance (30%) than the summer 2008 three-variable model A, correctly classifying the presence/absence of water shrews at only three out of the ten 'unseen' sites (see Table 3.12 and Figure 3.13). The model showed the same specificity (50%) as the three-variable model A but only half the sensitivity (25%). However, the area under the ROC curve was 0.64, which was higher than the summer 2008 three-variable model A. The same sites misclassified with the summer 2008 three-variable model A were also misclassified by the summer 2008 three-variable model B, with the addition of SI, which was incorrectly classified as not having water shrew presence.

Table 3.12 Summary of results for the summer 2008 validation models.

	Three-variable model A	**Three-variable model B**
Variables	Management intensity Dissolved oxygen Overhanging vegetation	Management intensity Dissolved oxygen Water depth
Predictive performance	50%	30%
Sensitivity	50%	25%
Specificity	50%	50%
Area under ROC curve	0.42	0.64

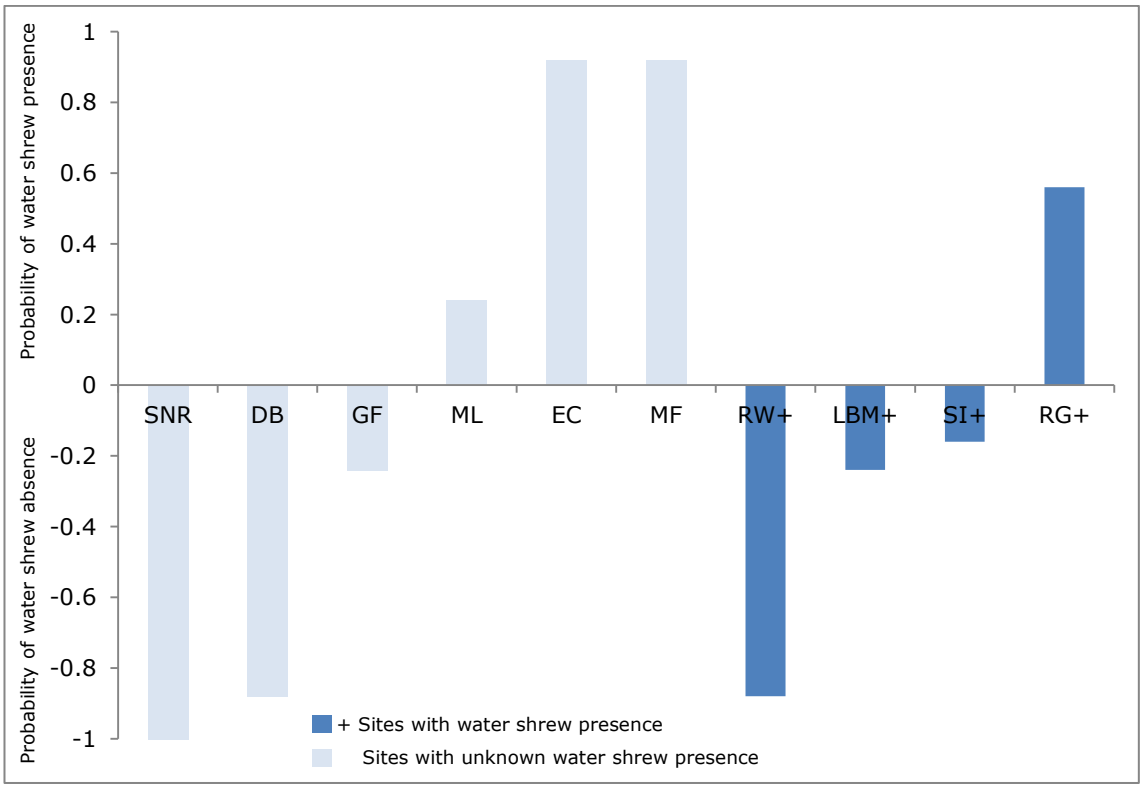


Figure 3.13 Summer 2008 three-variable model validation B predictions. Sites above zero are predicted to have water shrew presence and sites below zero are predicted not to have water shrew presence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

3.5. HSI model revision

The poor performance of the summer 2008 models, when applied to the summer 2009 validation data, could have been due to the HSI validation sites not being representative, or as an affect of different environmental factors in each year, consequently skewing the data. Therefore, both summer data sets were combined and reanalysed using ANNs to determine the important variables for the combined data set (see Figure 3.14 for the HSI modelling process). In order to create an 'unseen' data set with which to independently test the generalization ability of the revised models, prior to ANNs analysis ten sites (five from summer 2008 and five from summer 2009) were systematically selected (by alphabetising and selecting every other site) and excluded from the training data (see Table 3.13).

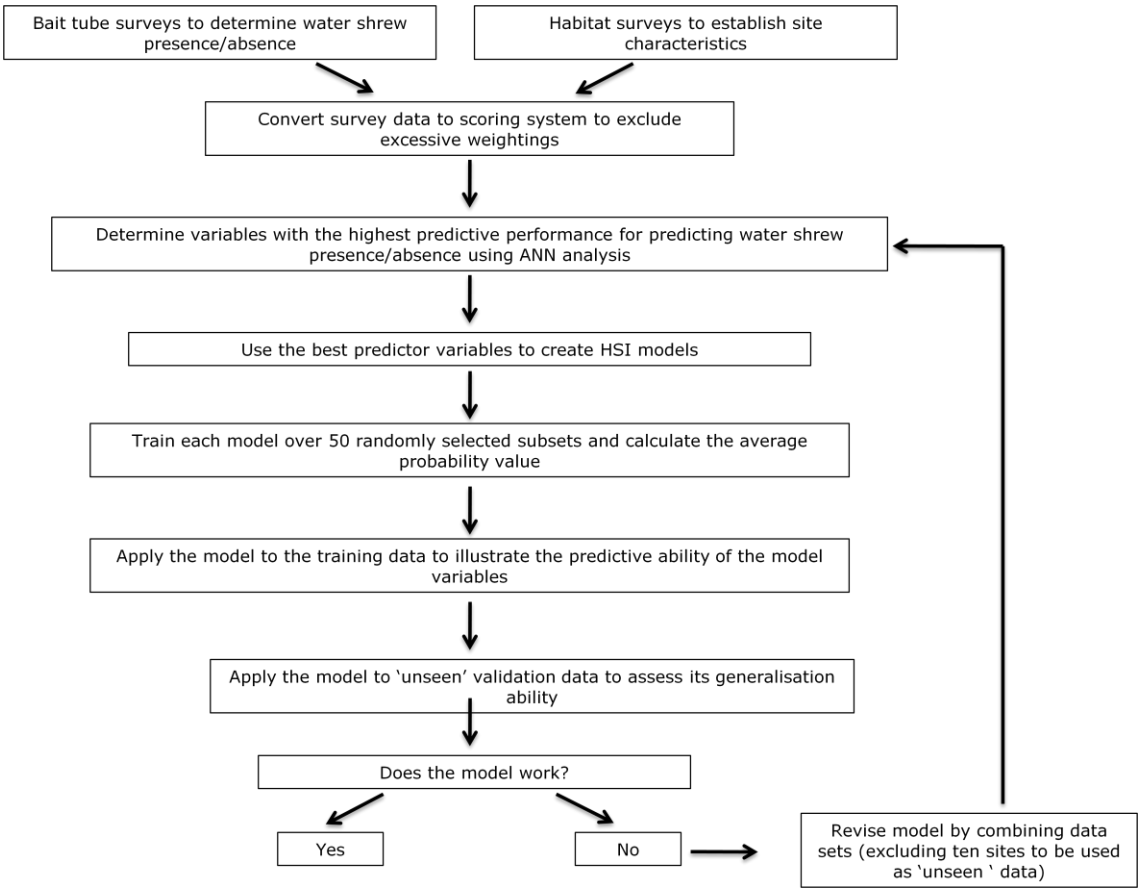


Figure 3.14 The HSI modelling process.

Table 3.13 Combined summer 2008/09 sites, showing in bold, the ten excluded 'unseen' validation sites.

National Grid Square	Grid reference	Site	Year of habitat survey	Water shrews
SK42	SK470225 – SK470225	Drypot Lane Pond (DLP)	2008	Present
	SK476240 – SK476240	Ash Spinney Pond (AS)	2008	Present
	SK492232 – SK492233	Whatton Brook (Mill House) (WBMH)	2008	Present
	SK484235 – SK485235	Whatton Brook (Mill Lane Bridge) (WBMLB)	2008	Present
SK43	SK406333-SK405333	**Elvaston Castle (EC)**	2009	Not detected
SK44	SK446501 – SK446501	Brinsley Flash (BF)	2008	Present
	SK457438– SK452438	American Adventure Pond (AAP)	2008	Not detected
	SK429441 – SK429440	Shipley Country Park Stream (SCPS)	2008	Present
	SK448433 – SK448432	American Adventure Stream (AAS)	2008	Not detected
SK52	SK558254-SK558254	Manor Farm (MF)	2009	Not detected
	SK537214-SK537215	**Loughborough Big Meadow (LBM)**	2009	Present
SK53	SK561338 – SK561337	Fairham Brook (Road End) (FBR)	2008	Present
	SK558333 – SK558334	**Fairham Brook (School End) (FBS)**	2008	Present
	SK573321 – SK573320	Rushcliffe Country Park Lake (RCPL)	2008	Present
	SK570324 – SK570324	Rushcliffe Country Park Pond (RCPP)	2008	Not detected
SK54	SK549480-SK549481	Mill Lakes (ML)	2009	Not detected
SK55	SK562566 – SK562566	Harlow Wood Pond (HWP)	2008	Not detected
	SK555564 – SK554564	**Harlow Wood Stream (HWS)**	2008	Not detected
	SK543534 – SK543534	Newstead Park Pond (NPP)	2008	Not detected
SK62	SK620222 – SK620221	Clock Farm Stream (CFS)	2008	Present
	SK611231 – SK611231	Wymeswold Meadows (WM)	2008	Present
	SK639211 – SK639211	Twenty-Acre Piece (TAP)	2008	Present
	SK636235 – SK636235	Ella's Pond (EP)	2008	Not detected
SK63	SK675367-SK676367	Spike's Island (SI)	2009	Present
	SK619391-SK619390	**Skylarks Nature Reserve (SNR)**	2009	Not detected
SK64	SK670435 – SK671435	Shelford Manor (River Pond) (SMRP)	2008	Not detected
	SK664431 – SK665432	River Trent (Shelford End) (RTSE)	2008	Not detected
	SK675427 – SK675427	Shelford Manor (Wood Pond) (SMWP)	2008	Present
	SK679436 – SK680436	River Trent (Gunthorpe Bridge) (RTGB)	2008	Not detected
SK65	SK622603-SK622603	Rainworth Water (RW)	2009	Present
	SK697557-SK697556	**River Greet (RG)**	2009	Present
	SK625502-SK624502	Dover Beck (DB)	2009	Not detected
SK72	SK754240-SK753239	**Grange Farm (GF)**	2009	Not detected
SK73	SK750382 – SK749381	Whatton Manor (Mink End) (WMME)	2008	Not detected
	SK742372 – SK743373	**Whatton Manor (Road End) (WMRE)**	2008	Not detected
	SK761320 – SK761321	Washdyke Farm (Railway Pond) (WFRP)	2008	Not detected
	SK757308 – SK758309	Washdyke Farm (Secret Pond) (WFSP)	2008	Not detected
SK75	SK774556 – SK775556	Kelham Hall (KH)	2008	Present
	SK718560 – SK718560	**Hockerton Pond (HP)**	2008	Present
	SK716562 – SK716561	Hockerton Stream (HS)	2008	Present
	SK758552 – SK758552	Sheepwalks Pond (SWP)	2008	Present

Models were, again, created from the variables identified by ANNs as having greatest predictive ability and applied initially to the training data, to illustrate how well they fitted the data, and then to the validation data set of 'unseen' sites to assess predictive performance.

3.5.1 Summer 2008/09 models

3.5.1.1 Summer 2008/09 ANN analysis

The best individual predictor for water shrew presence was water depth (see Table 3.14). The predictive ability of the ANNs model decreased (and the test error increased), after the fifth step (see Table 3.15). Therefore, a five-variable model of water depth, bank height, management intensity, floating vegetation and overhanging vegetation was created. The three variables (management intensity, dissolved oxygen and water depth) which together created the highest performing summer 2008 model also featured as high ranking predictors, either individually or in combination with each other, in the summer 2008/09 data set, therefore a second model consisting of these variables was also created.

Table 3.14 Step 1 summary of highest-ranking individual variables (see Appendix for complete list) for summer 2008/09.

Input ID	Average Test Error
Water depth	0.221471
Bank height	0.229469
Dissolved oxygen	0.237289
Management intensity	0.237716
pH	0.241970
Width	0.247348
Habitat type	0.249205
Floating vegetation	0.250226
Bankside grasses	0.253640
Aquatic plants	0.254246

Table 3.15 Overall summary of each step showing the highest-ranking combined variables for summer 2008/09.

Step	Input ID	Average Test Error
1	Water depth	0.221471
2	Bank height	0.182917
3	Management intensity	0.177444
4	Floating vegetation	0.158273
5	Overhanging vegetation	0.135107
6	Dissolved oxygen	0.138311
7	Bankside herbs	0.121346
8	Substrate complexity	0.129578
9	Human activity	0.126916
10	Bankside shrubs	0.132663

3.5.1.1.1 Effect of the variables on the summer 2008/09 five-variable model

The probability of water shrew presence was greatest when the bank sides were 'occasionally' managed, water depth was '<25cm' bank height was greater than '>2m', overhanging vegetation covered '>3/4' of the waterbody and floating vegetation was 'absent' (see Figure 3.15)

3.5.1.1.2 Effect of the variables on the summer 2008/09 three-variable model

The probability of water shrew presence was greatest when the bank sides were 'occasionally' managed, levels of dissolved oxygen were '0-2.99mg l^{-1}' and water depth was '<25cm' (see Figure 3.16).

3.5.1.2 Summer 2008/09 five-variable model

Overall, the five-variable model of water depth, bank height, management intensity, floating vegetation and overhanging vegetation had a predictive performance of 90%, i.e. correctly classifying the presence/absence of water shrews at 28 of the 31 sites (see Figure 3.17). The model showed the same sensitivity and specificity as the summer 2008 management intensity, dissolved oxygen and water depth model (88% and 93%) and the same sensitivity, but higher specificity, than the summer 2008 management intensity, dissolved oxygen and overhanging vegetation model.

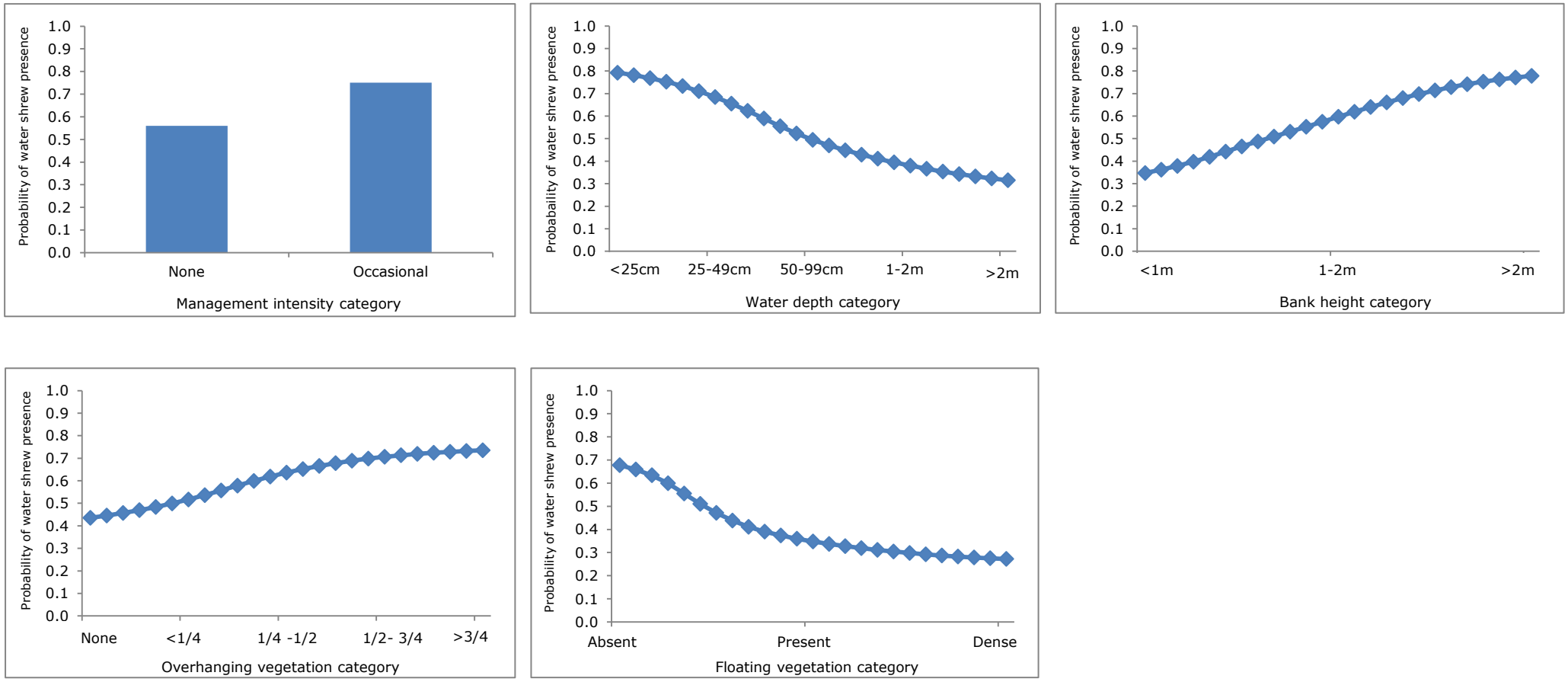
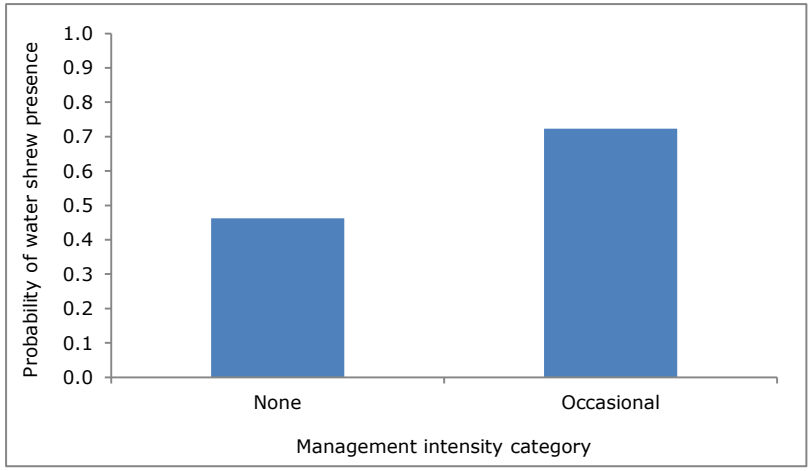
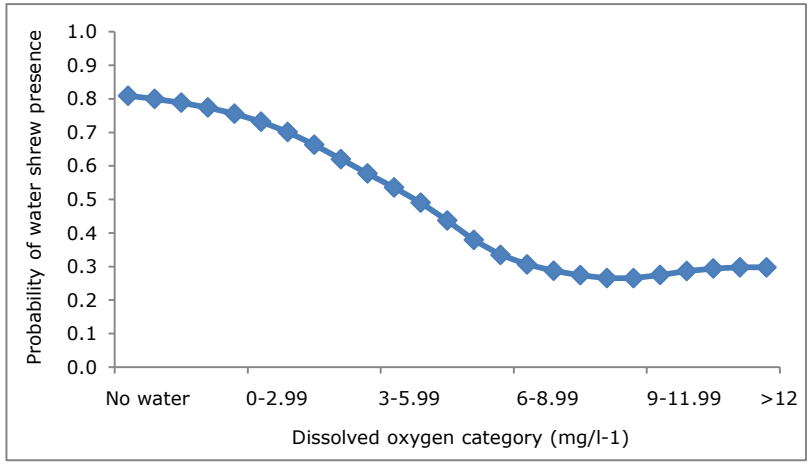


Figure 3.15 The effects of management intensity, water depth, bank height, overhanging vegetation and floating vegetation on probability of water shrew presence for the summer 2008/09 five-variable model.





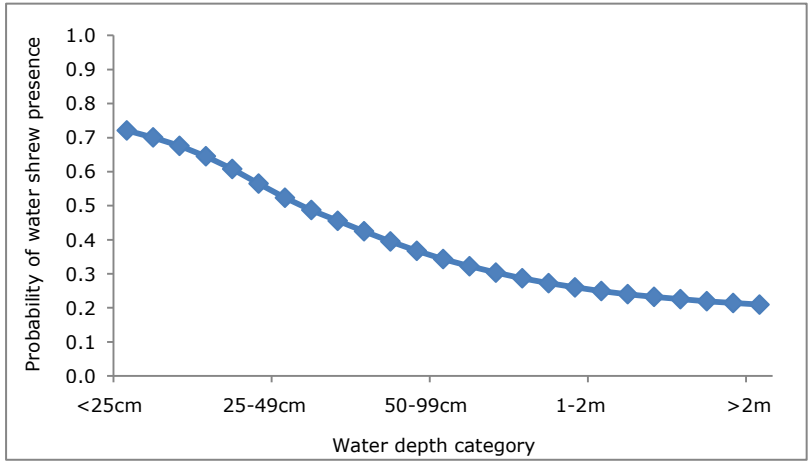


Figure 3.16 The effects of management intensity, dissolved oxygen and water depth on probability of water shrew presence for the summer 2008/09 three-variable model.

Of the three misclassified sites, one (ML) was a false positive (also misclassified by the summer 2008 validation models) and two were false negatives, DLP (also misclassified by the winter 2008 three-variable model and summer 2008 models) and RW (also misclassified by the summer 2008 validation models). The model's discrimination ability, as determined by the area under the ROC curve, was 0.82, which was the same as the winter 2008 four-variable model but lower than the winter 2008 three-variable model and summer 2008 models.

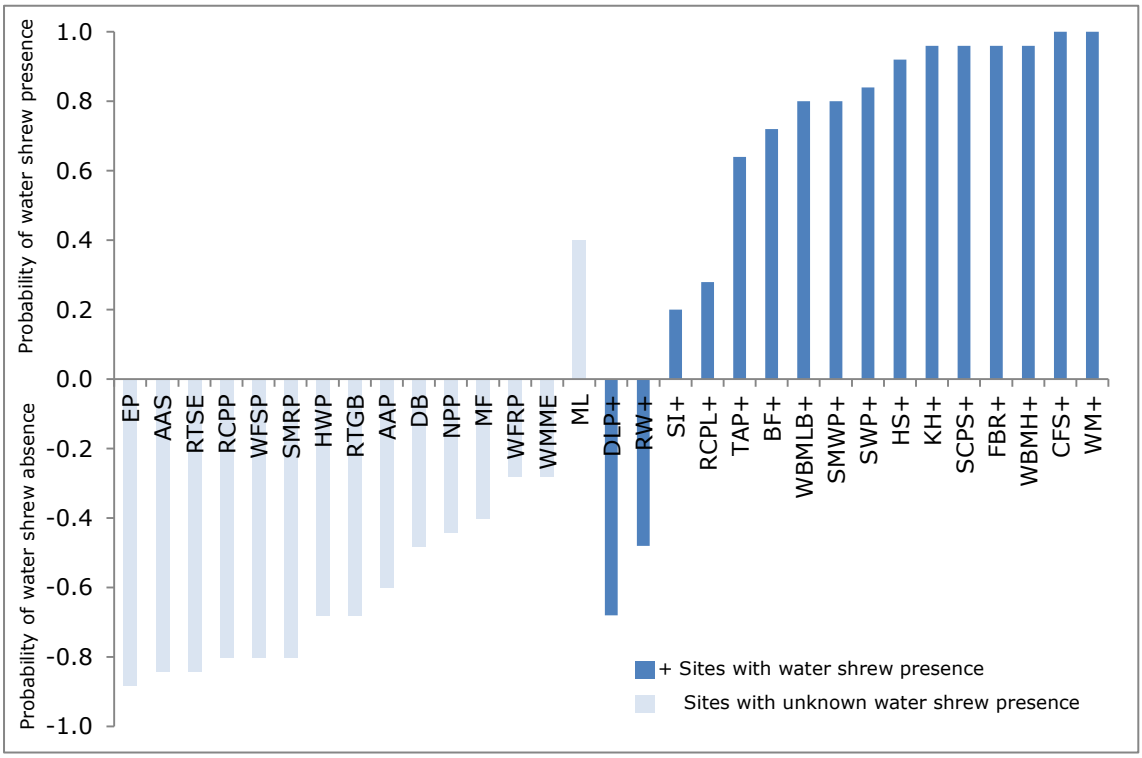


Figure 3.17 Summer 2008/09 five-variable model predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

3.5.1.3 Summer 2008/09 three-variable model

Overall, the three-variable model of management intensity, dissolved oxygen and water depth had a predictive performance of 87%, i.e. correctly classifying the presence/absence of water shrews at 27 of the 31 sites (see Figure 3.18 and Table 3.16). The model showed lower sensitivity (81%) than all other models, although specificity was the same as the summer

2008/09 five-variable model and summer 2008 three-variable model B (93%) but higher than the summer 2008 three-variable model A, and both winter 2008 models. ML, which was misclassified as having water shrew presence by the five-variable model, was correctly classified by the three-variable model. However, DLP and RW were also misclassified by the three-variable model as not having water shrew presence. In addition, MF, a site previously correctly classified by the five-variable model, was misclassified by the three-variable model. The model's discrimination ability, as determined by the area under the ROC curve, was 0.82, which was the same as for the five-variable model and the winter 2008 four-variable model, but lower than the winter 2008 three-variable model and summer 2008 models.

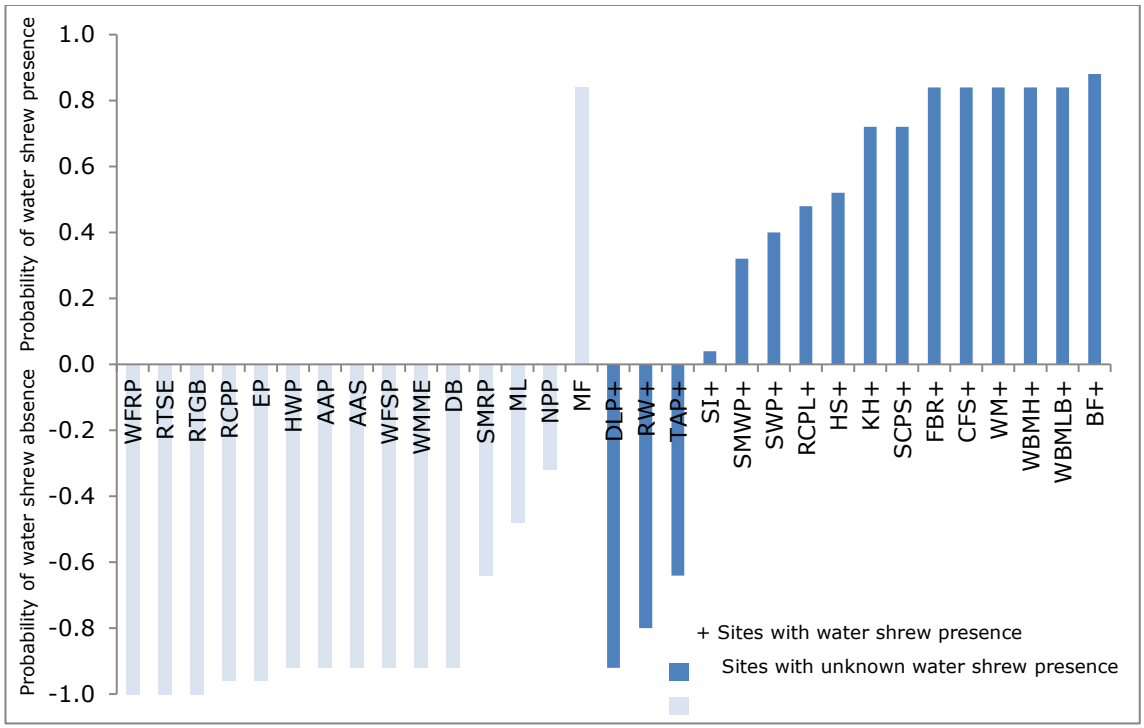


Figure 3.18 Summer 2008/09 three-variable model predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

Table 3.16 Summary of results for the summer 2008/09 models.

	Five-variable model	**Three-variable model**
Variables	Water depth Bank height Management intensity Floating vegetation Overhanging vegetation	Management intensity Dissolved oxygen Water depth
Predictive performance	90%	87%
Sensitivity	88%	81%
Specificity	93%	93%
Area under ROC curve	0.82	0.82

3.6 Revised HSI model validation

3.6.1 Effects of the variables on the validation models

3.6.1.1 Effect of the variables on the summer 2008/09 five-variable validation model

The effect of the five variables on the probability of water shrew presence was the same as for the summer 2008/09 training data. Probability was greatest when the bank sides were 'occasionally' managed, water depth was '<25cm' bank height was greater than '>2m', overhanging vegetation covered '>3/4' of the waterbody and floating vegetation was 'absent' (see Figure 3.15).

3.6.1.2 Effect of the variables on the summer 2008/09 three-variable validation model

The effect of the three variables on the probability of water shrew presence was the same as for the summer 2008/09 training data. The probability of water shrew presence was greatest when the bank sides were 'occasionally' managed, levels of dissolved oxygen were '0-2.99mg l^{-1}' and water depth was '<25cm' (see Figure 3.16).

3.6.2 Summer 2008/09 five-variable model validation

Overall, the five-variable model of water depth, bank height, management intensity, floating aquatic vegetation and overhanging vegetation had a predictive performance of 60% i.e. correctly classifying the presence/absence of water shrews at six out of the 10 'unseen' sites (see Figure 3.19). The model showed 100% sensitivity, which was the same as the winter four-variable model and higher than all of the other models, but only 40% specificity, which was the lowest of all the models. Of the four misclassified sites, all were false positives (EC, GF, HWS and WMRE). EC, a site from the summer 2009 data set was also misclassified previously by both of the summer 2008 validation models. GF, another summer 2009 site, was previously correctly classified by the summer 2008 validation models but misclassified by this model. HWS was misclassified for the first time with this summer 2008/09 five-variable model, whereas WMRE remained misclassified, as it has been throughout by every model. The area under the ROC curve (0.65) was higher than the summer 2008 validation models but lower than all other models.

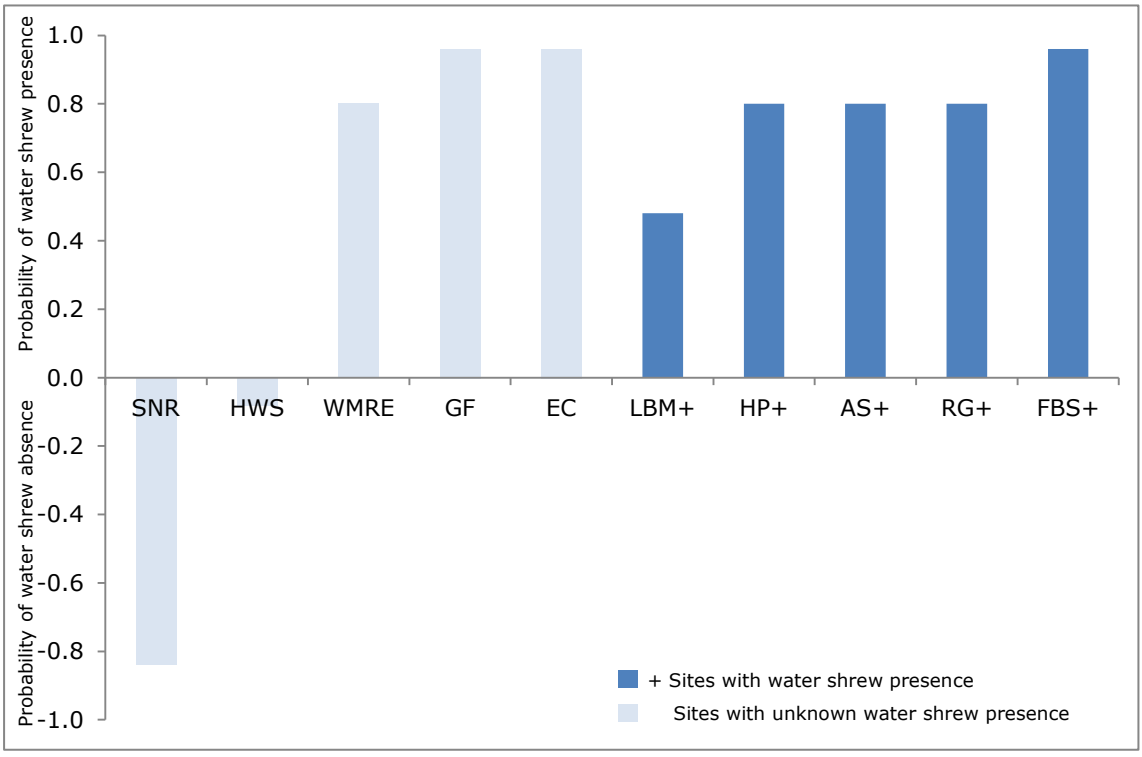


Figure 3.19 Summer 2008/09 five-variable model validation predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

3.6.3. Summer 2008/09 three-variable model validation

Overall the three-variable model of management intensity, dissolved oxygen and water depth had a greater predictive performance (80%) than the five-variable model, correctly classifying the presence/absence of water shrews at eight out of 10 sites (see Figure 3.20 and Table 3.17). The model showed the same sensitivity (100%) but higher specificity (60%) than the five-variable model. Specificity was better than both summer 2008 validation models although lower than all other models. The model still incorrectly classified EC and WMRE, as having water shrew presence however, GF and HWS, sites incorrectly classified by the five-variable model were correctly classified. The model's discrimination ability, as determined by the area under the ROC curve, was 0.85 which was higher than the five-variable model, both summer 2008 validation models, both 2008/09 training models, and the winter 2008 four-variable model.

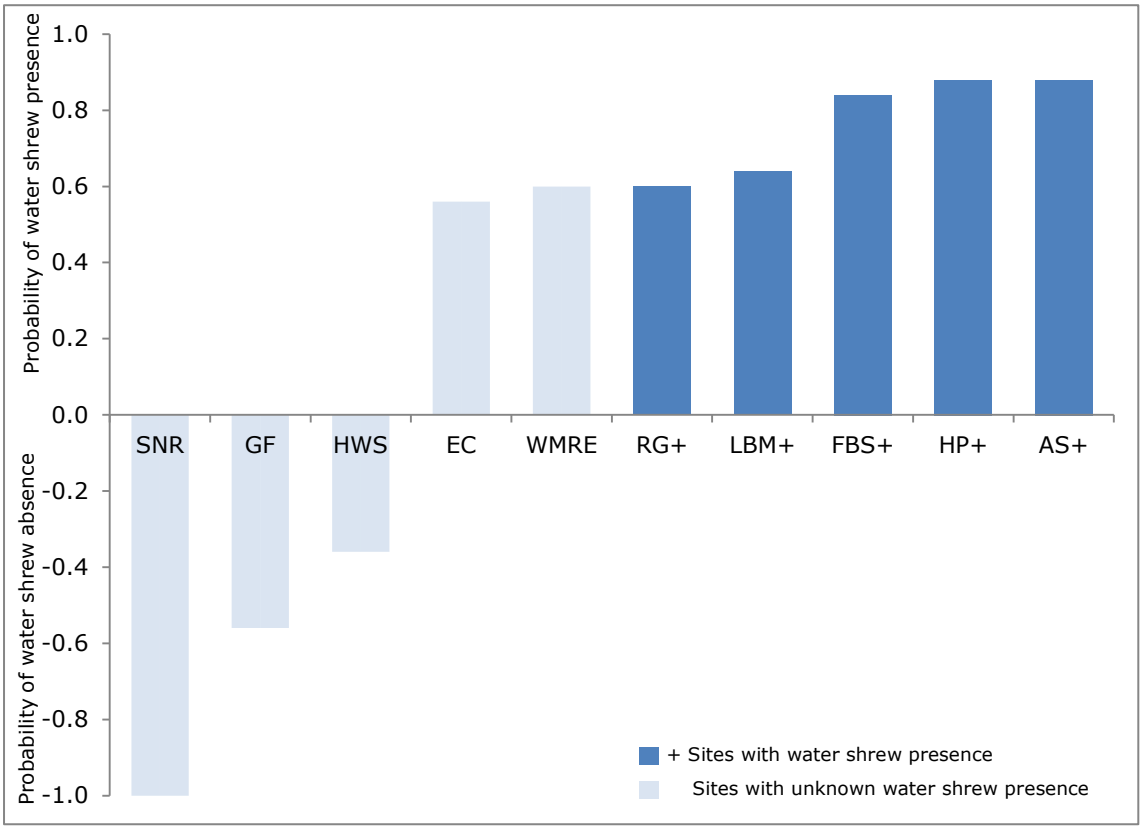


Figure 3.20 Summer 2008/09 three-variable model validation predictions. Sites above zero are predicted to show water shrew presence and sites below zero are predicted to show water shrew absence. The probability of presence or absence is greater the closer to 1 and -1 respectively.

Table 3.17 Summary of results for the summer 2008/09 validation models.

	Five-variable model	**Three-variable model**
Variables	Water depth Bank height Management intensity Floating vegetation Overhanging vegetation	Management intensity Dissolved oxygen Water depth
Predictive performance	60%	80%
Sensitivity	100%	100%
Specificity	40%	60%
Area under ROC curve	0.65	0.85

3.6.4. Summary of variable effects and model performance

3.6.4.1 Summary of variable effects on HSI models

Management intensity, bank height, water depth, dissolved oxygen, phosphates, overhanging vegetation, and floating vegetation were identified as the greatest predictors of water shrew presence. Water shrew presence was positively associated with occasional and frequent bankside management, bank heights of one to two metres or above, water depth of less than 25cm, dissolved oxygen levels of 0-2.99mg l^{-1}, phosphates levels of 0-0.99mg/l, vegetation overhanging at least three-quarters of the waterbody and little or no floating vegetation (see Table 3.18).

3.6.4.2 Summary of HSI models' performance

Overall, the model with the greatest discrimination ability, as determined by the area under the ROC curve value, was the summer 2008 three-variable model B of management intensity, dissolved oxygen and water depth (see Table 3.19 for HSI model summaries and Table 3.20 for HSI model validation summaries). However, the model performed poorly on the initial validation data. Nevertheless, when the model was trained on the combined summer 2008 and 2009 data sets, it showed good discrimination ability for 'unseen' sites.

Table 3.18 Summary of variable effects on the HSI models.

Winter 2008 models	**Summer 2008 models**	**Summer 2008/09 models**
The probability of water shrews was greatest when: banksides were 'occasionally' managed bank height was '1-2m' or above floating vegetation was either 'present' or 'absent' but not 'dense' phosphates were between '0-0.99mg/l' water depth was '<25cm'	The probability of water shrews was greatest when: banksides were 'occasionally' or 'frequently' managed dissolved oxygen levels were '0-2.99mg l^{-1}' overhanging vegetation covered '>3/4' of the waterbody water depth was '<25cm'	The probability of water shrews was greatest when: banksides were 'occasionally' or managed water depth was '<25cm' bank height was '1-2m' or above overhanging vegetation covered '>3/4' of the waterbody floating vegetation was 'absent' dissolved oxygen levels were '0-2.99mg l^{-1}'

Table 3.19 HSI model summaries.

	Winter 2008		Summer 2008		Summer 2008/09	
	Four-variable model	**Three-variable model**	**Three-variable model A**	**Three-variable model B**	**Five-variable model**	**Three-variable model**
Variables	Management intensity Bank height Floating vegetation Phosphates	Management intensity Bank height Water depth	Management intensity Dissolved oxygen Overhanging vegetation	Management intensity Dissolved oxygen Water depth	Water depth Bank height Management intensity Floating vegetation Overhanging vegetation	Management intensity Dissolved oxygen Water depth
Predictive performance	84%	77%	84%	90%	90%	87%
Sensitivity	100%	88%	88%	88%	88%	81%
Specificity	67%	67%	80%	93%	93%	93%
Area under ROC curve	0.82	0.86	0.87	0.88	0.82	0.82
False positives	AAP NPP WFRP WMME WMRE	AAP NPP WFRP WMME WMRE	EP SMRP WMRE	WMRE	ML	MF
False negatives		DLP	DLP SWP	DLP TAP	DLP RW	DLP RW TAP
Unclassified		RCPL TAP				

Table 3.20 HSI validation model summaries.

	Summer 2008 validation		Summer 2008/09 validation	
	Three-variable model A	**Three-variable model B**	**Five-variable model**	**Three-variable model**
Variables	Management intensity Dissolved oxygen Overhanging vegetation	Management intensity Dissolved oxygen Water depth	Water depth Bank height Management intensity Floating vegetation Overhanging vegetation	Management intensity Dissolved oxygen Water depth
Predictive performance	50%	30%	80%	80%
Sensitivity	50%	25%	100%	100%
Specificity	50%	50%	40%	60%
Area under ROC curve	0.42	0.64	0.65	0.85
False positives	EC MF ML	EC MF ML	EC GF HWS WMRE	EC WMRE
False negatives	LBM RW	LBM RW SI		
Unclassified				

3.6 Discussion

3.6.1 HSI Models

3.6.1.1 Winter 2008

Overall, the predictive performance of the four-variable model of management intensity, bank height, floating vegetation and phosphates, was better than the three-variable model of management intensity, bank height and water depth, predicting 100% of sites with known water shrew presence correctly. However, when the classification of sites is threshold-independent, as assessed using the area under the ROC curve, the three-variable model, had higher predictive ability indicating overall better discriminating ability. The winter models performed well considering dissolved oxygen, a variable identified as important in predicting water shrew presence, was excluded from the model.

3.6.1.2 Summer 2008

The summer 2008 training models gave the greatest predictive performances for both percentage predictability and area under the ROC curve. However, when tested using the summer 2009 validation data the models performed poorly. This could be due to a number of reasons. Primarily, it suggests that habitat suitability models created in one year cannot be applied to data collected in a different year. However, the water shrew presence/absence data on which the models were based was taken from summer 2007, and the habitat survey data from winter 2008 and summer 2008. Therefore, as the winter 2008 and summer 2008 models worked well, an effect of different years seems unlikely. It is possible that the failure of the models be due to the low number of validation sites in the sample, although the subsequent revised models worked well with only ten sites, so this too seems unlikely. However, if the sites selected in the summer 2009 data set were not representative, and therefore skewed the data, this could have been exacerbated by the low sample size. Initial findings suggest this to be the case, with water shrews displaying preferences to types of adjacent land use and intensity of human use

contradictory to the previous bait tube survey and to other studies (e.g. Carter and Churchfield, 2006a).

3.6.1.3 Summer 2008/09

Both training models performed very well and along with the summer 2008 model B had the highest specificity of all models, correctly classifying sites without water shrews 93% of the time. They were also showed high ability to predict presence. With respect to testing the models, the five-variable validation model performed better than most models for sensitivity, predicting water shrew presence correctly every time, although it had the lowest specificity correctly classifying only 40% of sites. However, the three-variable validation model had very high predictive ability correctly classifying water shrew presence at every site as well as predicting 'absence' 60% of the time. This model's discrimination ability was very good, performing better than all of the validation models and half of the training models.

3.6.1.4 Misclassified sites

WMRE was the only site consistently misclassified as having water shrew presence by all models, which may be explained by the presence of mink observed at the site during the bait tube survey. None of the models created took into account predation factors so, although the site was determined to be suitable for water shrews based on the habitat features identified as important, they may have been absent due to the presence of predators. Mink have been implicated in the accelerated decline of water voles (Strachan and Moorhouse, 2006) and is likely that they also predate on water shrews. However, the solitary lifestyle of water shrews may make the overall population less vulnerable to mink than the colonial water vole which has lost entire local populations in a very short space of time (Strachan and Moorhouse, 2006). Conversely, water shrews may have been present at the site but were simply not detected during the bait tube survey. As discussed in Chapter 2, such false absences may result in misrepresentation of habitat preferences (MacKenzie and Nichols, 2004; MacKenzie and Royle, 2005; Pagano and Arnold, 2009; Jeffress *et al*., 2011).

Absence data are difficult to obtain accurately as a species may not be detected for a number of reasons (Hirzel *et al*., 2002). For example, Kéry (2002) found that it took 34 unsuccessful visits to a given site before it could be assumed, with 95% confidence that the snake *Coronella austriaca* was actually absent from that site. In addition, there may be historical reasons why a species is absent from a site even though the habitat is suitable such as habitat fragmentation which has caused the site to be isolated from other populations.

Finally, the habitat may genuinely be unsuitable for the species. The prediction of water shrew absence at sites where presence was detected may be explained by the timing of the bait tube survey which took place during the breeding season (August). During the breeding season, the probability of finding water shrews is increased as population numbers and dispersal rate are at their highest (Churchfield, 1984b) and during this time water shrews are more likely to enter sub-optimal habitats even if they do not remain there (Greenwood *et al*. (2002). This would suggest that absence from a site during the breeding season is more likely to accurately indicate unsuitable water shrew habitat.

DLP was correctly classified as having water shrews by the winter 2008 two-variable model, but was consistently misclassified by all other models. TAP was incorrectly classified as not having water shrews by all models which included the variable water depth. This is probably because water depth at the site was greater (50-100cm) than the depth usually associated with water shrew presence (<25cm) however, there were shallower areas of the pond and it is likely that it is in these areas that the water shrews forage. RW, a summer 2009 site, was incorrectly classified by both the summer 2008 validation models and the summer 2008/09 validation models. RW was a pond but it was very close to an adjacent stream and therefore it is possible, that the water shrews may have come from the stream to enter the bait tubes. If this was the case, habitat data from the pond would give an inaccurate description of the area from which the water shrews came. The pond may have been suboptimal and the water shrews only visited it because they were attracted to the bait tubes.

3.6.2 Model variables

Variables identified as the most reliable predictors of water shrew presence were management intensity, bank height, water depth, dissolved oxygen, phosphates, overhanging vegetation, and floating vegetation. Presence was positively associated with both occasional and frequent bankside management, bank heights of one to two metres or more, low water depth, low dissolved oxygen and phosphate levels, vegetation overhanging at least three-quarters of the waterbody and lack of dense floating vegetation.

3.6.2.1 Management intensity

Bankside management was a key factor in determining water shrew presence, both alone and when combined with water depth and bank height. The most frequently recorded management intensity category was 'occasional' and each time the management practice undertaken was annual mowing or strimming of bankside vegetation, supporting evidence that water shrews prefer habitats with bankside management (Carter and Churchfield, 2006a). Annual weed cutting, strimming, mowing, tree trimming and pollarding are common management practices in riparian habitats (Carter and Churchfield, 2006a). Like water voles, water shrews use riparian vegetation to avoid predation and prefer well-vegetated banks without frequent management (Mason, 1995). Provision of vegetation cover is important for water vole conservation since this decreases detection and capture by mink (Strachan and Moorhouse, 2006). Therefore, frequent mowing may be detrimental to water shrews. Although, strimming bankside vegetation in early spring or late autumn bi-annually, stimulates a rich grass sward which provides plenty of cover and protection from predators (Strachan and Moorhouse, 2006). Strimming also removes overgrown vegetation that can shade the water negatively affecting aquatic invertebrate abundance and therefore water shrew prey availability (French *et al*., 2001). Only one of the study sites with water shrew presence was 'frequently' managed, being regularly mown throughout the summer. However, the dense marginal vegetation gives the water shrews areas of cover.

Destruction of good quality habitat has contributed to the loss of water voles (Strachan and Moorhouse, 2006) and has likely affected water shrews too (Carter and Churchfield, 2006a). For example, riverside development of floodplains for buildings and agriculture increases flood defence engineering causing river levels to rise during winter. This leaves riverbanks as unsuitable habitat for water voles and probably water shrews (Strachan and Moorhouse, 2006). Increasing water levels may prevent water shrews from reaching the substratum when foraging for benthic invertebrates (Churchfield, 1997b). Moreover, the clearing of ditches to prevent flooding removes crucial cover for water shrews and water voles (Strachan and Moorhouse, 2006). Similarly, loss of bankside vegetation through overgrazing, particularly by sheep, may have contributed to the decline of the water vole (Strachan and Moorhouse, 2006) and may negatively affect water shrews by removing ground cover. In addition, poaching of the banksides by livestock compacts the soil making it unsuitable for burrowing riparian mammals (Strachan and Moorhouse, 2006).

Sensitive bankside management and habitat enhancement has helped the conservation of water voles (Strachan and Moorhouse, 2006). Preventing overgrazing by fencing off the bankside results in the rapid regrowth of vegetation (Strachan and Moorhouse, 2006) and with carefully positioned fencing vegetation management can prevent scrub from establishing. In this study, livestock use did not affect the presence of water shrews. However, most sites with livestock only had 'light' use (e.g. a few rare breeds) so impact was minimal, in contrast the only site which scored 'heavy' for livestock use (a dairy farm) had no presence of water shrews. Water shrews would likely benefit from the same sensitive habitat management that has been effective for water voles (Carter and Churchfield, 2006a).

3.6.2.2 Bank height

Bank height was an important predictor of water shrews when combined with management intensity and when combined with water depth. Probability of water shrew presence was greatest when bank height was above one metre, supporting findings by Greenwood *et al.* (2002) who found bank heights below 1.5m had a negative effect on presence. Water

shrews make burrows in the banksides with entrances above water level (Churchfield, 1990) therefore preference for an increased bank height avoids water-logging and keeps nests dry (Greenwood *et al.,* 2002). In addition, water shrews have been found to occur only at sites with bank inclines of less than 45° (Greenwood *et al.,* 2002). However, no effect was seen in the current study with water shrews occurring at sites with a variety of bank inclines. Carter and Churchfield (2006a) found no effect of bank height or incline on water shrew presence and attributed the findings of Greenwood *et al.* (2002) to the types of habitats surveyed, which were mainly lotic. Lotic sites, by their nature, experience fluctuating water levels more often than lentic ones so water shrew burrows need to be located at sufficient height and incline to avoid risk of flooding (Carter and Churchfield, 2006a). In contrast, almost half of the sites in Carter and Churchfield's survey were lentic, where water levels are more stable. Therefore, bank height and incline may be less important, and mask any effect of either on water shrew presence at purely lotic sites.

3.6.2.3 Water depth

Water depth was an important predictor of water shrew presence both alone and when combined with management intensity and bank height and when combined with management intensity and dissolved oxygen. Probability of presence was greatest when water depth was low (<25cm) supporting evidence that although captive water shrews can dive to depths in excess of 200cm (Vogel, 1998), in the wild they generally favour depths of less than 30-40cm when foraging for benthic invertebrates (Schloeth, 1980; Illing *et al*, 1981; Churchfield, 1998; Lardet 1988). Reaching the substratum in very deep water would be difficult and energetically expensive (Churchfield, 1997b). Despite water shrews being positively associated with low water depth in the National Water Shrew Survey they were also recorded at sites with water depths up to 2m. However, at these sites they would probably only exploit the shallower edges while foraging for prey (Carter and Churchfield, 2006a). In addition, water shrews are probably unable to swim against the strong currents associated with deep, swiftly flowing water (Carter and Churchfield, 2006a). Moreover, shallow water, especially in ponds, is generally much more valuable to aquatic invertebrates than deep

water (English Nature, 1997b), with the majority of species occurring in the edges in very shallow water (Williams *et al*., 1999).

3.6.2.4 Dissolved oxygen

Dissolved oxygen was an important variable in predicting water shrew presence when combined with management intensity. However, unlike previous research, probability of presence was greatest when levels of dissolved oxygen were low. For example, low levels of dissolved oxygen and high levels of Biochemical Oxygen Demand (BOD) had a negative effect on water shrew occurrence (Southgate, 2006; Carter and Churchfield, 2006a). BOD is the amount of oxygen used by microorganisms in the process of breaking down organic matter in water, and is a good indicator of organic pollution (Mason, 2002; Dodds, 2002). Large amounts of organic matter lead to a greater number of microbes and greater need for oxygen. Hence, high values of BOD indicate high rates of decomposition of organic matter, and a reduction in the oxygen available to aquatic invertebrates, on which water shrews depend. Furthermore, Greenwood *et al.* (2002), found water shrews were absent from sites with poor water quality.

Little data exists on water shrew presence at lentic sites and currently no information on how pond water quality affects occurrence is available. Previous studies have concentrated on water quality in lotic environments, such as streams and rivers (French *et al*., 2001, Greenwood *et al*., 2002, Southgate, 2006), where dissolved oxygen levels are naturally higher (Jeffries and Mills, 1990). Approximately half of the sites with water shrew presence in the current study were ponds, where dissolved oxygen is often naturally low or variable and the aquatic invertebrates inhabiting them are well adapted to such conditions (Williams *et al*., 1999). For example, Bazzanti *et al*. (2003) found no difference between numbers of macroinvertebrates in temporary ponds with highly variable dissolved oxygen levels and permanent ponds with more stable levels. Therefore, low levels of dissolved oxygen, in lentic environments, may not necessarily be detrimental to invertebrates and consequently water shrews.

3.6.2.5 Phosphates

Phosphate concentrations were an important predictor when combined with management intensity, bank height and floating vegetation, with probability of water shrews greatest when phosphate concentrations were low. This supports evidence that water shrews appear to be affected by high nutrient concentrations. For example, Carter and Churchfield (2006) found water shrews were more likely to be occur at sites where nitrate levels were low. Water shrews are probably affected indirectly by phosphate concentrations through the impact on their aquatic invertebrate prey. High phosphate concentrations can result in excessive growth of algae, reducing light penetration below the water surface, leading to the death of submerged plants. Decomposition of dead plant material de-oxygenates the water leading to eutrophication, causing changes in the diversity and biomass of aquatic invertebrates and detrimentally affecting many invertebrate species and consequently their predators (Jeffries and Mills, 1990).

3.6.2.6 Overhanging vegetation

Overhanging vegetation was an important predictor of water shrews when combined with management intensity and dissolved oxygen and when combined with water depth, bank height, management intensity and floating vegetation.

The probability that water shrews would occur was when overhanging vegetation covered more than 3/4 of the water body. These findings are contrary to those of Greenwood *et al.* (2002) who found water shrews at relatively fewer sites with dense tree cover and to Carter and Churchfield (2006a) who found no significant difference in water shrew presence at sites with and without trees. Heavily shaded waterbodies can have reduced bankside and aquatic vegetation due to the lack of sunlight, resulting in a lack of food and habitat for aquatic and terrestrial invertebrates (Williams *et al.*, 1999). Thus, pond management guides often recommend the removal of fallen branches from the water and pond side trees to allow sunlight to penetrate. However, overhanging vegetation is not necessarily detrimental to wildlife and may be beneficial. Shade from trees helps maintain constant water temperatures in summer and fallen leaves from bankside trees

provide habitat and food for a wide range of aquatic and terrestrial invertebrates, many of which are water shrew prey (Carter and Churchfield, 2006a). In addition, submerged portions of wetland trees, such as alder and willow, provide excellent underwater habitat used by newts and many aquatic invertebrates (Williams *et al*., 1999).

Although the removal of some trees can be beneficial and encourage species diversity, the increased light allows the domination of vigorous plant species such as duckweed. Ponds in long-established woodland with mature trees probably have a specialised fauna adapted to woodland conditions (Williams *et al*., 1999). Rotting trunks in the water provide egg-laying sites for dragonflies and food for aquatic beetle larvae. In addition, caddis larvae use leaves and tree bark to build their cases, and the muddy edges of shaded ponds provide an important habitat for a wide range of insect larvae (Williams *et al*., 1999). Therefore, ponds potentially provide a rich source of food for water shrews.

3.6.2.7 Floating vegetation

Floating vegetation was an important predictor of water shrew presence when combined with management intensity, bank height and phosphates and when combined with water depth, bank height, management intensity and overhanging vegetation.

With the winter 2008 model, water shrew probability was the same for sites where floating vegetation was either 'absent' or 'present' suggesting probability was unaffected by absence or presence of floating vegetation. In contrast, the summer 2008/09 model showed probability to be greatest when floating vegetation was only absent. However, the results of the winter 2008 stepwise analysis show floating vegetation, when combined with management intensity and bank height, to influence water shrew probability. In addition, the absence of a 'dense' category on the response graph shows that the one site which had 'dense' vegetation was not selected during the process of random resampling when the model was constructed. If the 'dense' site had been included, water shrew probability would probably be greater at sites with 'absent' or present' floating

vegetation. This is highly likely as the 'dense' site was included in the summer 2008/09 model and water shrew probability was greatest when floating vegetation was absent.

French *et al*. (2001) also found water shrews absent from sites with the presence of floating vegetation. Aquatic plants are a crucial feature in waterbodies, providing habitat, egg laying sites and food, particularly for invertebrates (Williams *et al.*, 1999). However, floating vegetation such as duckweed and algae may be indicative of nutrient-enriched water (French *et al*., 2001). These plants are very tolerant of nutrients (especially nitrate and phosphate) and if concentrations in the water are high, they can grow without restriction, resulting in blooms of vegetation (Williams *et al*., 1999). Furthermore, if duckweed or algae form a thick blanket on the water surface, light is blocked to submerged plants and the exchange of gasses with the atmosphere is prevented and the water can become de-oxygenated and harmful to aquatic organisms (Williams *et al*., 1999). In the current study, duckweed was the most frequently observed floating vegetation, which could explain the negative effect on water shrew probability.

3.6.3 Summary

Several variables were identified as being important predictors of water shrew presence but management intensity, dissolved oxygen and water depth were shown to be the most important. A positive association was found between water shrew presence and occasional or frequent bankside management, low levels of dissolved oxygen and low water depth. A positive association was also found between water shrew presence and bank heights above one metre, low levels of phosphates, overhanging vegetation and absence of dense, floating vegetation. Of all the factors investigated that potentially influence water shrew occurrence, bankside management was the most important for predicting presence. This suggests that factors such as adjacent habitat, type of waterbody, proximity to human habitation and water quality are not important factors for water shrews in habitats with sufficient bankside ground cover.

Chapter 4
Analysis of Water Shrew Prey Availability

The aim of this chapter is to investigate whether there is an association between water shrew presence and prey availability by comparing numbers and diversity of terrestrial and aquatic invertebrates at sites with and without known water shrew presence.

4.1 Introduction

Whether the distribution and abundance of organisms is regulated by resources (bottom-up control) or predators (top-down control) has been a subject of much debate (Power, 1992; Hunter and Price, 2002; Meserve *et al*., 2003). Cases where organisms occupying the higher trophic levels (predators) affect the abundance, biomass and diversity of the organisms at lower trophic levels (prey) are referred to as top-down control (Hairston *et al*., 1960; Meserve *et al*., 2003). Bottom-up control refers to the situation where the abundance, biomass and diversity of organisms on each trophic level are food limited (Hunter and Price, 2002). Resource availability is the key process in bottom-up control and therefore populations within the trophic levels are affected mainly by competition rather than predation (Power, 1992).

Food availability is one of the main factors determining the distribution and abundance of populations (Cassini and Krebs, 1994) and in birds it is the main limiting factor (Strong and Sherry, 2000). In mammals, Kager and Fietz (2009) found edible dormice *Glis glis* numbers and proportions of reproductively active females, as well as litter sizes, were positively correlated with beech mast. In addition, Cassini and Krebs (1994) found food addition to affect abundance of hedgehog populations with an increase in density following food supplementation.

In contrast, many studies using food supplementation have generally shown only a limited increase in density (Boutin, 1990). Other factors such as

habitat structure, water and temperature may play a more important role. For example, Churchfield *et al*., (1997) found no overall correlation between abundance of shrews and invertebrate prey in the central Siberian taiga. Here, despite there being abundant prey in certain habitats such as bush-meadow, there was a paucity of shrews in those areas, indicating that habitat structure may have the greater influence over shrew distribution. Conversely, Getz (1961) concluded that the main factor in determining the distribution of shrews *Blarina brevicauda* and *S. cinereus* in Michigan was food availability, which was affected by moisture and vegetation cover. Similarly, in grey squirrels *Sciurus carolinensis* tree seed availability is the most important factor limiting population densities however this is affected positively and negatively by the severity of winter weather (Gurnell, 1996).

Species which specialise in certain types of prey are more likely to be affected by food availability than generalists. For example, Wickramasinghe *et al*. (2004) found that the activity of bat species that mainly ate Lepidoptera was significantly correlated with the abundance of this order.

Opportunistic feeders such as Soricine shrews which feed on a wide variety of invertebrate prey may not be particularly affected by the lack of availability of any one prey type, which may explain their wide distribution (Churchfield, 2008). However, the general lack of food availability at very high latitudes and elevations may limit their distribution in such areas (Hanski and Kaikusalo, 1989). Moreover, the high energy requirement of shrews (Crowcroft, 1957; Hawkins and Jewell, 1962; Churchfield, 1990) necessitates them eating every few hours, thus a more or less constant supply of food is crucial to their survival. This vulnerability to temporal variation in food availability is overcome by having a diverse diet and changing the emphasis to alternative prey when necessary (Churchfield, 1993).

With respect to seasonal abundance, DuPasquier and Cantoni, (1992) observed an annual reduction in water shrew populations on a Swiss river during the winter. However, aquatic invertebrate populations in the river were only slightly smaller during the winter than the in summer.

Furthermore, during one winter even though the river was frozen, faecal analysis revealed shrews still consumed mainly aquatic prey (86%). Therefore, lack of food was not the cause for the winter reduction in water shrew population size. Similarly, Dineen *et al*. (2007) found the highest densities of benthic invertebrates (e.g. Gammaridae) during the autumn in streams in grassland and closed canopy habitats in Ireland.

Water shrews are generalist feeders whose diets encompass a wide range of aquatic and terrestrial prey. However, certain types of prey have been found to have an impact on shrew distribution. For instance, French *et al.* (2001) found the presence of *Asellus aquaticus* and *Gammarus pulex* had a positive effect on water shrew occurrence. Churchfield (1997b) suggested that seasonal or annual declines in prey availability may be a limiting factor affecting water shrew numbers and occurrence at particular sites. Therefore, the impact of invertebrate prey availability on water shrew presence, as a bottom-up regulator is an important factor requiring investigation.

4.1.1 Investigating water shrew prey availability

Analysis of feeding ecology can be undertaken either directly, by looking at the feeding habits of the animal, or indirectly, by undertaking surveys to assess prey availability.

Although the feeding habits of water shrews have previously been investigated (e.g. Wolk, 1976; Churchfield, 1984b; DuPasquier and Cantoni, 1992; Castien, 1995), with the exception of Churchfield (1984b), who looked at watercress beds, none of the studies have looked at prey availability in lentic environments such as ponds, but instead have concentrated on lotic habitats such as streams and rivers. The techniques used include collection and examination of food remains left on artificial rafts (Wolk, 1976), analysis of stomach contents (e.g. Castien, 1995) and faecal analysis of live-trapped animals (e.g. Churchfield, 1984b; DuPasquier and Cantoni, 1992). Wolk (1976) found that water shrews inhabiting drainage ditches in Poland left the remains of their prey on the edges of

pipe outlets and on pieces of wood carried in on the water, so installed artificial rafts and inspected the remains. Analysis of stomach contents involves killing the animal and is therefore not a good choice for an uncommon species of conservation concern, occurring in low densities, as removing even a small number of water shrews could have a devastating effect on the local population (Churchfield, 1985).

Assessing prey availability involves undertaking invertebrate surveys where the method used is dependent on the type of invertebrates to be surveyed. Water shrews eat both terrestrial and aquatic invertebrate prey, so methods of surveying both types of invertebrate are required. The method used for surveying aquatic invertebrates depends upon the type of habitat. For example, in lotic habitats, such as streams and shallow rivers, kick sampling is a simple, commonly used method (Williams, 1991), where sampling involves kicking an area of stream substrate for a short period of time (usually three minutes) and collecting the dislodged animals downstream into a net (New, 1998).

Since aquatic invertebrates are often used to assess water quality at a site by using the Biological Monitoring Working Party (BMWP) score system (Hawkes, 1998), BMWP scores can give an insight into prey availability. Samples of macro-invertebrates are used since they are found in nearly all fresh waters and respond to physical and chemical changes to their habitat (Environment Agency, 2010). Samples are collected from a water body and assigned a set of simple numerical values according to their tolerance to pollution. This method can detect very low concentrations of pollution which may be missed by chemical sampling (Environment Agency, 2010). High scores are associated with species, such as mayflies and stoneflies, which are pollution intolerant and therefore the biological condition of the water body tends to be good. Conversely, low scores, associated with species such as worms, which are pollution tolerant, are indicative of poor water quality (Hawkes, 1998). Such bodies of water might be expected to have low prey availability.

Beetles, spiders, centipedes and molluscs are typical terrestrial prey of the water shrew (Carter and Churchfield, 2006b) and therefore a method of surveying ground dwelling terrestrial invertebrates is required. Such methods include pitfall trapping, soil core sampling and vacuum sampling (New, 1998). Pitfall trapping for invertebrates is similar to a sampling method for small mammals, invertebrates simply fall into containers placed into the soil with the rim flush to the surface (New, 1998). Pitfall traps can be used in a wide variety of habitats, are cheap to make and can be used in large numbers. They are particularly effective for sampling larger invertebrates such as beetles, spiders and ants (New, 1998). Unlike sampling for aquatic invertebrates, which is an active method, pitfall trapping is passive and relies on animals being active on the ground surface in order to fall into the traps (Terrell-Nield, 1986).

4.1.2 Aim and objectives

The aim of this part of the study is to investigate the effect of prey availability on water shrew presence at a subset of sites where water shrews were detected and a subset where they were not detected. The objectives are to:

- Select eight sites (four with and four without evidence of water shrew presence)
- Undertake aquatic and terrestrial invertebrate surveys at the eight sites
- Investigate the difference in invertebrate numbers and diversity at sites with and without evidence of water shrews

4.2 Methods

4.2.1 Site selection

In order to determine water shrew prey availability, aquatic and terrestrial invertebrate surveys were undertaken at eight sites (four sites with known water shrew presence and four sites where no water shrew presence was detected, with which to compare; see Figure 4.1 and Table 4.1). Lentic sites were chosen as it gave the opportunity to look more closely at water shrews in ponds and lakes, as records from such habitats are scarce (Greenwood *et al*, 2002; Carter and Churchfield, 2006a) and previous studies have often concentrated on lotic environments such as streams and rivers (French *et al*., 2001; DuPasquier and Cantoni, 1992; Castien, 1995). Ponds are very dynamic environments and an important habitat for a diverse range of animals and plants (Williams *et al.,* 1999). At a regional level ponds have been found to contribute more to biodiversity than streams, rivers and ditches (Williams *et al*., 2003; Davies *et al*., 2008) and therefore may be an important resource for water shrews. However, over 75% of ponds that existed at the beginning of the twentieth century have been destroyed and pond numbers in Europe are at an all-time low (Hull, 1997; Keeble *et al*., 2009). The main causes of loss are drainage or infilling for agricultural reasons as well as through urban development (English Nature, 1997a). In addition, ponds are affected by the same impacts as other freshwater habitats such as degradation through pollution from their surroundings, overstocking with fish and unnaturally high numbers of waterfowl, but have limited capacity for buffering due to their small size (Keeble *et al*., 2009).

As the same four sites with water shrew presence were also to be used for live-trapping (see Chapter 5) sites were selected on the basis of having minimum public access, so as to reduce the risk of disturbance or theft of the traps.

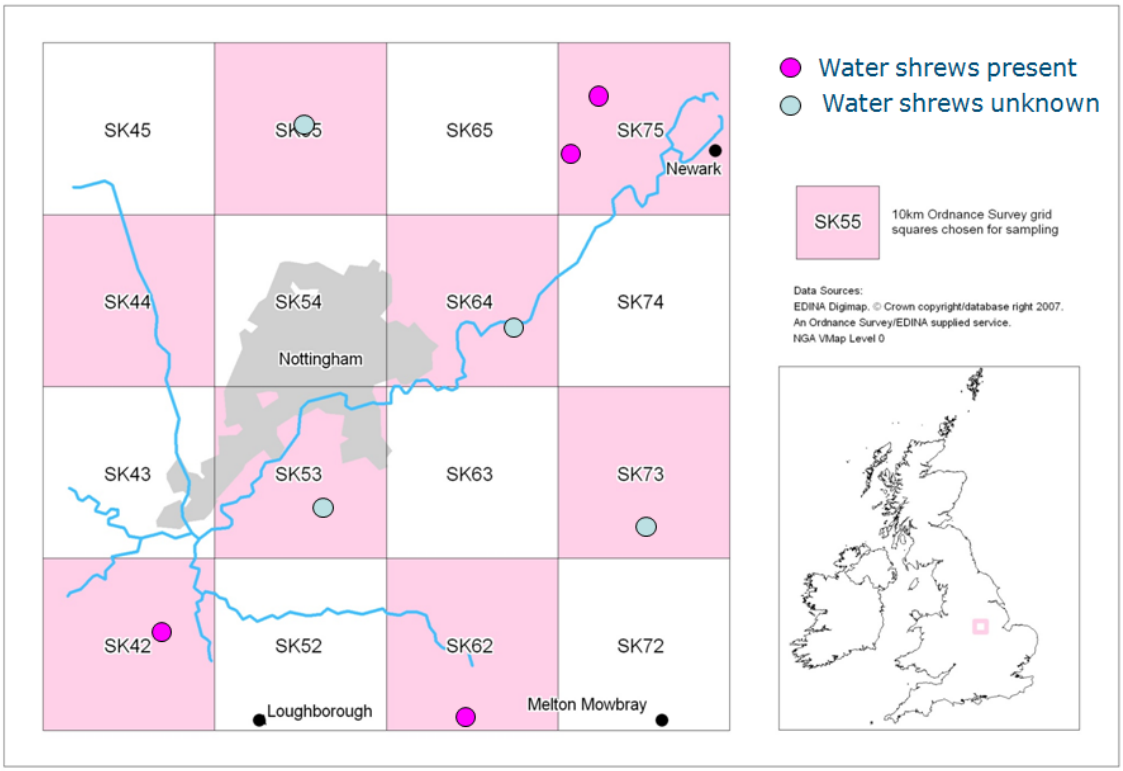


Figure 4.1 Map showing the four sites with known and four sites with unknown water shrew presence where invertebrate surveys were undertaken.

Table 4.1 Ponds which were surveyed for invertebrates.

Water shrews	Site	Surveyed	Habitat type
Water shrew presence known	Ash Spinney Pond	Autumn 2008	Woodland
	Twenty-Acre Piece	Autumn 2008	Woodland
	Sheepwalks Pond	Autumn 2008	Grassland
	Hockerton Pond	Autumn 2008	Grassland
Water shrew presence unknown	Newstead Park Pond	Autumn 2009	Grassland
	Rushcliffe Country Park Lake	Autumn 2009	Grassland
	Shelford Manor River Pond	Autumn 2009	Woodland
	Washdyke Farm Railway Pond	Autumn 2009	Scrub

The four ponds with known water shrew presence were Ash Spinney, Hockerton, Sheepwalks and Twenty-Acre Piece. Ash Spinney Pond is located in a deciduous woodland habitat situated adjacent to arable farmland and a low use recreational sports ground. The pond was manmade approximately 20 years ago and is one of three in the woodland. Hockerton Pond is situated in an 'eco' housing project and was created approximately 10 years ago. It is surrounded by reeds and an encircling stone path. Adjacent land consists of a combination of mown and uncut grassland, willow plantation, a larger pond and housing. Sheepwalks Pond is situated in pasture land and was created approximately ten years ago. The pond is surrounded by reeds, grassland and a regularly coppiced willow plantation. Twenty-Acre Piece is a SSSI, due to its acidic clay grassland, which encompasses a variety of habitats including grass, scrub and secondary woodland. It is within the woodland that the study pond is situated. The pond is not made-made and therefore its exact age is unknown, however it is at least 50 years old.

The four ponds were water shrew presence was not detected were Newstead Park Pond, Rushcliffe Country Park Lake, Shelford Manor River Pond and Washdyke Farm Railway Pond. The ponds were selected on the basis of being similar to the ponds with known water shrew presence in terms of: habitat types, management and water depth. Newstead Park Pond was created approximately seven years ago and is located in grassland grazed by rare breed sheep close to the River Leen. Rushcliffe Country Park Lake is a large manmade pond created in 2007 surrounded by grassland. Nearby land consists of a mix of scrub, woodland and amenity grassland. Shelford Manor River Pond is a naturally created waterbody in a small deciduous woodland on the floodplain of the River Trent and is therefore subject to occasional inundation of river water. Adjacent habitat consists of improved grassland grazed by cows. Washdyke Farm Railway Pond is a large pond on an intensive dairy farm, located beside a disused railway. The pond is at least 50 years old and is surrounded by scrub with adjacent habitat pasture.

Surveying was undertaken twice (autumn 08 and spring 09) at the four sites positive for water shrew presence and once (autumn 09) at four sites

where no evidence of water shrews were found as a comparison. However, in the interests of consistency only the results of the autumn surveys were used in the analysis.

4.2.2 Aquatic invertebrate sampling

The National Pond Monitoring Network method for sampling aquatic invertebrates (Biggs *et al*., 1998) was used at each of the eight sites. Kick sampling was not undertaken as the method is specifically for lotic habitats and depends on the flowing water to flush the invertebrates into the stationary net. However, an equivalent method, designed for sampling aquatic invertebrates in ponds was undertaken which, unlike kick sampling, relies on sweeping a net through the water to catch the invertebrates. Aquatic invertebrate sampling was undertaken using a long handled fine mesh net at each site for a total of three minutes. Six samples were taken, two each from the pond edge, the pond centre and the emergent vegetation. Each of these mesohabitats was sampled for 30 seconds by netting vigorously through the water column to collect the invertebrates. After each sampling period the contents of the net were carefully rinsed through with some water taken from the pond to clean away any silt or sediment and then emptied into a labelled clear plastic bag containing a small amount of pond water. Into this bag all samples were accumulated the aim being to collect a representative sample of invertebrates from that pond (Hawkes, 1998; Biggs *et al*., 1998). This process was repeated for the remaining seven ponds. Back at the laboratory the contents of each bag were emptied into a white tray and the captured invertebrates identified to family level, where possible, using keys (see Appendix 2 for list of invertebrate keys) and counted and BMWP scores calculated. Identification of captures was confirmed by Dr Chris Terrell-Nield and, following identification, animals were released at the site where they were caught.

4.2.3 Terrestrial invertebrate surveys

Pitfall trapping was undertaken at each of the four main sites within the 100 m sampling unit used in the bait tube survey. The pitfall traps were

constructed from double-walled plastic disposable coffee cups (7cm diameter x 8.5 cm deep) with a 2 cm layer of slightly dampened lightweight cat litter in the bottom, to prevent captured animals from drying out and to give them a place to hide from captured potential predators (Terrell-Nield, 1986). Several small holes were pierced in the bottom of each cup to allow water to escape and stop any captures from drowning if it rained. Twelve traps were placed at each site in holes dug into the ground with a bulb planter within 3 metres of the water's edge since this is where water shrews spend most of their time foraging (Churchfield, 1985). The soil core removed when making the hole was left nearby and replaced following the trapping period. The traps were placed approximately 2 metres apart. A square piece of mesh (chicken wire) was placed over the top of each trap to stop any non-target species (e.g. small mammals) or debris from falling into the trap. The traps were left out for three days and then collected and taken to the laboratory for identification of the captures. The contents of each trap were emptied into a white tray and carefully inspected for invertebrates. Captures were then sorted, identified to family level using keys (See Appendix 2 for list of invertebrate keys) and counted. Again, identification of captures was confirmed by Dr Chris Terrell-Nield and all captures returned and released at the site where they were trapped.

Ideally, surveys of both water shrew faeces and of invertebrates would be undertaken. However, an accurate assessment of prey from faecal sampling from the bait tubes was not possible, due to the amount of blowfly pupae bait they had eaten. Therefore, only the detection of aquatic invertebrates, to determine whether or not it was water shrew faeces, was possible. In addition, surveying invertebrates in the field, as opposed to their remains in the stomachs or faeces of water shrews, allows easier identification of species as they are whole and not degraded or digested. Furthermore, surveying for invertebrates to determine water shrew prey availability can give an indication of suitability of a site for water shrews.

4.2.4 Data analysis

Calculating the invertebrate diversity is useful when estimating the likelihood that water shrews will use a site because high diversity is generally regarded as a measurement of good habitat quality. However, the numbers of invertebrates of certain species, which water shrews are known to favour, may be of more use. Consequently, it was decided to examine both diversity and numbers. Invertebrate species diversity was measured using a Shannon Diversity Index (H') based on numbers in each species converted to $\log_{10}$ to give an indication of habitat quality. Diversity indices take into account relative abundance as well as species richness. The difference between total numbers of invertebrate individuals caught at sites with known and unknown water shrew presence was analysed using Mann-Whitney U. As a third measure of habitat quality BMWP scores were calculated for each site and differences between the scores at sites with known and unknown water shrew presence were analysed using Mann-Whitney U. The Shannon Diversity Index was calculated using Biodiversity Pro Version 2 and all other analyses were undertaken on Minitab Student Release 14.

Ash Spinney was excluded from all aquatic invertebrate analyses as the pond was dry on the day of the survey. The combined terrestrial and aquatic invertebrate data sets were analysed first together and then separately since there was a huge difference between numbers of invertebrates caught during the terrestrial and aquatic surveys, which may have skewed the results.

4.3 Results

A wide range of terrestrial and aquatic invertebrate taxa were caught during surveying (see Figure 4.2 and Appendix 3 for complete species list). Coleoptera (beetle) species were the most frequently caught terrestrial invertebrates followed by Collembola (springtails), Araneae (spiders) and Diplopoda (millipedes), whereas the most frequently caught aquatic invertebrates were Diptera larvae, Hemiptera, Odonata larvae (dragonflies and damselflies), Amphipoda (*Gammaridae* spp.) and Gastropoda. All other species were only caught infrequently.

4.3.1 Combined terrestrial and aquatic invertebrates

Despite higher total numbers of terrestrial and aquatic invertebrate individuals at sites with known water shrew presence compared with sites with unknown presence (see Table 4.2) this difference was not significant (U = 21.0, $n_{1 =}$ 4, $n_{2 =}$ 4, p = 0.4705).

4.3.1.1 Relative abundance of taxa

Greater numbers of terrestrial invertebrates such as of Collembola, Diplopoda and Gastropoda individuals were caught at sites with known water shrew presence, whereas greater numbers of Coleoptera, Araneae and Opiliones individuals were caught at sites with unknown water shrew presence (see Figure 4.3). The same numbers of Hemiptera and Hymenoptera individuals were caught at sites with known and unknown water shrew presence. However, Diptera larvae and Isopoda were only caught at sites with known water shrew presence and Lepidoptera larvae were only caught at sites with unknown water shrew presence.

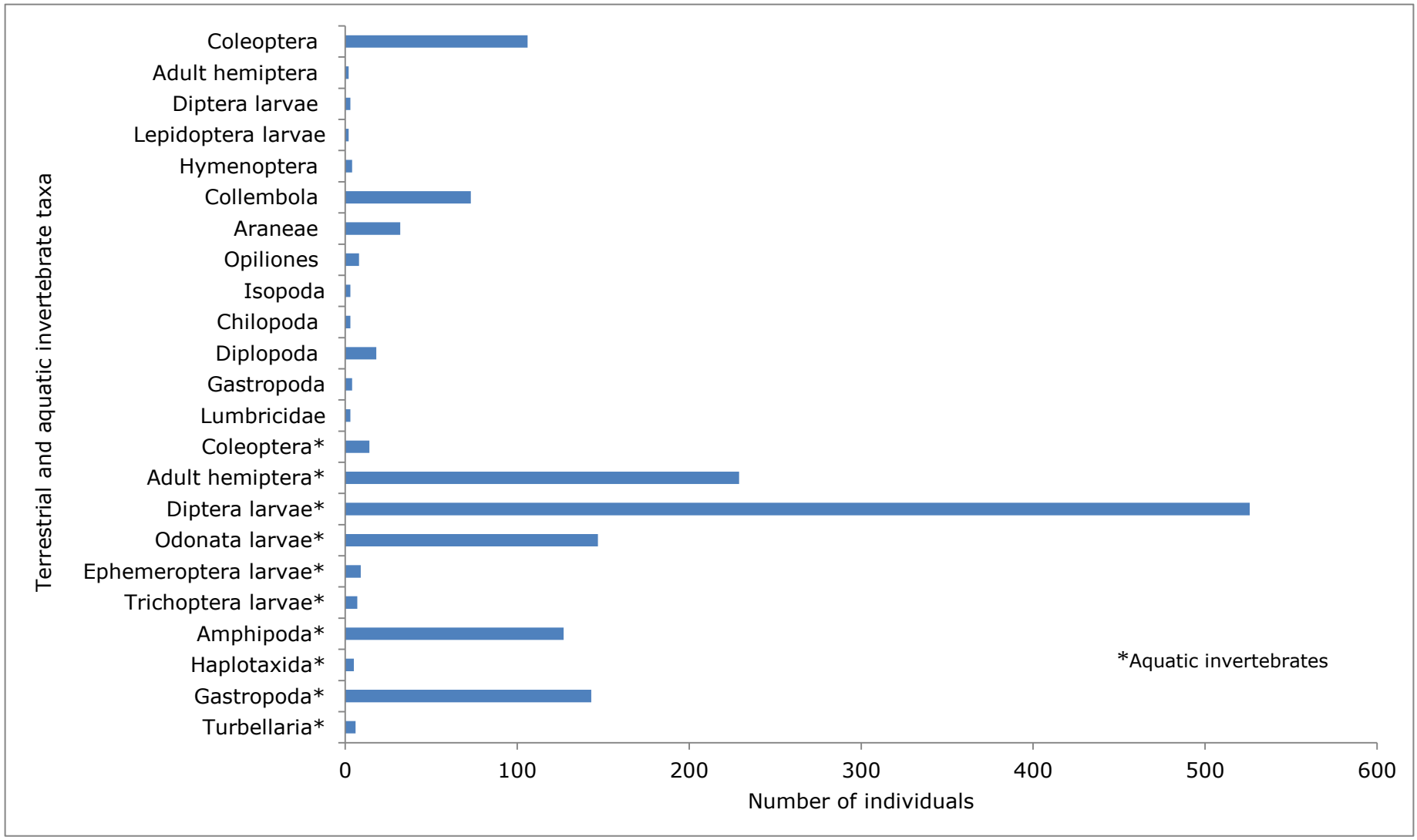


Figure 4.2 Overall numbers of terrestrial and aquatic invertebrate individuals caught at all sites.

Greater numbers of aquatic invertebrates such as Hemiptera and Diptera individuals were caught at sites with known water shrew presence, whereas greater numbers of Odonata, Amphipoda and aquatic Gastropoda individuals were caught at sites with unknown water shrew presence (see Figure 4.3). Similar numbers of aquatic Coleoptera were caught at sites with known and unknown water shrew presence. However, Ephemeroptera, Trichoptera larvae, and Turbellaria were only caught at sites with known water shrew presence and aquatic Haplotaxida were only caught at sites with unknown water shrew presence.

4.3.1.2 Species diversity

Overall, there was virtually no difference between Shannon (H') diversity of combined terrestrial and aquatic invertebrate species at sites with known and unknown water shrew presence (see Figure 4.4), although the composition of species varied.

Table 4.2 Combined numbers of terrestrial and aquatic invertebrate individuals at sites with known and unknown water shrew presence.

	Site	**Number of individuals**
Sites with known water shrew presence	Ash Spinney Pond	34
	Hockerton Pond	149
	Sheepwalks Pond	155
	Twenty-Acre Piece	651
Total		**989**
Sites with unknown water shrew presence	Newstead Park Pond	140
	Rushcliffe Country Park Lake	238
	Shelford Manor River Pond	76
	Washdyke Farm River Pond	31
Total		**485**

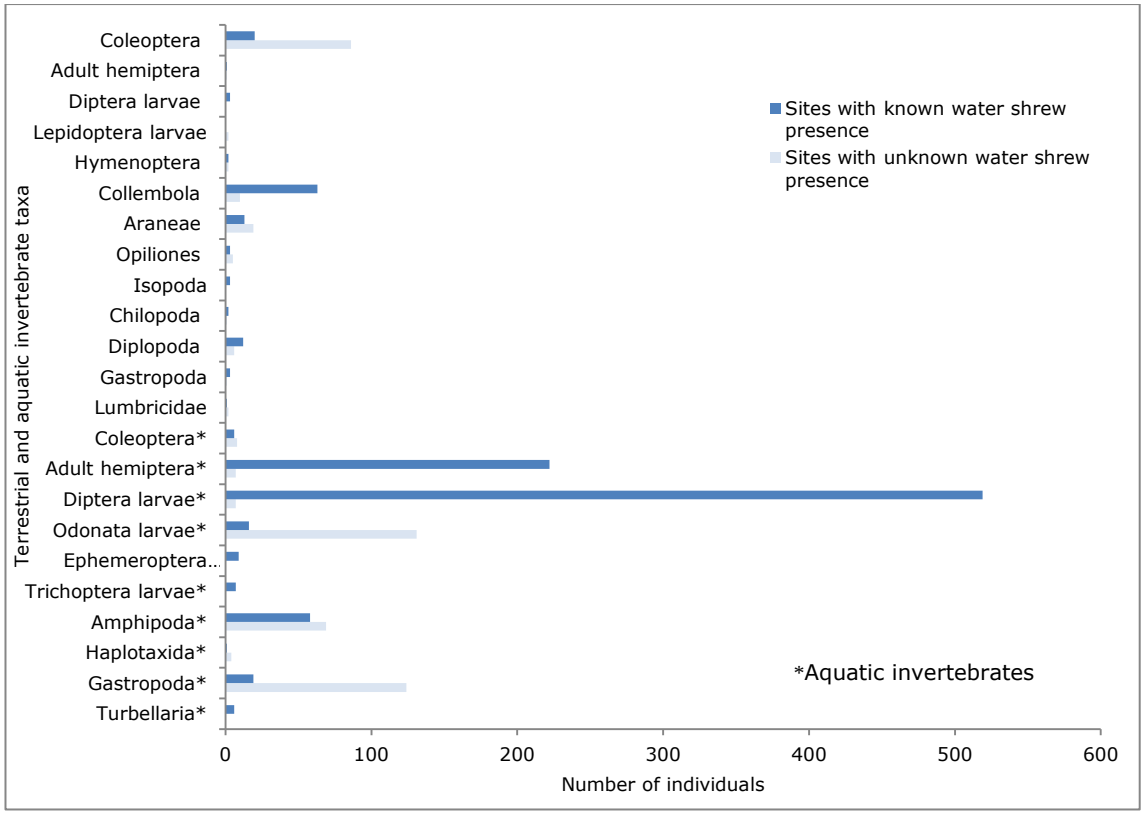


Figure 4.3 Numbers in each taxon of terrestrial and aquatic invertebrate individuals at sites with known and unknown water shrew presence.

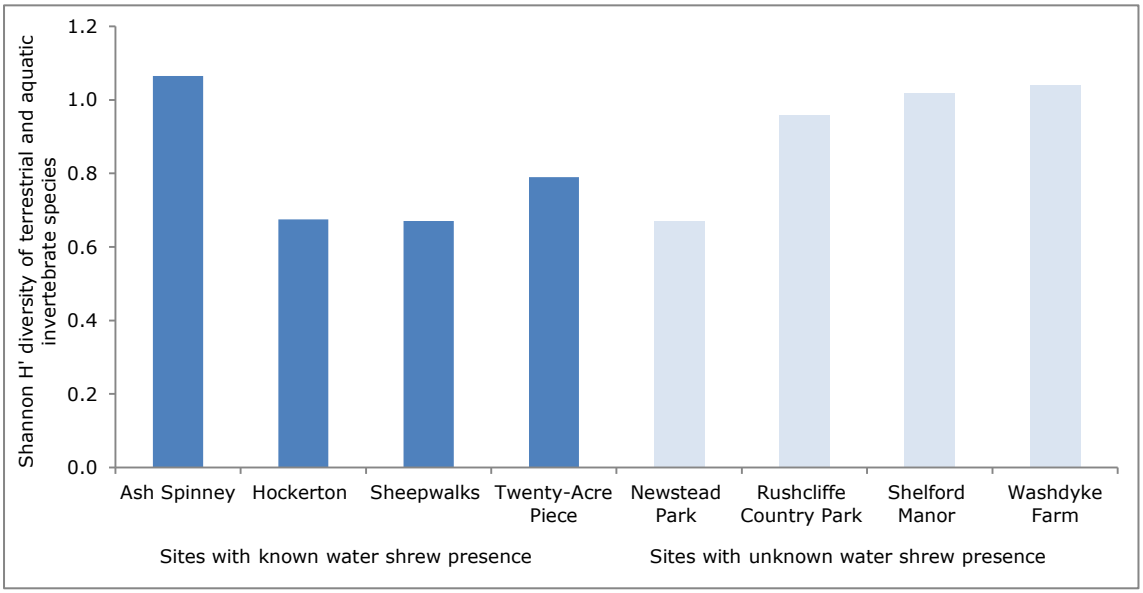


Figure 4.4 Shannon (H') diversity of terrestrial and aquatic invertebrate species at sites with known and unknown water shrew presence.

4.3.2 Terrestrial invertebrates

Although the numbers of terrestrial invertebrate individuals varied between sites (see Table 4.3) the difference between sites with known and unknown water shrew presence was not significant (U = 21.0, n_1 = 4, n_2 = 4, p = 0.4705).

Table 4.3 Numbers of terrestrial invertebrate individuals at sites with known and unknown water shrew presence.

	Site	**Number of individuals**
Sites with known water shrew presence	Ash Spinney Pond	34
	Hockerton Pond	15
	Sheepwalks Pond	6
	Twenty-Acre Piece	71
Total		**126**
Sites with unknown water shrew presence	Newstead Park Pond	21
	Rushcliffe Country Park Lake	21
	Shelford Manor River Pond	62
	Washdyke Farm Railway Pond	31
Total		**135**

4.3.2.1 Relative abundance of taxa and species diversity

Overall, there was virtually no difference between Shannon (H') diversity of terrestrial invertebrate species at sites with known and unknown water shrew presence (see Figure 4.5), although the composition of species varied (see Figure 4.6).

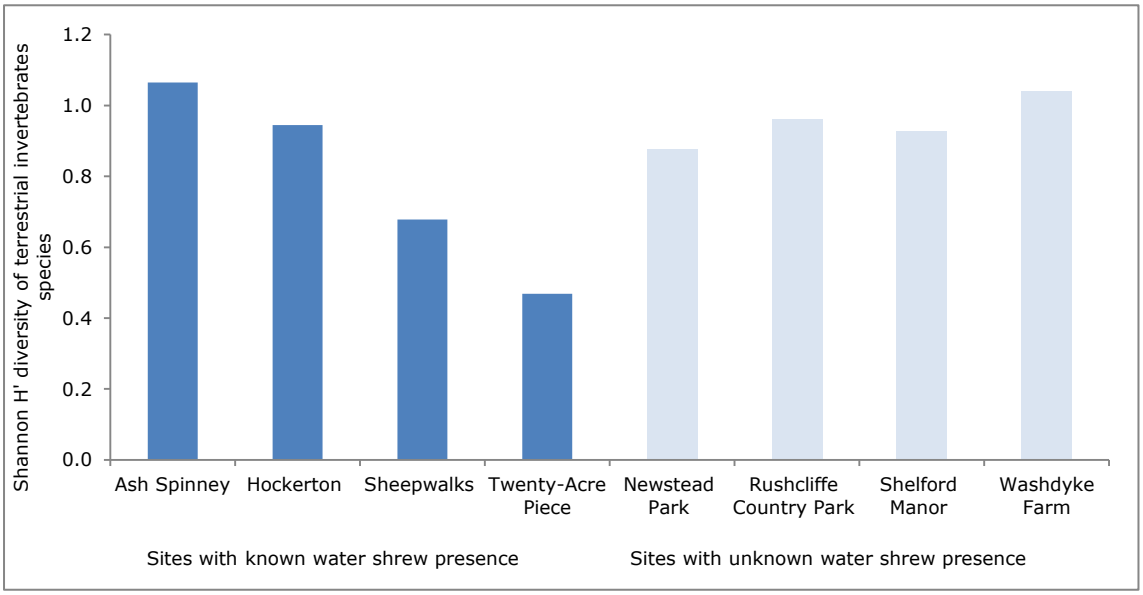


Figure 4.5 Shannon (H') diversity of terrestrial invertebrate species at sites with known and unknown water shrew presence.

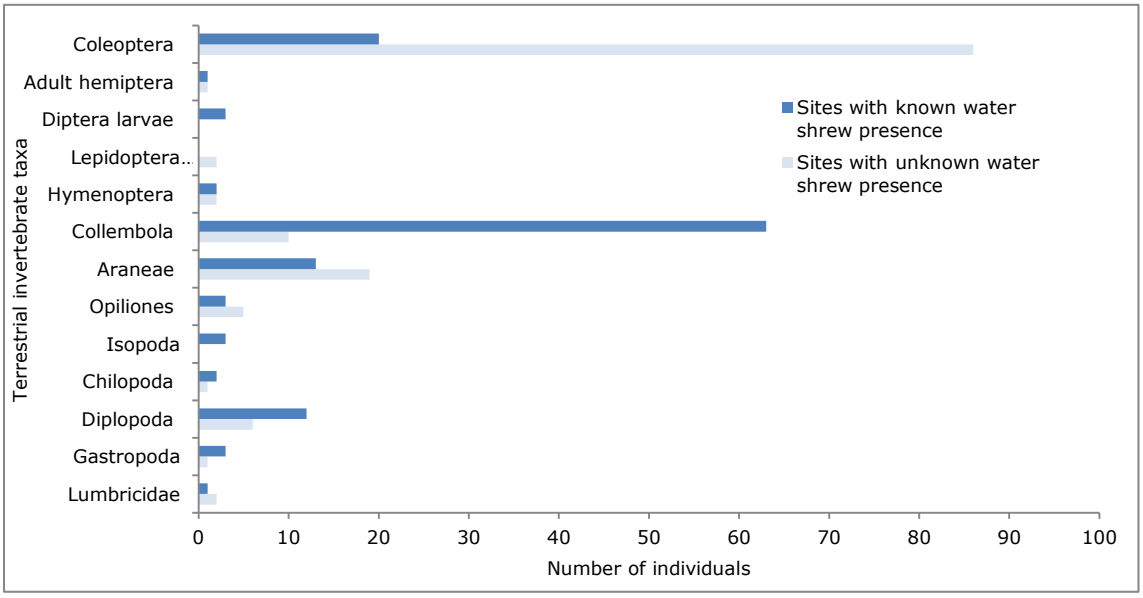


Figure 4.6 Numbers in each taxon of terrestrial invertebrate individuals at sites with known and unknown water shrew presence.

4.3.3 Aquatic invertebrates

Although greater numbers of aquatic invertebrate individuals were caught at sites with water shrew presence compared with sites with unknown presence (see Table 4.4) this difference was not significant (U = 16.0, n_1 = 3, n_2 = 4, p = 0.2159).

4.3.3.1 Relative abundance of taxa and species diversity

Overall, there was virtually no difference between Shannon (H') diversity of aquatic invertebrate species at sites with known and unknown water shrew (see Figure 4.7), although the composition of species varied (see Figure 4.8).

Table 4.4 Numbers of aquatic invertebrate individuals at sites with known and unknown water shrew presence.

	Site	**Number of individuals**
Sites with known water shrew presence	Ash Spinney Pond	-
	Hockerton Pond	134
	Sheepwalks Pond	149
	Twenty-Acre Piece	580
Total		**863**
Sites with unknown water shrew presence	Newstead Park Pond	119
	Rushcliffe Country Park Pond	217
	Shelford Manor River Pond	14
	Washdyke Farm Railway Pond	0
Total		**350**

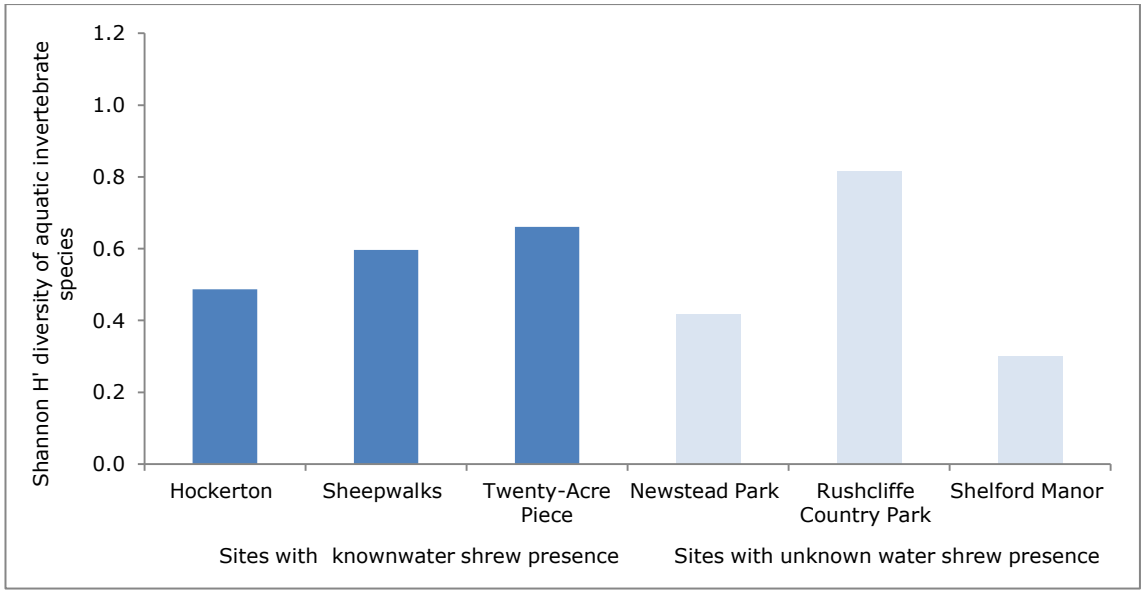


Figure 4.7 Shannon (H') diversity of aquatic invertebrate species at sites with known and unknown water shrew presence

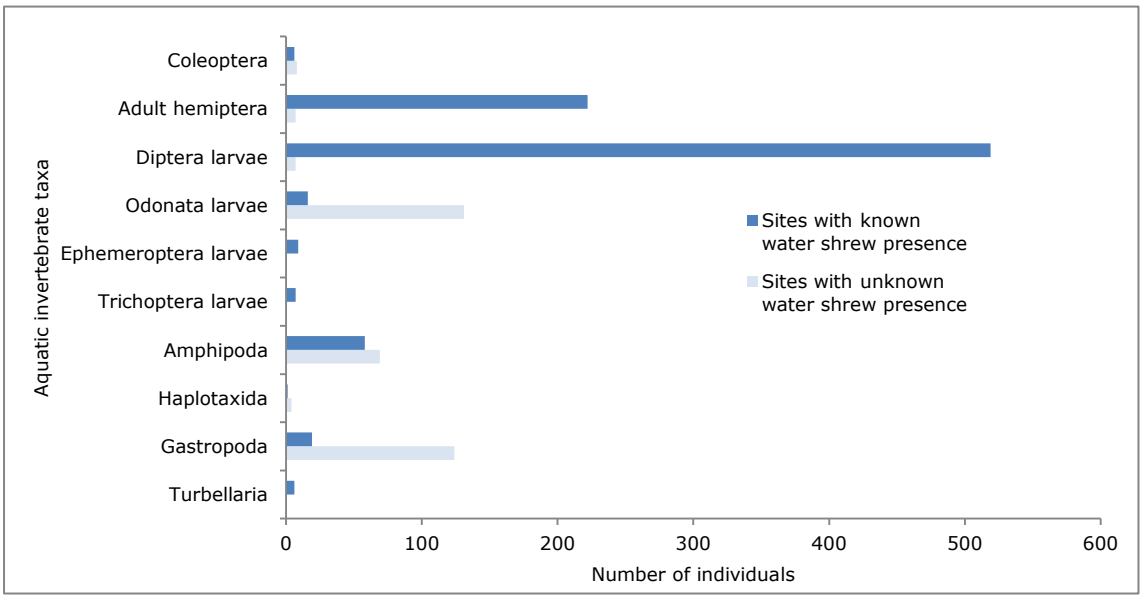


Figure 4.8 Numbers in each taxon of aquatic invertebrates at sites with known and unknown water shrew presence

4.3.3.2 BMWP

There was no significant difference between overall BMWP score at sites with known and unknown water shrew presence (U = 14.0, n_1 = 3, n_2 = 4, p = 0.595; see Figure 4.9).

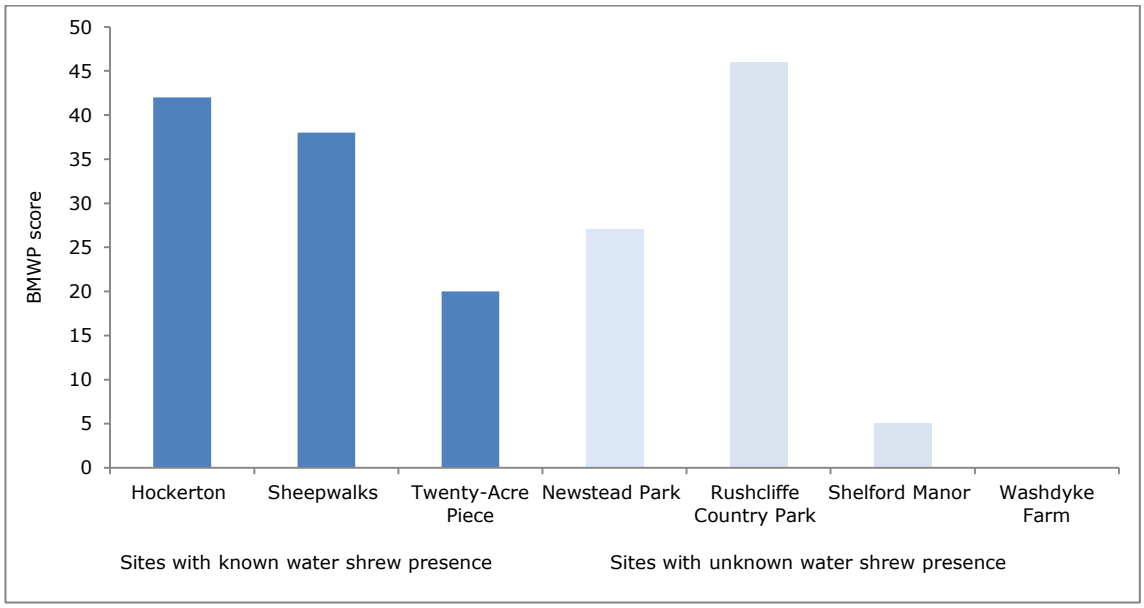


Figure 4.9 BMWP scores at sites with known and unknown water shrew presence.

4.4 Discussion

Overall, there was no significant difference between the total numbers of terrestrial and aquatic invertebrate individuals, either combined or separately, at sites with known and unknown water shrew presence, although fewer numbers of terrestrial invertebrates and greater numbers of aquatic invertebrates were caught at sites with known water shrew presence.

4.4.1 Terrestrial invertebrate taxa

More terrestrial invertebrate individuals were caught at sites with unknown water shrew presence than known water shrew presence and there were differences in the composition of taxa. Gastropoda and Diplopoda were caught in greater numbers, and Diptera larvae and Isopoda caught exclusively, at sites with known water shrew presence suggesting they may be an important potential source of terrestrial prey. These findings are supported by Churchfield (1984b) who found Gastropoda the most frequently eaten terrestrial prey item in faecal samples of water shrews

from watercress beds. Greater numbers of Collembola were caught at sites with known water shrew presence, although water shrews rarely eat them (Churchfield, 1984b), and they have not previously been identified as an important food source. However, Collembola may be a food source for other larger invertebrate species such as spiders (Agusti *et al*., 2003) on which water shrews do feed (Churchfield, 1984b), which could explain why they were found in greater numbers at sites with water shrews. However, greater numbers of Coleoptera, Araneae and Opiliones, which are major water shrew prey types (Churchfield, 1985), were caught at sites with unknown water shrew presence, suggesting that prey availability is not the only factor affecting water shrew occurrence. Further evidence for this is the fact that Hemiptera, another important food source, was caught in equal numbers at sites with known and unknown water shrew presence.

The proportion of terrestrial and aquatic prey taken by water shrews varies according to habitat, as well as geographically. For example, aquatic invertebrates comprised, on average, 50% of the prey of water shrews inhabiting watercress beds in southern England (Churchfield, 1984b) but 80% of the diet of water shrews at a Swiss river (DuPasquier and Cantoni, 1992) and up to 95% along a brook in the Austrian Alps (Niethammer, 1978). In addition, the fauna of aquatic invertebrate habitats varies greatly depending on the environmental conditions. For example, ponds support very different assemblages of plants and invertebrates compared to streams, rivers and ditches (Williams *et al*., 2003). With the exception of Churchfield (1984b), the majority of studies investigating water shrew diet have been undertaken in lotic habitats (e.g.; Wolk, 1976; DuPasquier and Cantoni, 1992; Castien, 1995) and are therefore bound to have a different range of invertebrate species compared with the lentic sites in the current study. In addition, adjacent habitat type, such as grassland or woodland, further affects the diversity and abundance of invertebrate species.

4.4.2 Terrestrial invertebrate numbers

Overall, greater numbers of terrestrial invertebrate individuals were caught at the woodland sites (Ash Spinney, Twenty-Acre Piece, Shelford Manor and Washdyke Farm) than the grassland sites (Hockerton, Sheepwalks, Newstead Park and Rushcliffe Country Park). Greater numbers of Coleoptera, Collembola, Opiliones, Diplopoda, Isopoda, Gastropoda and Lumbricidae were caught at the woodland sites, many of which (e.g. Isopoda, Leiodidae, and Julidae) were fairly typical of the woodland habitat surveyed, although the species of Gastropoda and Lumbricidae caught were not particularly associated with woodland habitats. However, the damp areas near woodland ponds provide ideal conditions and likely explain their occurrence at the woodland sites. The only taxon occurring in greater numbers at the grassland sites were Hymenoptera which comprised only Formicidae. However, this is likely to be due to one of the pitfall traps inadvertently being close to an ants nest. The greater numbers of terrestrial invertebrates caught at the woodland sites is likely to be due to the greater species richness usually found at woodlands compared with grasslands, especially improved grassland (the grassland sites in this study), because of the greater number of microhabitats and niches for species to exploit (Harris and Harris, 1997). For example, dead wood from fallen trees and leaf litter provide a diverse habitat and food supply for a wide range of invertebrate species. However, although greater numbers of individuals were found at the woodland sites there was virtually no difference in species diversity overall. In addition, because the purpose of the invertebrate survey was to investigate water shrew prey availability, the area which was surveyed was within 3 metres of the water's edge and therefore, the types of species caught may not necessarily be typical to woodlands or grasslands but those associated with riparian habitats.

4.4.3 Aquatic invertebrate taxa

Greater numbers of aquatic invertebrate individuals were caught at sites with known water shrew presence which could suggest that higher abundance of aquatic invertebrates positively affects water shrew

occurrence. The proportion of aquatic invertebrate prey in the diet of water shrews varies greatly and can constitute up to 95% of their diet (Niethammer, 1978) which could explain the difference between numbers of aquatic invertebrates at sites with known and unknown water shrew presence. Ephemeroptera larvae, Trichoptera larvae and Turbellaria were caught only at sites with known water shrew presence. Ephemeroptera larvae and Trichoptera larvae are known to be important sources of water shrew prey with Ephemeroptera accounting for 9-17% of their diet and Trichoptera larvae 12-17% (Niethammer, 1978; Carter and Churchfield, 2006b). In addition, Churchfield (1984b) found that although Turbellarians were common in watercress beds, their remains were rarely seen in the faecal pellets of water shrews. Nevertheless, during food preference tests with captive water shrews (Churchfield, 1984b), Turbellarians were in fact eaten, but only when other more preferred food items, such as *Gammarus sp*. (Amphipoda) and *Asellus sp*. (Isopda) were not available. However, Turbellarians are entirely soft-bodied (Barnes, 1980) so even if they were an important source of prey it is likely that they would leave little remains in water shrew faeces to show this. Greater numbers of adult aquatic Hemiptera and Diptera larvae individuals were caught at sites with known water shrew presence. Adult aquatic Hemiptera are not major prey items for water shrews, however aquatic Diptera larvae are known to be an important food source (Churchfield, 1985). Coleoptera were caught in similar numbers at sites with known and unknown water shrew presence suggesting they are not a particularly important food source which is supported by evidence that aquatic Coleoptera do not feature highly in the diet of water shrews (Churchfield, 1985). Greater numbers of aquatic Gastropoda, Odonata and Haplotixida were caught at sites with unknown water shrew presence which makes sense as none feature as major prey items in the diet of the water shrew. In addition, Churchfield (1984b), found although aquatic Gastropods were common in the watercress beds, their remains were not found in the faeces of water shrews nor were they taken in food tests. However, Amphipoda (e.g. *Gammarus sp*.) are known to be an important source of food for water shrews and is their preferred food in some cases. For example, DuPasquier and Cantoni (1992) found water shrews, on a river in Switzerland, to prefer Amphipoda whereas Churchfield

(1984b) found water shrews, inhabiting watercress beds, to prefer Isopoda (e.g. A*sellus sp.*). However, the abundance of Amphipoda in the Swiss river was much greater than that of Isopoda which probably explains the difference and led DuPasquier and Cantoni (1992) to conclude that the water shrew is an opportunistic feeder, choosing its prey according to abundance.

4.4.4 Aquatic invertebrate numbers

Greater numbers of Diptera larvae and Trichoptera larvae were caught at the woodland sites. Woodland ponds are an important habitat for Trichoptera species, since many caddis fly larvae use leaves and tree bark to build their cases (Williams *et al*., 1999). In addition, the types of Diptera larvae caught (e.g. Chironomidae and Culicidae) are often found in shady pools typical of woodland (Davies, 1988) where leaf litter provides many species with an abundant food source (Williams *et al.,* 1999). Amphipoda (e.g. Gammaridae), which are known to be an important source of prey for water shrews (Churchfield, 1984b), were caught in similar numbers at woodland and grassland sites, which could explain water shrews occurring equally at both habitat types. A number of taxa which were only caught at the grassland sites, such as Odonata larvae and Ephemeroptera larvae, are associated with the open water typical of grassland ponds and therefore their presence only at the grassland sites is not surprising.

4.4.5 BMWP

The lack of a significant difference in the BMWP scores at sites with and without water shrews would suggest that they are not necessarily as sensitive to water quality as previously thought (e.g. French *et al*., 2001; Carter and Churchfield, 2006a). For example, Twenty-Acre Piece, a site with known water shrew presence, scored lower than two of the sites with unknown water shrew presence. In addition, some of the invertebrates such as Chironomidae larvae, caught at Twenty-Acre Piece, such as Chironomidae larvae, are allocated very low BMWP scores, indicating tolerance to poor water quality (Hawkes, 1998). However, low scoring

aquatic invertebrates are also found at high quality sites. Furthermore, the transient nature of water shrews (Churchfield, 1990) could account for their appearance at suboptimal habitats and therefore their presence at such sites may not necessarily indicate a lack of association with water quality. Although diversity and BMWP are good measures of habitat quality, results have shown that it is not the site quality that determines site suitability but maybe the presence of a range of species that water shrews have been demonstrated to eat.

4.4.6. Critique of sampling methods

A significant limitation in the experimental design of the prey availability investigation is its reliance on the results of the bait tube survey. As previously discussed (see Chapter 2), the issue of imperfect detection was not addressed therefore, it is likely that there were a number of sites where water shrews were not detected but were in fact present. It is not known whether any of the four sites were water shrews were not detected were actually false absences. This has obvious implications when looking for an association between water shrew presence and prey availability. Consequently, the results of this investigation and subsequent inferences regarding habitat selection must be interpreted with caution. It is recommended that for future work any such investigation is based on presence data which has taken into account detection probability and therefore obtained with a higher degree of certainty.

The experimental design of the aquatic invertebrate survey was based on the assumption that the home range of a water shrew (60- 468 m^2, Illing *et al*., 1981; Cantoni, 1993) was about the same size as the ponds used in the survey, so consequently the shrew would have access to all areas of this habitat. For this reason the samples taken from each pond were pooled prior to analysis (the standard methods of the Pond Monitoring Network (Biggs *et al*., 1998) and BMWP (Hawkes, 1998)) since this was the area over which an animal might be expected to forage. However, as water shrew territories typically only encompass a portion of the waterbody adjacent to the bankside this assumption may have been misguided.

Furthermore, samples could have been kept separately to examine variability within sites to obtain information on the distribution of prey. For example, prey which is clustered may be exploited more efficiently by water shrews than food more randomly distributed (DuPasquier and Cantoni, 1992). This could have an effect on water shrew presence at a given site as clustered distribution of prey could lead to increased competition within the water shrew population. Therefore, for future studies of prey availability it is recommended that samples from within a single waterbody are analysed separately.

Chapter 5
Estimating Water Shrew Abundance

The aim of this chapter is to estimate the abundance of water shrews and other small mammal species and to investigate any apparent relationships at a subset of sites with known water shrew presence.

5.1 Introduction

The estimation of abundance plays an important role in ecology (Loreau, 1992; He and Gaston, 2000; Nichols and Mackenzie, 2004; Conn *et al*., 2006; Wiewel *et al*., 2009) particularly with respect to rare or vulnerable species (Rosenberg *et al*., 1995; Gregory and Gaston, 2000; Magurran and Henderson, 2003). Determining whether a species population is too small, too large or changing requires counting animals (Sinclair *et al*., 2006). However, assessing numbers of rare species can be particularly problematic by the very nature of their scarcity (Mackenzie *et al*., 2005; Williams and Thomas, 2009). This can result in abundances being inferred or given a maximal value because sample sizes may be too low for more accurate estimates (Gaston, 1994; Mills *et al*., 2000). In addition, the more biased the abundance estimates, due to low sample sizes, the less the reliability of categorising an assemblage into rare or common species (Thompson, 2004). Abundance estimates of individual species that are classified as regionally or globally rare are often conservative and the estimates often much smaller than actual numbers of individuals in the population (Gaston, 1994). Conversely, abundance estimates of rare species are sometimes overestimated as a result of their rarity. For example, this has been documented in areas with high numbers of bird watchers who are more likely to record the sighting of a rare species than a common one (Bock and Root, 1981; Booth *et al*., 2011). Furthermore, insufficient sampling may result in a species being recorded as absent when it is in fact present. There are many examples of species, including large organisms such as birds and mammals, which have been declared as globally extinct only to be later rediscovered (Diamond, 1985; Ladle, 2011).

In order to manage wildlife populations effectively knowledge of the abundance of that population is necessary. The decline in numbers of many mammal species due to abiotic factors such as climate change, habitat loss and degradation makes assessing and monitoring populations essential for their conservation and management (Morris, 2011). There are many biotic factors which affect species abundance including interspecific competition (e.g. Munger and Brown, 1981; Heske *et al*., 1994; Zhang and Zhang, 2012) which is an important factor in the structure of small mammal communities (Eccard and Ylonen, 2003; Liesenjohann *et al*., 2011).

5.1.1 Estimating species abundance

It is practically impossible to undertake a complete count of small mammals therefore the numbers caught at a site are only a proportion of the actual population size (Pocock *et al*., 2004; Conn *et al*., 2004). Capture-mark-recapture (CMR) techniques are standard ecological methodology for estimating population sizes of species (Seber, 1982; Morley, 2002). A sample of the population is taken (e.g. through live-trapping), counted, marked and then released back into the population. Further samples are then taken and the size of the population is calculated from the proportion of marked and unmarked individuals subsequently caught. In order to accurately estimate population size at least 20% of the population must be captured (Henderson, 2003). This can be calculated by plotting the rate of decline of new captures.

Population size estimators using CMR only work when a number of assumptions are true (Henderson, 2003):

- marks are durable and correctly recorded
- the behaviour or life expectancy of the animal is not affected by being marked
- the chances of the animal being caught are not affected by trapping, handling or marking

- the chances of an animal leaving the population (through death or emigration) are not affected by trapping, handling or marking
- all animals have an equal chance to leave the population
- marked animals must become completely mixed when released back into the population
- the probability of capturing a marked animal is the same for any member of the population (equal catchability) and they are sampled at random

In addition to population estimation, CMR provides information necessary to estimate the likelihood of detection i.e. capture probabilities (Conn *et al*., 2006). Variation in capture probability is a limitation for the accuracy of population estimates using CMR (Pledger and Efford, 1998) and is caused by a number of factors such as time, behavioural response and individual heterogeneity (Menkens and Anderson, 1988). For example, capture probabilities may vary between trapping sessions because season and time of day affect activity patterns of animals (Hammond and Anthony, 2006). In addition, different behavioural responses of individuals to traps will affect capture probability with trap-happy and trap-shy animals increasing and decreasing capture probability, respectively. For instance, individuals of some species are more likely to enter traps previously occupied by themselves or by conspecifics, particularly those of the opposite sex (e.g. Townsend's vole *Microtus townsendii*, Boonstra and Krebs, 1976 and white footed mice *Peromyscus leucopus*, Wolf and Batzli, 2002). Conversely, some individuals may be less likely to enter traps previously occupied by other species or by dominant conspecifics (e.g. Meadow voles *Microtus pennsylvanicus* Boonstra *et al*., 1982 and house mice *Mus musculus and* prairie deer mice *Peromyscus maniculatus bairdii*, Wuensch, 1982). Finally, individual heterogeneity (e.g. age, sex, social status etc.) will also cause variation in capture probability.

There are a number of methods of varying complexity for estimating population numbers. Simple enumeration methods such as minimum number alive (the number of distinguishable individuals caught during a

capture session; Krebs, 1966), are widely used (e.g. Bates and Harris, 2009; Pedersen *et al*., 2010; Fischer *et al*., 2011; Horn *et al*., 2012) despite two significant problems associated with such techniques (Rosenberg *et al*., 1995; Pocock *et al*., 2004). Firstly, enumeration methods tend to considerably underestimate actual population size because they are based on the minimum number alive (Macdonald *et al*., 1998; Bryja *et al*., 2001; Conn *et al*., 2006) and secondly, they work on the assumption of equal trappability between individuals and between captures (Jolly and Dickson, 1983; Pocock *et al*., 2004). Therefore, such methods are typically used when numbers of animals captured are too low to undertake more complex CMR methods (e.g. Tattersall *et al*., 2000; Deitloff *et al*., 2010; Renwick and Lambin, 2011). However, there are ways to reduce these negative biases. For example, one way of minimising the underestimation of population size is by ensuring a high proportion of the population is trapped (e.g. by using a large number of traps over a large area) and by extending the trapping period so that animals caught later in the trapping period are mainly recaptured animals (Gurnell and Flowerdew, 2006). Furthermore, with respect to the assumption of equal capture probability, it is possible to lessen such biased estimates by taking into account trappability estimates when analysing mark-recapture data.

Nevertheless, there is evidence that in some circumstances the minimum number alive do reflect population size estimates. For example, Pryde *et al*. (2005) found that the minimum number alive closely followed estimates derived from both recapture rates and predictions in population viability analyses in a population of long-tailed bats *Chalinolobus tuberculatus.* Similarly, a correlation between minimum number alive and population estimates has been reported in studies of sitka mice *Peromyscus keeni* (Hanley and Barnard, 1999) and house mice *Mus musculus domesticus* (Ruscoe *et al*., 2001).

CMR models can be based on closed or open populations. Closed models such as the Lincoln-Petersen index rely on the number of individuals in a population remaining constant over the period of study and can only

estimate population size at one point in time (Menkens and Anderson, 1988). For open populations, more complex models such as the Jolly-Seber can be used, although such methods deal with small subsets of data which are prone to sampling error and therefore require a large sample size which could be problematic when sampling species with low population numbers (Schwarz and Seber, 1999). However, in addition to estimates of population size, open models can be used to estimate survival, recruitment and population growth (Pryde, 2003). The Program MARK (White and Burnham, 1999) is a software package which provides population parameter estimates (e.g. survival, population size and capture probability) by fitting a series of powerful statistical models to CMR data and is widely used in a range of species (e.g. house mice *Mus musculus* Conn *et al*., 2006, Kaboodvandpour *et al*., 2010; whale sharks *Rhincodon typus* Rowat *et al*., 2009; humpback whales *Megaptera novaeangliae* Constantine *et al*., 2010; small mammals Arlettaz *et al*., 2010; field voles, Renwick and Lambin, 2011; red-backed salamanders *Plethodon cinereus* Buderman and Liebgold, 2012).

Although water shrew abundance has been estimated at a number of freshwater habitats such as canals (Cantoni, 1993), watercress beds (Churchfield, 1984a) and marshland (Aulak, 1970) there is a lack of studies investigating the abundance of water shrews inhabiting ponds. Furthermore, despite previous studies on the relative abundance of shrews in multi-species communities (e.g. Cotgreave and Stockley, 1994; Churchfield *et al*., 1997; Dickman, 1998; Brannon, 2000; Sheftel and Hanski, 2002) there has been no specific work in the UK on the relationship between water shrews and other small mammal species in pond habitats. Deriving accurate abundance estimates of rare species is key to conserving wildlife populations. CMR protocols commonly used to estimate abundance of small mammals are difficult with rare species (Williams and Thomas, 2009) so MNA is often used (Mills *et al*., 2000), although validity is seldom tested. This study compares MNA with estimates derived by Jolly-Seber (POPAN in MARK).

5.1.2 Aim and objectives

The aim of this part of the study is to estimate abundance of water shrews and other small mammals at a subset of sites with known water shrew presence using live-trapping methods. The objectives were to:

- Select four sites where evidence of water shrew presence was found
- Undertake live-trapping over a number of seasons
- Estimate abundance of water shrews and other small mammal species
- Investigate the relationships between abundance of water shrews and other small mammal species

5.2 Methods

The four sites with water shrew presence which were used previously for determining prey availability (Ash Spinney Pond, Hockerton Pond, Sheepwalks Pond and Twenty-Acre Piece; see Figure 5.1) were selected for live-trapping sampling to estimate abundance and investigate relationships between water shrews and other small mammals. Considering the intensive nature of live-trapping, the use of four sites was considered a feasible number for further study and provides sufficient replicates to allow for site variation. Sites were selected on the basis of having minimum public access, so as to reduce the risk of disturbance or theft of the traps, and for easy accessibility, as traps require checking three times per day.

Live-trapping was undertaken, under Natural England Licence, using standardised methodology (Gurnell and Flowerdew, 2006) at each of the four selected sites where evidence of water shrews was found following the bait tube survey. Trapping was undertaken twice a year during autumn/winter (October-December) and spring/summer (April-June) for two years (2007-2009). Generally, small mammals undergo marked changes in population size throughout the year where populations tend to be low in the spring, followed by autumn/winter peaks after the summer breeding season (Flowerdew, 1993).

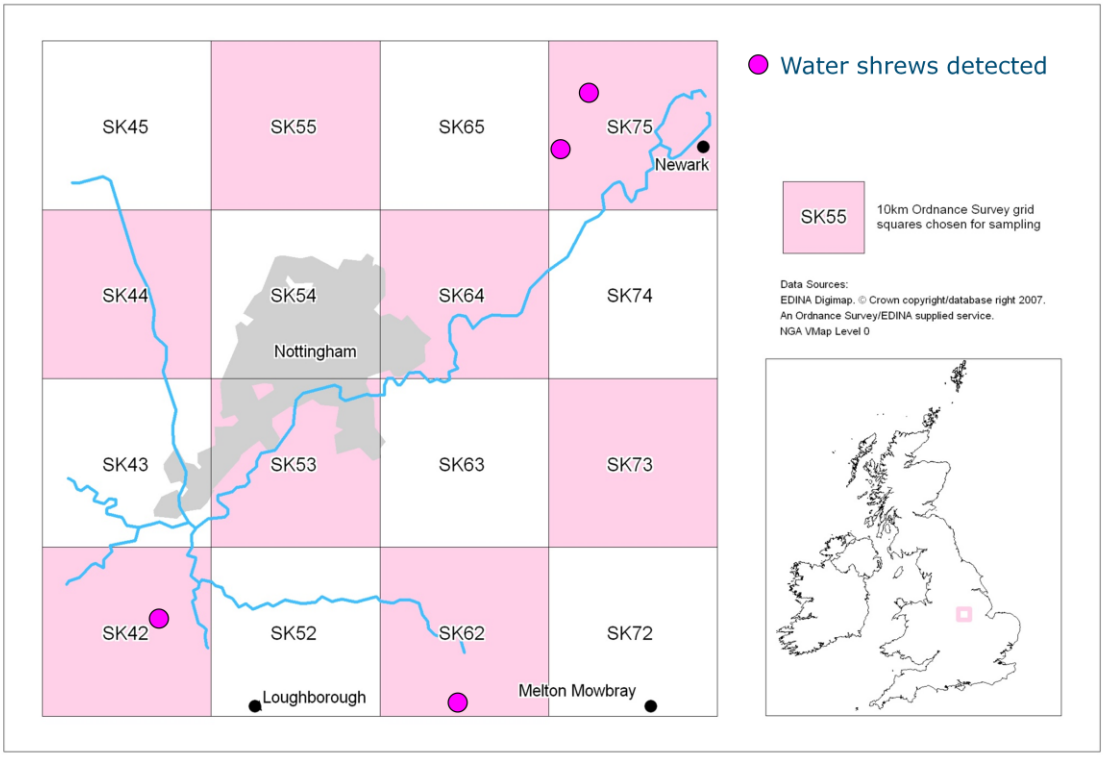


Figure 5.1 Map showing the four sites with water shrew presence subject to live-trapping

Trapping twice a year therefore allows the population to be sampled during two distinct phases. Trapping was replicated over two years to reduce the effects of variability e.g. weather. The sequence in which each site was trapped each season was varied where possible, although there were constraints to the timing of trapping at Ash Spinney imposed by the gamekeeper due to the pheasant shooting season. This ensures that sites which were trapped early in the season one year were trapped later in the season the following year, enabling order effects to be reduced (see Table 5.1 in Results for trapping dates) and a similar opportunity to trap animals across the sites.

Fifty Longworth traps (Chitty and Kempson, 1949) baited with appropriate food (small handfuls of oats, blow-fly pupae and a small piece of apple) and bedding (hay) were placed at ground level at each site within 3 metres of the water body. Traps were positioned with the tunnel flush to the ground and the nest box sloping up at the back to prevent rain from entering the

nest box and to ensure that urine and condensation drained away (Chitty and Kempson, 1949). Pre-selected trapping points/stations were marked with a short cane with coloured tape attached to the top to ensure visibility. Traps were situated amongst vegetation in both obvious surface runs and along likely runs, such as along fallen logs, in order to maximise capture success. The traps were placed in groups of three (except at two points where they were placed in groups of four due to using 50 traps), at approximately five metre intervals and were in position for a period of seven days. For CMR studies three trapping days and nights are usually recommended, but in this case to increase the chances of catching water shrews trapping was carried out over five days and nights. This proved to be effective as on more than one trapping session a water shrew was caught for the first time on the final (seventh) day of trapping.

For the first two days the traps were on a pre-bait catch, letting the animals enter and leave the traps freely, thus allowing familiarisation to take place. For the following five days the traps were set to catch. Traps were checked three times per day (6am, 1pm and 8pm) where trapped animals were processed then released at the point of capture (see Figure 5.2). Mark-recapture methods (fur-clipping) were applied to determine individual recaptures and estimate abundance during the trapping session. However, since water shrews do not possess darker underfur like other small mammals (Sargent and Morris, 2003) identification through fur clipping can lead to errors. Therefore, the number of captured water shrews was also ascertained through individual genotyping (see Chapter 6 for details).

Traps were opened into a large (60 x 45cm) polythene bag, to prevent captured animals from escaping and the trap and bedding carefully removed. The species was identified (by physical characteristics) and examined (through the bag) for previous fur clips. If the animal was a recapture its unique clip was recorded and it was released immediately. New animals were manoeuvred (head first) into the corner of the bag and gently held in one hand from the outside of the bag.

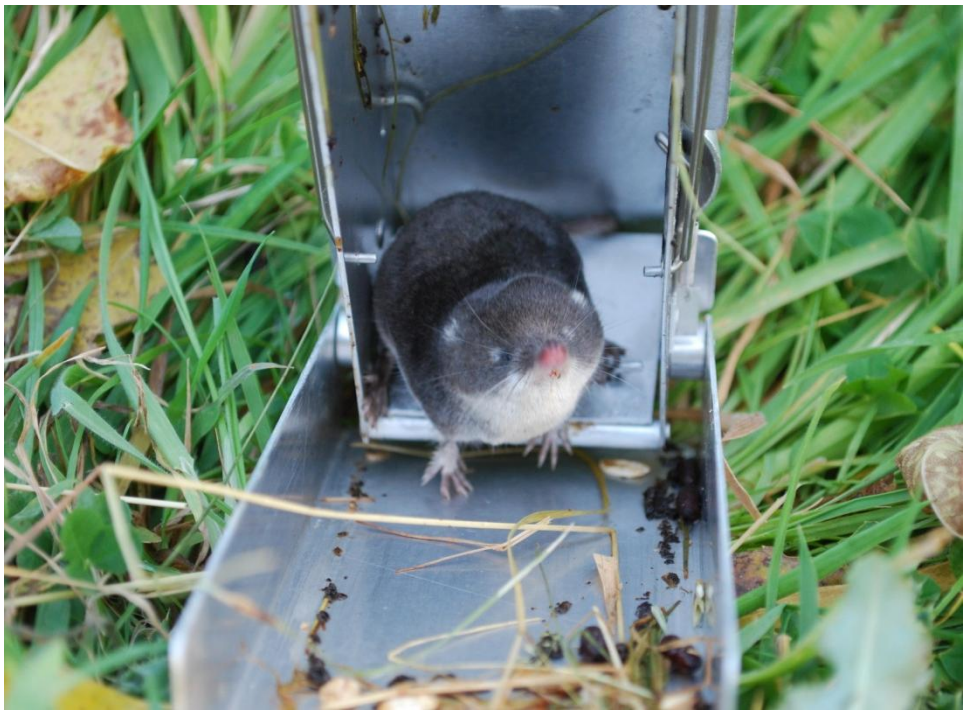


Figure 5.2 Water shrew being released from a Longworth trap.

Figure 5.3 Water shrew being held by the scruff.

Using the other hand the animals were removed from the bag (holding them by the scruff; see Figure 5.3), transferred into a smaller bag (25 x 30cm) (calibrated with the scales) and weighed using a Pesola Light-Line 50g spring balance. The sex of rodents (voles and mice) was easily recorded but shrews have internal sex organs making sexing difficult in the field (Churchfield, 1990). Some sexing techniques such as observation of nipples in young shrews (Searle, 1985) and the sound of their call (Crowcroft, 1957) have been suggested but require a high level of skill and therefore a high risk of misidentification (Matsubara, 2001). Therefore shrews were not sexed.

5.2.1 Data analysis

Abundance and capture probabilities of all species were estimated using open population POPAN models (a robust parameterisation of the Jolly-Seber; Schwarz and Arnason 1996) implemented in program MARK version 6.1 (White and Burnham, 1999). POPAN assumes that both marked and unmarked animals have equal capture probabilities and that animals captured during the surveying period represent a component of a larger 'super-population'. The model derives a probability of entry of animals from the 'super population' into the survey areas (Schwarz and Arnason 1996). Models were fitted using a sine link function for survival φ and capture probability p, the multinomial logit link function for entry probability β and a logarithm link function for N. For all models data were grouped by site and session for population estimates, while apparent survival and entry probability were assumed to be constant (time-independent). Data for capture probability were grouped by site, session, habitat type and season to determine the best fitting model. Models were selected using the Akaike Information Criteria corrected for small sample size (AICc) (Burnham and Anderson, 2002).

Relationships between water shrews and other species were investigated using Pearson's correlation coefficient and a false discovery rate (FDR; Narum, 2006) correction was applied.

5.3 Results

Overall, 614 individuals from seven small mammal species were caught at the four sites (see Appendix 4). Bank voles and wood mice were the most frequently caught species followed by common shrews. Field voles, water shrews, pygmy shrews and harvest mice were caught in much fewer numbers.

5.3.1 POPAN abundance estimation

The best fitting models for each species (see Table 5.1) were used to produce abundance estimates for each species (see Table 5.2). Models were constrained to have constant survival and probability of entry because small sample sizes led to convergence problems. These are reasonable assumptions, given the short trapping intervals. In general, models suggested that capture probabilities were constant across sites and seasons with the exception of bank voles which showed variation in capture probability across seasons (i.e. autumn/winter versus spring/summer). Common shrews and wood mice had the highest capture probabilities of all species (0.91) and pygmy shrews and water shrews the lowest (0.48 and 0.64, respectively; see Table 5.2). Numbers of harvest mice were too low for analysis therefore abundance was taken to be the minimum number alive. POPAN abundance estimates across all species showed a significant positive correlation with the minimum number alive ($r = 0.998$, $p < 0.000$, $n = 6$; see Figure 5.4).

Table 5.1 Model selection for POPAN abundance estimation for each species (φ, survival; β, entry probability; (.) constant; *K*, number of parameters). Only models which converged are shown. The best-fitting models are shown in blue.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	*K*	Deviance
a) Pygmy shrews						
N(site*sess)*p*(.)φ(.)β(.)	353	0	1.00	1.00	19	34
N(site*sess)*p*(habitat)φ(.)β(.)	521	168	0.00	0.00	20	32
b) Common shrews						
N(site*sess)*p*(.)φ(.)β(.)	38186	0	0.96	1.00	19	37752
N(site*sess)*p*(sess)φ(.)β(.)	38192	6	0.04	0.04	22	37751
N(site*sess)*p*(habitat)φ(.)β(.)	43468	5282	0.00	0.00	20	43031
N(site*sess)*p*(season)φ(.)β(.)	48684	10498	0.00	0.00	20	48248
c) Water shrews						
N(site*sess)*p*(.)φ(.)β(.)	9904	0	1.00	1.00	19	9811
d) Field voles						
N(site*sess)*p*(.)φ(.)β(.)	19705	0	1.00	1.00	19	19572
e) Bank voles						
N(site*sess)*p*(season)φ(.)β(.)	3578	0	0.97	1.00	20	1994
N(site*sess)*p*(habitat)φ(.)β(.)	3585	7	0.03	0.03	20	2001
N(site*sess)*p*(site) φ(.)β(.)	3622	44	0.00	0.00	22	2034
N(site*sess)*p*(.)φ(.)β(.)	164963	161385	0.00	0.00	19	163382
f) Wood mice						
N(site*sess)*p*(.)φ(.)β(.)	73132	0	1.00	1.00	19	71959

Table 5.2 POPAN abundance estimates (± s.e.) and capture probabilities of species caught at each site during the four trapping sessions. Numbers of harvest mice were too low for analysis. AW = autumn/winter, SS = spring/summer.

Site	Date	Common shrew	Pygmy shrew	Water shrew	Bank vole	Field vole	Wood mouse	Total
Ash Spinney	21/09/2007	18.2 ± 1.38	7.0 ± 3.61	3.4 ± 1.94	44.3 ± 1.78	0.0 ± 0.00	30.6 ± 1.86	103.4
	03/05/2008	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	5.0 ± 0.00	0.0 ± 0.00	19.5 ± 1.50	24.5
	08/12/2008	7.1 ± 0.90	0.0 ± 0.00	0.0 ± 0.00	29.3 ± 1.44	0.0 ± 0.00	32.8 ± 1.93	69.3
	30/04/2009	9.4 ± 1.02	4.5 ± 2.89	0.0 ± 0.00	27.8 ± 1.22	0.0 ± 0.00	29.5 ± 1.83	71.1
Hockerton	22/10/2007	1.0 ± 0.00	0.0 ± 0.00	1.4 ± 1.39	3.0 ± 0.00	1.3 ± 1.30	8.3 ± 1.02	15.0
	01/06/2008	0.0 ± 0.00	0.0 ± 0.00	5.3 ± 2.38	0.0 ± 0.00	0.0 ± 0.00	3.0 ± 0.00	8.3
	16/10/2008	5.0 ± 0.03	2.0 ± 2.02	1.4 ± 1.39	0.0 ± 0.00	1.3 ± 1.30	10.6 ± 1.13	28.2
	08/05/2009	3.0 ± 0.00	0.0 ± 0.00	1.4 ± 1.39	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	4.4
Sheepwalks	01/12/2007	8.3 ± 0.96	0.0 ± 0.00	3.4 ± 1.94	15.5 ± 1.06	21.9 ± 4.47	11.7 ± 1.18	60.6
	22/05/2008	5.0 ± 0.02	0.0 ± 0.00	1.4 ± 1.39	0.0 ± 0.00	0.0 ± 0.00	1.0 ± 0.00	7.4
	23/10/2008	2.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	3.0 ± 0.00	5.1 ± 2.22	8.3 ± 1.02	26.4
	02/04/2009	4.0 ± 0.01	7.0 ± 3.61	0.0 ± 0.00	0.0 ± 0.00	5.1 ± 2.22	1.0 ± 0.00	17.1
Twenty-Acre Piece	12/11/2007	7.1 ± 0.90	2.0 ± 2.02	1.4 ± 1.39	53.9 ± 1.97	8.8 ± 2.86	17.3 ± 1.41	90.4
	24/06/2008	7.1 ± 0.90	0.0 ± 0.00	0.0 ± 0.00	15.2 ± 0.91	0.0 ± 0.00	2.0 ± 0.00	24.3
	31/10/2008	7.1 ± 0.90	0.0 ± 0.00	0.0 ± 0.00	21.9 ± 1.25	0.0 ± 0.00	26.1 ± 1.73	55.2
	20/04/2009	13.8 ± 1.21	14.6 ± 5.38	0.0 ± 0.00	27.8 ± 1.22	0.0 ± 0.00	26.0 ± 0.00	82.2
Total		98.1	37.1	19.0	246.6	43.4	227.7	**687.9**
Capture probability *p*		0.91 ± 0.00	0.48 ± 0.07	0.64 ± 0.02	0.32 ± 0.01 (AW) 0.38 ± 0.02(SS)	0.67 ± 0.01	0.91 ± 0.00	

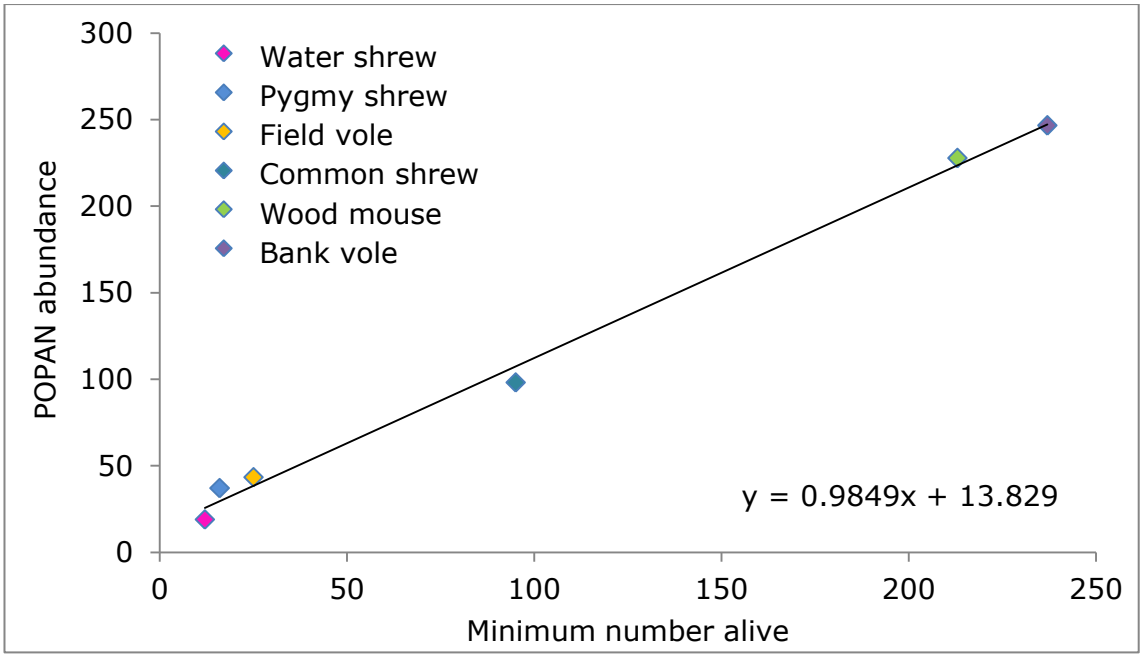


Figure 5.4 The minimum number alive versus POPAN abundance estimates of all species (except harvest mice) caught at all sites and trapping sessions (r = 0.998, p <0.000).

5.3.1.1 Sites

Although overall species abundance was higher at Ash Spinney and Twenty-Acre Piece, water shrew abundance was greatest at Hockerton and Sheepwalks, the grassland sites (see Figure 5.5). Fewer species were found at Ash Spinney and Twenty-Acre Piece, the woodland sites, which were dominated by wood mice and bank voles, compared to Hockerton and Sheepwalks, the grassland sites, which had more species and greater evenness. Relative water shrew abundance was greatest at Hockerton (16.92%) and lowest at Twenty-Acre Piece (0.5%; see Table 5.3). Water shrews constituted almost half of shrew abundance at Hockerton (46%) but only 2.6% at Twenty-Acre Piece (see Table 5.4).

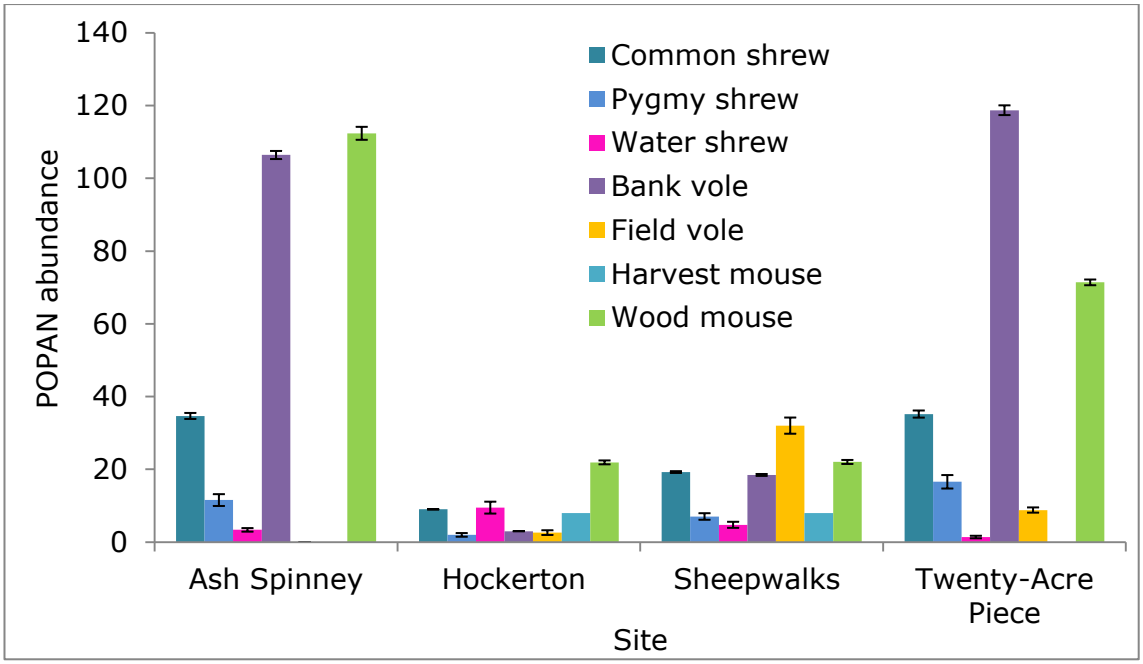


Figure 5.5 POPAN species abundance estimates (± s.e.) of all species (except harvest mice which are the MNA) caught at the four sites.

Table 5.3 Relative POPAN abundance (%) of species caught at each of the four sites (harvest mice abundance is MNA).

Site	Common shrew	Pygmy shrew	Water shrew	Bank vole	Field vole	Harvest mouse	Wood mouse
Ash Spinney	12.92	4.30	1.25	39.65	0.00	0.00	41.87
Hockerton	16.08	3.53	16.92	5.36	4.65	14.30	39.16
Sheepwalks	17.26	6.31	4.25	16.55	28.70	7.17	19.75
Twenty-Acre Piece	13.96	6.58	0.55	47.10	3.49	0.00	28.32
Mean relative abundance (± s.e.)	15.06 ± 0.99	5.18 ± 0.75	5.74 ± 3.81	27.16 ± 9.75	9.21 ± 6.57	5.37 ± 3.42	32.28 ± 5.10

Table 5.4 Relative POPAN abundance (%) of shrews caught at each of the four sites.

Site	Common shrew	Pygmy shrew	Water shrew
Ash Spinney	69.93	23.30	6.77
Hockerton	44.02	9.66	46.32
Sheepwalks	62.03	22.68	15.29
Twenty-Acre Piece	66.21	31.18	2.61
Mean relative abundance (± s.e.)	60.55 ± 5.74	21.71 ± 4.46	17.75 ± 9.88

5.3.1.2 Sessions

Overall species abundance was highest during the first trapping session and lowest during the second (see Figure 5.6). Abundance during the third and fourth sessions was similar. Abundance of water shrews decreased over the four trapping sessions. Common shrew abundance was relatively stable and bank voles and wood mice dominated each session whereas field voles were only caught during autumn/winter sessions, and harvest mice only during autumn/winter 2008. The relative abundance of water shrews was greatest during spring/summer 08 (10.39%) and lowest during autumn/winter 08 (0.77%; see Table 5.5). Water shrews constituted 35.55% of shrew abundance during spring/summer 08 but only 2.40% during spring/summer 09 (see Table 5.6).

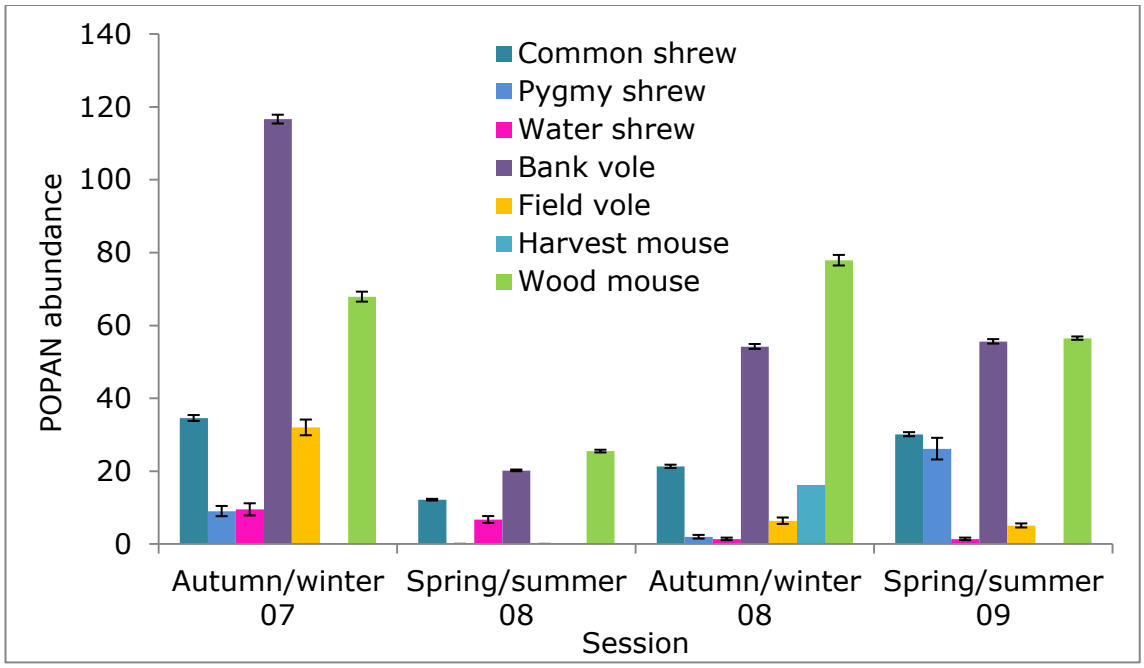


Figure 5.6 POPAN species abundance estimates (± s.e.) of all species (except harvest mice which are the MNA) caught during each of the four trapping sessions.

Table 5.5 Relative POPAN abundance (%) of species caught during each of the four trapping sessions (harvest mice abundance is MNA).

Site	Common shrew	Pygmy shrew	Water shrew	Bank vole	Field vole	Harvest mouse	Wood mouse
AW07	12.83	3.34	3.52	43.26	11.87	0.00	25.18
SS08	18.83	0.00	10.39	31.30	0.00	0.00	39.49
AW08	11.89	1.10	0.77	30.26	3.56	8.93	43.48
SS09	17.23	14.96	0.79	31.81	2.90	0.00	32.31
Mean relative abundance (± s.e.)	15.20 ± 1.68	4.85 ± 3.44	3.87 ± 2.27	34.16 ± 3.05	4.58 ± 2.55	2.23 ± 2.23	35.11 ± 4.04

Table 5.6 Relative POPAN abundance (%) of shrews caught during each of the four trapping sessions.

Site	Common shrew	Pygmy shrew	Water shrew
AW07	65.14	16.99	17.88
SS08	64.45	0.00	35.55
AW08	86.37	8.02	5.62
SS09	52.24	45.36	2.40
Mean relative abundance (± s.e.)	67.05 ± 7.09	17.59 ± 9.88	15.36 ± 7.51

5.3.1.3 Water shrews versus other species

A negative relationship was found between total abundance per site of water shrews and common shrews although this was not significant (Table 5.7 and Figure 5.7). A negative relationship was also found between water shrews and pygmy shrews (see Table 5.7 and Figure 5.8) although this was not statistically significant when FDR correction was applied. No other relationships were found between water shrews and other species.

To further investigate the negative relationships between water shrews and the terrestrial shrews, correlations across both sites and sessions were undertaken. However, there were no relationships between water shrew and

pygmy shrew abundance (r = -0.152, p = 0.575, d.f. = 15) or water shrew and common shrew abundance (r = 0.015, p = 0.955, d.f. = 15) across sites and sessions combined.

Table 5.7 Pearson's correlation of total abundance of water shrews and other small mammal species at each of the four sites (FDR correction applied significant at $p < 0.02041$, d.f. = 3).

Species	Correlation	P value
Water shrew v common shrew	-0.938	0.062
Water shrew v pygmy shrew	-0.965	0.035
Water shrew v bank vole	-0.866	0.134
Water shrew v wood mouse	-0.645	0.355
Water shrew v field vole	-0.113	0.887
Water shrew v harvest mouse	0.794	0.206

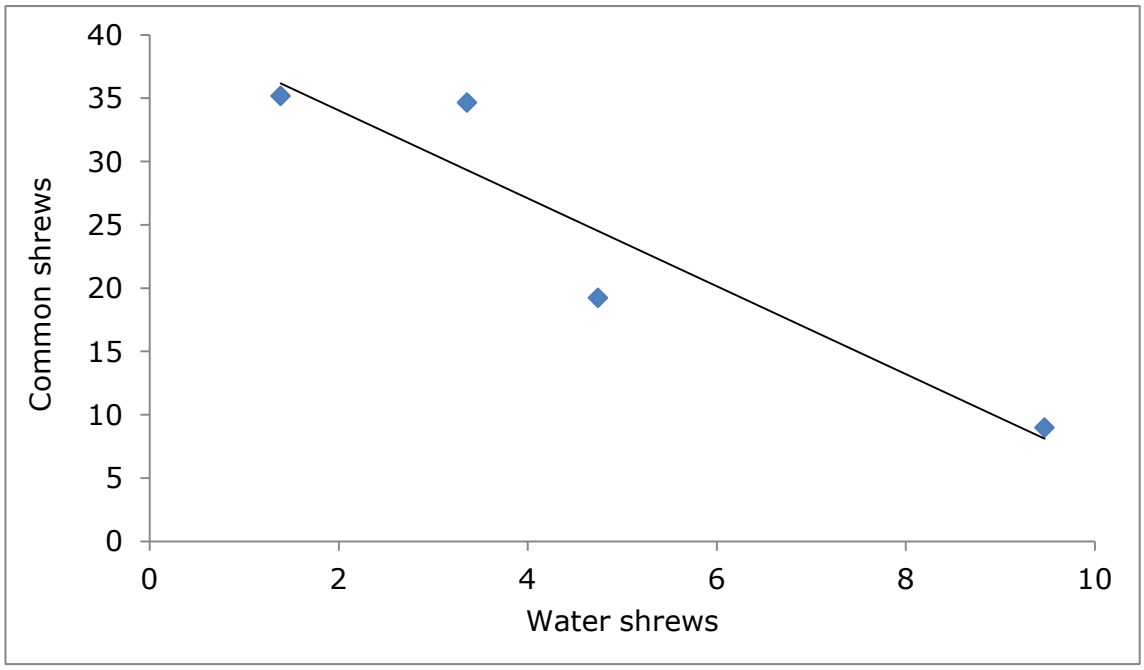


Figure 5.7 Relationship between water shrew and common shrew abundance at each of the four sites (r = -0.938, p = 0.062, d.f. = 3).

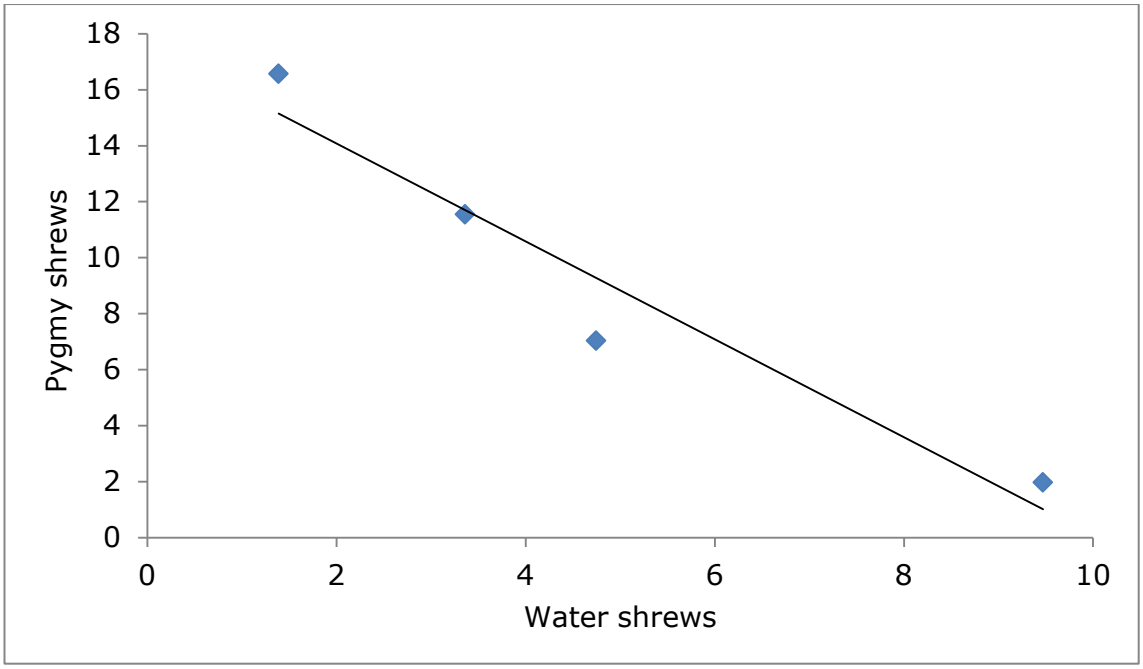


Figure 5.8 Relationship between water shrew and pygmy shrew abundance at each of the four sites (r = -0.965, p = 0.035, d.f. = 3).

5.4 Discussion

Bank voles and wood mice were the most frequently caught species overall followed by common shrews whereas water shrews, pygmy shrews and harvest mice were caught in much fewer numbers (see Appendix 4), reflecting their comparatively lower population sizes (Harris and Yalden, 2008). The POPAN abundance estimates reflected the minimum number alive (see Figure 5.4), supporting findings from other studies of species such as sitka mice (Hanley and Barnard, 1999), house mice (Ruscoe *et al*., 2001), long-tailed bats (Pryde *et al*., 2005) and black-footed ferrets (Grenier *et al*., 2009). However, estimates were most accurate for species caught in higher numbers such as wood mice and bank voles compared with the less frequently caught water shrews, pygmy shrews and field voles. Despite this, the minimum number alive for water shrews during each trapping session was within the 95% confidence limits of the POPAN abundance estimates. Therefore, for studies of water shrews when numbers are too low for more complex analysis, minimum number alive may not be as negatively biased as previously thought.

The high capture probabilities of common shrews and wood mice (see Table 5.2) may be due to differences in behaviour such as being more inquisitive (Churchfield, 1990) or trap-happy (Montgomery, 1979), or because the habitats were optimal for the species so they were occurring at high population densities (Flowerdew *et al*., 2004; Flowerdew and Tattersall, 2008). The low capture probabilities of pygmy shrews may also be due to differences in behaviour (e.g. trap-shyness), or a reflection of their relatively lower population densities. Although population density should not determine capture probability *per se*, large home ranges (equating to low population densities) might result in low trappability if traps are only in part of their home range. The low capture probabilities of field voles is unusual as they typically exhibit high recapture rates (e.g. 0.89; Renwick and Lambin, 2011). For example, Krebs and Boonstra (1984) estimated trappability for four species of *Microtus* and found mean capture probablilites of 0.63 (*M. pennsylvanicus*), 0.64 (*M. californicus*), 0.66 (*M. townsendii*) and 0.86 (*M. ochrogaster*). This was probably due to the study sites not being their preferred habitat and the voles were just visiting from adjacent grassland.

Capture probabilities of bank voles were relatively low compared with other vole species (e.g. Krebs and Boonstra, 1984; Renwick and Lambin, 2011). However, Jensen (1975) found that the majority of bank voles (53.6%) trapped in forest habitat in Denmark, had capture probabilities of less than 0.33 and with only 6.6% greater than 0.66. Despite this, abundance estimates of bank voles were similar to the minimum number alive. The seasonal variation in bank vole capture probability may be a result of variation in food availability at different times of the year, which makes them more or less likely to enter traps in search of food (Tanton, 1965), or due to a change in behaviour, such as an increase in activity during the breeding season (Ylönen and Viitala, 1991).

The relatively low capture probability of water shrews is reflected by the fact that they were often not caught until several days into the trapping session. For example, during the spring/summer 08 trapping at Sheepwalks

a water shrew was not caught until day six of trapping and at Hockerton in spring/summer 09 a water shrew was only caught for the first time during the final trap round on the final day of trapping. Standard small mammal trapping methodology usually recommends trapping for three days as after this time numbers of new captures tails off (Gurnell and Flowerdew, 2006). However, when studying an elusive species which occurs at such low densities it may be beneficial to trap over a longer period as, unlike species such as wood mice and bank voles which can occur in very high numbers (Harris *et al*., 1995), there may be a water shrew population of only one or two animals at a site (Carter and Churchfield, 2006b).

5.4.1 Sites

The overall higher abundance of species at Ash Spinney and Twenty-Acre Piece (see Table 5.2 and Figure 5.5) was due to the large numbers of wood mice and bank voles caught in these woodland habitats. Wood mice and bank voles are principally woodland species and therefore the highest population densities occur in woodland (wood mice 1-40/ha and bank voles 11-34/ha; Flowerdew *et al*., 2004; Flowerdew and Tattersall, 2008). Both species favour mixed and deciduous mature woodland (Flowerdew, 1993; Flowerdew and Tattersall, 2008) with thick ground cover, a particularly important feature for bank voles (Shore and Hare, 2008).

Hockerton had the highest abundance of water shrews and was the only site where they were found during every trapping session. In addition, at Hockerton water shrews constituted the greatest component of shrew abundance (see Table 5.4). The higher abundance and relative abundance of water shrews at the grassland sites could be due to the difference in availability of aquatic prey between grassland and woodland ponds. Ponds which are heavily shaded by the tree canopy have reduced herb and grass cover, due to the lack of sunlight, and therefore lack food and habitat for aquatic and marginal invertebrates (Williams *et al.*, 1999). This could explain why water shrews were caught more frequently at the grassland

ponds which potentially have a higher diversity and abundance of aquatic invertebrates.

Common shrews comprised the greatest contribution to shrew abundance at all sites, apart from Hockerton, with abundance generally highest at Ash Spinney and Twenty-Acre Piece (see Table 5.4). Pygmy shrews represented 14% of shrew abundance at Ash Spinney and Twenty-Acre Piece, which is much higher than the proportion of 4% of this species typically found in deciduous woodland (Crowcroft, 1957; Churchfield and Brown, 1987). However, like common shrews, pygmy shrews are widespread and occur in all types of habitat with good ground cover, such as thick grassland, hedgerows and woodlands (Churchfield and Searle, 2008). Twenty-Acre Piece and Ash Spinney both have low vegetation cover with moss and leaf litter covering the ground which probably explains high numbers of common and pygmy shrews at these sites. In addition, both woodland sites are surrounded by grassland and arable land so it is possible that some of the shrews came from outside of the woodland.

5.4.2 Sessions

A considerable difference was found in total abundance during the different trapping sessions with higher abundance during autumn/winter than spring/summer (see Figure 5.6). However, such a difference is to be expected as populations of small mammals usually tend to be low in the spring, as many animals will have died over the winter, followed by autumn/winter peaks after the summer breeding season (Flowerdew and Tattersall, 2008).

The appearance of harvest mice at Hockerton and Sheepwalks during autumn/winter 08 was interesting as they had not been caught during the previous trapping sessions and were not caught subsequently. For much of the year trapping at ground level fails to catch harvest mice because they inhabit the stalk zone (Trout and Harris, 2008) which would explain their absence from the traps in spring/summer sessions (see Figure 5.6).

However, during winter, as the annually growing vegetation dies back, they abandon tall vegetation instead using the runways of other small mammals and make temporary nests in grass tussocks (Harris, 1979). Harvest mice were not caught during the previous autumn/winter 07 at Hockerton pond even though trapping was undertaken at a similar time (late October). However, the trapping at Sheepwalks took place in early December which may have been too late for the peak time (September and October) for catching harvest mice (Buckley, 1977; Trout, 1978).

Common shrews comprised the greatest proportion of shrew abundance during all sessions, whereas water shrews comprised a higher proportion than pygmy shrews during autumn/winter 07 and spring/summer 08, but a lower proportion during autumn/winter 08 and spring/summer 09 (see Table 5.6). The relative abundance of pygmy shrews was similar to water shrews during both autumn/winter trapping sessions. However, whereas water shrew abundance increased during spring/summer 08 pygmy shrew abundance declined to zero. Furthermore, pygmy shrew abundance increased greatly in spring/summer 09 whereas water shrew abundance dropped to just over 2%. This suggests there may be some form of seasonal competition between the two species.

The apparent decreasing abundance of water shrews over the trapping sessions (see Figure 5.6) may be a consequence of their transient nature (Churchfield, 1990) and the animals had simply moved on or it could be due to the deaths of the individuals caught. However, as abundance was so low any real patterns are hard to detect.

5.4.3 Water shrews versus other species

Competition between shrew species has been well documented (e.g. Croin Michielsen, 1966; Churchfield, 1980; Voesenek and Bemmel, 1984; Sheftel, 1989; Sheftel and Hanski, 2002) and may be the cause of the negative relationship found to occur between abundances of water shrews and the terrestrial shrews at each site (see Table 5.7, Figures 5.7 and 5.8).

On the wide-scale, competition between soricine and crocidurine shrews may be responsible for their geographical distributions. These two subfamilies have largely complementary distributions within Europe which may be caused by differences in habitat requirements but may be the results of broad-scale competitive exclusion (Hanski and Kaikusalo, 1989).

An example of complementary distributions at species level can be seen in Europe with the visually indistinguishable common shrew and Millet's shrew *S. coronatus*. These two species only co-exist when there is some level of habitat segregation with common shrews preferring more humid habitats with a thicker vegetation layer than Millet's shrew (Churchfield, 1990). However, during removal experiments of either species (Neet and Hausser, 1990), previously seen habitat segregation disappeared with the unmanipulated species widening its habitat distribution and niche to cover habitats previously inhabited by the competitor. This suggests that habitat segregation was in fact a consequence of interspecific interactions between the species and not habitat preference.

Common, pygmy and water shrews regularly co-exist in woodlands and grasslands throughout Europe with the addition of Laxmann's shrew *S. caecutiens* and southern water shrew *Neomys anomalus* in places (Churchfield, 1990). Such multi-species communities of shrews are common and have been studied throughout the world. For example, communities comprising six or more species in a single habitat have been examined in North America (Buckner, 1966), up to nine species in the Siberian taiga (Dokuchaev, 1989; Churchfield *et al*., 1997) and 25 species belonging to five different genera in Zaire (Dieterlen and Heim de Balsac, 1979). However, abundance of individual species of shrews, despite their high species richness, is generally low in relation to other small mammals in an area and, as previously mentioned, often comprises only a small proportion of captures (Crowcroft, 1957; Churchfield and Brown, 1987). In addition, although such communities contain many shrew species they are often dominated by a single species which may reflect strong interspecific competition (Dokuchaev, 1989). For example, Churchfield *et al*., (1997)

found differences in numbers of certain shrew species in the presence or absence of other shrew species in a multi-species community of shrews in Siberia and suggested that interspecific competition may be having an effect on habitat selection. A similar effect can be seen in south eastern Manitoba in Canada. Where, if populations of arctic shrews *S. arcticus* and masked shrews *S. cinereus* occur together, their populations have found to vary inversely with each other (Buckner, 1966).

Despite co-existence of related species in similar environments, habitat use varies both spatially and temporally which maintains some level of segregation. Although no relationship was found between the abundances of water shrews and other species per trapping session, differential seasonal changes in the activity patterns of shrew species have been documented. For example, Churchfield (1984a) found seasonal changes in the activity patterns of water shrews which were not displayed in common shrews. For much of the year common shrews and water shrews showed similar day and night time activity patterns with captures of both species occurring more often during the night (water shrews 66% and common shrews 69%) than the day. However, during the summer water shrew activity was at its highest during the day time and night time activity was at a minimum. In contrast, common shrew activity remained the same. This difference in behaviour may reduce competition at a time when overall activity and population are at their highest (Churchfield, 1984a). A similar difference in activity patterns between water shrews and common shrews was seen during April to July by Voesenek and Van Bemmel (1984). Water shrews were caught more frequently between 7am and 12pm whereas common shrews were caught more often between sunset and 2am.

Croin Michielsen (1966) found vertical segregation was the basis of slight differences in niche occupancy between common and pygmy shrews with pygmy shrews being more active on the surface and common shrews underground. Again, this partial segregation reduced competition between the two species.

A more obvious difference in habitat preference can be seen in France. Yalden *et al*., (1973) investigated small mammal habitat preferences in France and found amongst the five species of shrews the dominant Millet's shrew occurred mainly in grassland and marshland, pygmy shrew and white-toothed shrew *Crocidura leucodon* mainly grassland and stonewalls, water shrews mostly in marshy areas and pond edges and white-toothed shrew *C. russula* mainly found around stonewalls and inhabited buildings.

All three British mainland shrew species have a fairly large niche overlap (Churchfield, 1984b) and particularly in times of poor aquatic prey availability, the smaller common shrew is likely to be competing directly with the larger water shrew for terrestrial invertebrate prey. Churchfield (1984b), investigated shrew diets, through faecal analysis, and found a large dietary overlap between water shrews and common shrews. However, the study was undertaken around watercress beds, a favoured habitat of water shrews and one where the highest population densities have been recorded (Churchfield, 1984a). It could be that aquatic prey availability in this optimum habitat is higher than in less favourable environments and therefore competition for terrestrial invertebrate prey is actually greater in other habitats.

Conversely, competition for prey may not be the cause for the negative relationships between water shrews and common and pygmy shrews seen in the current study but other factors such as habitat suitability could be playing a role. However, it must be stressed that in this survey the area of trapping was relatively small and it is possible that the negative relationships found could be due to sampling within the home ranges of either common, pygmy or water shrews. The negative relationship between overall abundance per site of water shrews and common and pygmy shrews and the lack of a relationship between these species across all sites and sessions suggest that habitat suitability, rather than direct competition, may be the cause.

No significant relationships were found between abundances of water shrews and of any of the other species either per site (see Table 5.7). Unlike the three shrew species which have a large niche overlap this is much less evident between water shrews and other small mammal species. In terms of dietary overlap with water shrews, none of the other species take aquatic invertebrate prey (Churchfield, 1984b) and although wood mice will eat terrestrial invertebrates, as will bank voles (Hansson, 1985) and harvest mice (Dickman, 1986) to a lesser extent, field voles are entirely herbivorous (Evans, 1973). In addition, each of the different small mammal species prefer and occupy distinct habitats or at least different areas of habitat. Therefore, the lack of significant relationships found between water shrews and other species is probably due to their very different diet and habitat preferences.

Chapter 6
Genetic Identification of Individual Water Shrews

The aim of this chapter is to develop a technique to distinguish between different water shrews and identify individuals using a minimally invasive method. Microsatellites are the most suitable markers to distinguish between individuals when small quantities of DNA are available, as typically acquired from non-invasive sampling. Therefore, new microsatellite markers were isolated from the water shrew and tested alongside some existing markers from other related species for their suitability for genotyping water shrews. Buccal swab sampling was also investigated to determine whether it was an efficient method to obtain DNA of suitable quality and quantity for microsatellite genotyping. If successful, this approach would allow the determination of actual numbers of live-trapped water shrews and individual recaptures, and the estimation of abundance during the trapping sessions.

6.1 Introduction

Genetic techniques are being increasingly used in wildlife conservation. One of the main applications is to reduce extinction risk by minimising inbreeding and loss of genetic diversity (Frankham *et al*., 2009). For example, the introduction of genetically unrelated individuals into a small and long-isolated population of Florida panthers *Puma concolor coryi* alleviated low genetic diversity and inbreeding depression (Pimm *et al*., 2006). Similarly, genetic approaches enable species or populations at risk of reduced genetic diversity to be identified. The critically endangered Asiatic lion is a species with low genetic variability, and DNA profiling has allowed the identification of individuals with high genetic variability to be used in conservation breeding programs (Shankaranarayanan *et al*., 1997). Another related application is resolving fragmented population structures by using information on the extent of gene flow among vulnerable populations, to intervene if necessary and exchange individuals to minimise inbreeding, as performed for the Seychelles warbler *Acrocephalus sechellensis* (Richardson

et al., 2006) and proposed for the management of the grey wolf *Canis lupus* in Scandinavia (Hansen *et al*., 2011). In summary, DNA techniques can be used as a method of non-invasive sampling for genetic analyses to identify species and individuals using molecular markers (Waits and Paetkau, 2005).

6.1.1 Individual identification using molecular markers

The identification of individuals within a species is important for a number of reasons including estimating the number of individuals in a population (Wilson *et al*., 2003; Frantz *et al*., 2004; Miller *et al*., 2005), examining genetic diversity and gene flow between populations (Edwards *et al*., 1992), evaluating social structure (Morin *et al*., 1994) and assigning parentage (Constable *et al*., 2001; Griffith *et al*., 2010). Molecular markers can also be used to discern gender in organisms such as shrews (Matsubara *et al*., 2001) which possess internal sex organs (Churchfield, 1990) and hence cannot be identified in the field. The gender can then be used to help identify individuals and determine parentage, calculate sex ratios and in captive breeding programmes.

Typical methods of obtaining DNA from vertebrates have included blood sampling and tissue sampling from the partial amputation of body parts (e.g. tail, toe or ear clipping; Mitrečić *et al.*, 2008). Despite the ethical and legal considerations of these sampling techniques, they also involve the capture and handling of organisms which is often impractical depending on the nature of the species. The application of non-invasive DNA sampling to wild animals is an approach which has been utilised over the past decade involving extracting genetic material from sources such as hair or faeces, enabling samples to be collected without the need to handle or observe individuals (Waits and Paetkau, 2005). Non-invasive sampling techniques have been employed for a number of reasons including to identify the presence of rare or elusive species such as brown bears *Ursus arctos* (Taberlet and Bouvet, 1992), or to calculate numbers and identify individuals and/or differentiate between species such as grey seals *Halichoerus gryphus* and harbour seals *Phoca vitulina* (Reed *et al*., 1997).

6.1.2 Application of microsatellite markers

DNA profiling allows the variation in the genotype of an animal to be used as a natural molecular marker for individual identification (Jeffreys *et al*., 1985a). Individual identification techniques have employed a range of molecular markers since the discovery of multilocus minisatellite regions of DNA in humans, frequently referred to as 'DNA fingerprints' (Jeffreys *et al*., 1985a, b, c) and their application to wild animals (e.g. Burke and Bruford, 1987). Single-locus minisatellites have additionally been employed to evaluate specific loci (Burke *et al*., 1991). The use of single and multi-locus minisatellites as genetic markers have largely been replaced following the development of PCR technology (Saiki *et al*., 1988) and the discovery of microsatellites (Tautz, 1989).

The most common molecular markers currently used in population studies are microsatellites (Waits and Paetkau, 2005; see Figure 4.1), also known as simple tandem repeats. These are tandem repeats of short segments of DNA, typically 1-5 base pairs in length, usually occurring in non-coding (junk) DNA (Tautz, 1989; Li *et al*., 2002).

Figure 6.1 Example of a microsatellite sequence with $(CA)_n$ repeat motif.

Microsatellite markers can be used to detect polymorphisms in loci that are neutral and consequently not subject to selection (Frankham *et al.*, 2009). Due to mutation processes, the length of a microsatellite can vary between individuals owing to different numbers of repeat units in different individuals (Goldstein and Schlötterer, 1999). The number of repeat units in a microsatellite sequence can vary in individuals by as many as ten or more (Goldstein and Schlötterer, 1999). This difference in length of a microsatellite sequence can be assessed visually using gel electrophoresis or by using an ABI Sequencer. By examining several polymorphic

microsatellite sequences it is possible to build up a unique genetic profile of an individual animal (Bruford and Wayne, 1993). Microsatellites can be used to determine the identification of species, individuals, gender, parentage and population structure (Waits and Paetkau, 2005). The use of microsatellites has been applied to genetic studies of a wide range of species including large mammals such as chimpanzees *Pan troglodytes* (e.g. Morin *et al*., 1994), San Joaquin kit foxes *Vulpes macrotis mutica* (e.g. Bremner-Harrison *et al*., 2006), wolves *Canis lupus* (e.g. Sundqvist *et al*., 2001), and badgers *Meles meles* (e.g. Wilson *et al*., 2003). Microsatellites have also been utilised in studies of small mammals (Moran *et al.*, 2008), including shrew species belonging to the genus *Sorex* (e.g. Matsubara *et al*., 2001; Basset and Hausser, 2003).

One of the main advantages to using microsatellites is that they are the only molecular marker that can be used when utilising small amounts of DNA (Bruford and Wayne, 1993). Microsatellite analysis is typically undertaken using Polymerase Chain Reaction (PCR) techniques to amplify a specific region of DNA including a region of tandem repeats. Before the region of DNA can be amplified the flanking regions of each microsatellite are identified, and primer sets (specific invariant sequences corresponding to the flanking regions) are designed whereby the PCR reaction amplifies the microsatellite region of study (Beebee and Rowe, 2004). For most vertebrate taxa including mammals, a single pair of primers will amplify for every individual as the regions of DNA flanking the repeat are generally conserved within a species (McGregor and Peake, 1998).

Another advantage of using microsatellites as molecular markers is that they are the most polymorphic markers per locus and therefore provide the highest discriminating power to differentiate between individuals (Bruford and Wayne, 1993; Anderson *et al.*, 2006). However, in mammals, new primer sets usually have to be developed specifically for each study species, although occasionally a subset of primer sets will amplify in closely related species such as individuals within the same genus (e.g. Naitoh *et al.*, 2002) or family (e.g. Wyttenbach *et al*., 1997). Recent methods have included

utilising sequenced genomes to create conserved microsatellite marker sets suitable for genotyping a wide range of species from different families, enabling comparisons between species (Dawson *et al*., 2010). For example, conserved markers have been developed using this approach for passerine birds (Dawson *et al*., 2010) and Vespertilionidae bats (Jan *et al*., 2012).

The number of loci needed to identify individuals varies depending on the locus, study species and purpose of study. According to Mills *et al*. (2000), in order to be useful in population size estimations, genetic profiles should consist of enough microsatellite loci to distinguish between individuals with 99% certainty. Estimating the required number of loci can be achieved by computing probability of identity statistics. However, most studies use between seven and twelve microsatellite loci for estimating population sizes using individual identity in mammals (e.g. Eggert *et al*., 2003; Frantz *et al*., 2003).

6.1.3 Sampling techniques

The most commonly used minimally-invasive sampling techniques currently used in wild animals for DNA profiling are hair and faecal sampling (see Waits and Paetkau, 2005). DNA profiling using hair samples has been successfully used in a number of species including sex determination in pandas *Ailuropoda melanoleuca* (Durnin *et al.*, 2007) and otters (Anderson *et al.*, 2006), and estimating social group size in badgers (Frantz *et al*., 2004; Scheppers *et al.*, 2007). Faecal DNA profiling has been used to study species including mammalian carnivores that are difficult to survey using traditional techniques such as live-trapping (Ruell and Crooks, 2007). For example, the technique has been used for determining individual identity, sex and abundance in badgers (Wilson *et al*., 2003; Frantz *et al.*, 2006) and otters (Arrendal *et al.*, 2007; Lampa *et al.*, 2008). Recently, both methods have been identified as potentially useful techniques for monitoring small mammal species (Moran *et al.*, 2008), such as water shrews which can be difficult to survey and trap due to their elusive nature.

It is possible to collect hair samples from small mammals for DNA profiling remotely using hair tubes which have a sticky membrane to pluck a number of hairs from an organism (Moran *et al.*, 2008). However, this method is not ideal for sampling water shrews due to their short dense fur which would be unlikely to stick or be removed using such apparatus. Furthermore, plucking the 25 hairs necessary for obtaining sufficient DNA (Henry *et al*., 2011) directly from live-trapped water shrews may be possible but it is not clear how much stress this may cause the animal. Furthermore, DNA profiling of faecal samples is a particularly useful technique for species which use latrines or leave obvious droppings such as Canids (e.g. Paxinos *et al*., 1997) or primates (e.g. Morin *et al*., 1994). However, in species such as the water shrew, which leave few field signs, faecal sampling can only be undertaken using bait tubes or live-trapping techniques (see Chapter 5). Therefore, although DNA extraction from small mammal faeces is possible (Vege and McCracken, 2001; Zeale *et al*., 2011), nuclear DNA extraction from water shrew faecal samples is yet to be perfected (Moran *et al.*, 2008).

Minimally-invasive DNA profiling using buccal swabs is an alternative, reliable method of sampling individuals, unlike large scale hair and faecal sampling where the DNA from many individuals might be present in each sample, requiring only a small amount of biological material (e.g. epithelial cells) (Seki, 2003; Poschadel and Möller, 2004; Broquet, 2007; Yannic *et al*., 2011). The technique has been used extensively in humans (e.g. Thomson *et al*., 1992) and has more recently been applied to a number of other species, for example laboratory mice (Mitrečić *et al.*, 2008), birds (Seki 2003; Handel *et al.*, 2006; Yannic *et al*., 2011), reptiles (Poschadel and Möller, 2004; Miller, 2006) and amphibians (Pidancier *et al.*, 2003; Poschadel and Möller, 2004; Broquet *et al*., 2007). Although the method has been employed in wild mammals such as bonobos *Pan paniscus* (Hashimoto *et al*., 1996), genetic identification using buccal swabs has yet to be used in wild small mammals.

6.1.4 Limitations and sources of genotyping error

Minimally-invasive DNA sampling methods such as hair, faecal and buccal swab collection often contain small quantities of DNA and/or degraded DNA (Yannic *et al*., 2011). For example, studies assessing buccal swabbing in birds have produced yields ranging from 1.4mg/extraction at concentrations of 2.7 ± 3.69 ng/µl (Handel *et al*., 2006) to 1.8-2.4 mg/extraction at concentrations of 11.76 ± 18.10 ng/µl (Yannic *et al*., 2011), whereas blood sampling from the same species produced yields ranging from 3.3-4.4mg/extraction at concentrations of 22.05 ± 7.59 ng/µl (Yannic *et al*., 2011) to 129mg/extraction at concentrations 257 ± 202 ng/µl (Handel *et al*., 2006). The small quantities of DNA obtained via minimally-invasive DNA sampling methods can consequently make it difficult to obtain reliable genotypes of individuals due to lack of amplification for some individuals, allelic dropout and the occurrence of genotyping errors (Taberlet *et al*., 1996).

Genotyping errors such as allelic dropouts, false alleles and contaminants occur due to the sensitive nature of PCR techniques when using small quantities of DNA. Allelic dropouts arise when one allele of a heterozygous individual is not amplified during a PCR, leading to the retyping of the individual as a homozygote (Taberlet *et al*., 1996); false alleles occur when an miscellaneous artefact allele is generated due to a PCR error; and contaminants arise due to the amplification of DNA present from other species contamination (Miller *et al*., 2002). Of the three genotyping errors, allelic dropout has been reported as the most serious (Gagneux *et al*., 1997a). Genotyping errors have been encountered in a number of microsatellite studies using minimally-invasive DNA sampling techniques (e.g. Gerloff *et al*., 1995; Taberlet *et al*., 1996; Gagneux *et al*., 1997a; Bayes *et al*., 2000; Constable *et al*., 2001) which has consequences such as the miscalculation of population sizes and misidentification of parentage. Most notably, Gagneux *et al*. (1997b) genotyped a number of individual chimpanzees from hair samples to examine female mating strategies. However, in a reanalysis of this study Vigilant *et al*. (2001) found that 10 of

66 alleles and 9 of 33 individuals were incorrectly genotyped, mainly due to allelic dropout (Gagneux *et al*., 2001), which consequently affected the original reported mating strategies.

A multiple tubes approach (distributing the DNA extract between several tubes to create multiple repeat PCRs) has been suggested as one method to provide a more reliable genotype (Navidi *et al*., 1992; Taberlet *et al*., 1996). Although Taberlet *et al*. (1996) recommend seven multiples for homozygous and three for heterozygous genotypes with at least 5U template DNA/locus (~35 pg in mammals), more recently Bayes *et al*. (2000) used only three per homozygous and two per heterozygous genotype.

Although DNA yields from buccal swabs are low in comparison to those from blood or tissue samples (Seki, 2003; Handel *et al*., 2006; Yannic *et al*., 2011), they have been found to be sufficient in terms of quantity and quality for molecular studies using PCR techniques (Seki, 2003; Poschadel and Möller, 2004; Handel *et al*., 2006; Miller, 2006; Broquet *et al*., 2007; Yannic *et al*., 2011). Indeed, Gagneux *et al*. (1997) suggest allelic dropout only becomes a problem when the DNA concentration in the PCR reaction falls below 0.005ng/µl. Thus, probably due to the higher amounts of DNA in the PCR, previous studies utilising buccal swabs have found little evidence of allelic dropout (Miller, 2006; Broquet *et al*., 2007; Yannic *et al*., 2011) and little or no evidence of false alleles (Broquet *et al*., 2007; Yannic *et al*., 2011). Certainly, genotyping errors appear more prevalent in samples such as hair, faecal and shed feather samples which may be subject to environmental factors, causing DNA degradation (Waits and Paetkau, 2005; Gagneux *et al*., 2001), unlike buccal swabs which are retained in storage vials immediately. Consequently, Yannic *et al*.'s (2011) study of genotyping accuracy revealed that buccal swabs produced particularly reliable results with a quality index of 0.998 for genotyping performance, thus requiring only two repetitions for 100% genotyping accuracy. This is in accordance with Broquet *et al*.'s (2007) study reporting 99.65% accuracy with two repetitions and 100% accuracy with three.

6.1.5 Estimating abundance

Reliable estimates of population size are necessary to assess the conservation status of a population or species (Rosenberg *et al*., 1995; Gregory and Gaston, 2000; Magurran and Henderson, 2003), yet censusing a population can be difficult, especially in species that are small and elusive such as the water shrew (Aybes and Sargent, 1997; Churchfield *et al*., 2000; Greenwood *et al*., 2002). In order get an accurate estimation of abundance individuals need to be identified. Many field research studies have used PIT tagging for identification of animals including mammals such as squirrels (*Urocitellus townsendii*), voles (*Microtus spp*.) and badgers (*Meles meles*) (Schooley *et al.*, 1993; Harper and Batzli, 1996; Rogers *et al.* 2002); birds (Ballard *et al.*, 2001); reptiles (Mills *et al*., 1995); amphibians (Perret and Joly, 2002) and invertebrates (Pengilly and Watson, 1994). The use of PIT tagging to uniquely identify individual water shrews was considered. However, water shrews lack distinct loose skin between the shoulder blades, the ideal place for PIT tags to be implanted (Rathbun and Rathbun, 2006). In addition, unlike mice and voles, which are generally easy to handle when caught as they remain fairly still, shrews often wriggle a great deal. This movement during capture makes the implantation of a PIT tag extremely difficult without some sort of sedation which is not practical in the field (S. Churchfield, pers. comm.).

The recent application of minimally-invasive genetic sampling to abundance estimates, whereby the number of animals in a population can be estimated from the number of individually distinct genetic profiles, has furthered conservation research. Estimates of population abundance determined via genotyping hair and faecal samples have been obtained for a number of species including Eurasian badgers (Frantz *et al*., 2003; Wilson *et al*., 2003; Frantz *et al*., 2004) forest elephants *Loxodonta cyclotis* (Eggert *et al*., 2003) and grey wolves (Creel *et al*., 2003) using mark-recapture or rarefaction techniques.

6.1.6 Non-invasive sampling of water shrews

Genetic identification of small mammals from non-invasive samples has been identified as a potentially useful monitoring technique for species that are difficult to survey using other methods (Battersby and Greenwood 2004), and accurate estimates of water shrew populations are required as the species is of conservation concern. Although DNA has previously been extracted from hair and faecal samples in water shrews (Moran *et al*., 2008) population estimates are difficult to acquire from these non-invasive sampling methods as an individual is not physically confined at any one time so may leave several hair tufts or scats at many locations, creating multiple observations of an individual (Miller *et al*., 2005). As buccal swabbing produces single observations of individuals it can provide reliable population estimates, however it does involve the actual capture of individuals.

Genomic DNA has yet to be extracted from the buccal swabs of water shrews or used for individual identification. Therefore, to evaluate this DNA sampling approach and assess if suitable for genotyping, buccal swabs were taken from live-trapped water shrews. The quantity and quality of the genotype data obtained was assessed for use in individual identification and for the estimation of population sizes.

Water shrews, like all shrews, have internal sex organs making sexing in the field difficult (Churchfield, 1990). Therefore, any new loci identified were assessed for sex-linkage to check if useful for determining the gender of genotyped water shrews. The utility of a published Sorex shrew Y-linked marker was also assessed for sex-typing water shrews.

6.1.6.1 Availability of published microsatellite markers suitable for genotyping water shrews

Currently, based on a search of the EMBL and GenBank sequence databases, no nuclear microsatellite markers are available specifically for the water shrew or any species within the genus *Neomys*. However,

microsatellite markers are available for the closely related genus *Sorex* (e.g. Wyttenbach *et al*., 1997), belonging to the same sub-family (*Soricinae*), some of which have been shown to cross-amplify in other shrew species of the same genus. For example, *S. unguiculatus* primers amplify in *S. caecutiens* (Naitoh *et al*., 2002), however Wyttenbach *et al.* (1997) found cross-amplification between genera to be unsuccessful in shrews, with no primer sets designed from *Sorex* sequences amplifying in the genus *Crocidura* (white-toothed shrews) despite similar divergence times between the genera (Repening, 1967).

6.1.6.2 Availability of published markers for sex-typing water shrews

Although there are no primers currently available specifically for determining sex in the water shrew or other Neomys species, a Y-linked primer set is available which was isolated from a related Sorex species (*SRY* HMG box, Matsubara *et al*., 2001). The *SRY* HMG box (hereby referred to as *SRY*) primer set designed from the *S. unguiculatus* sequence (Matsubara *et al*. 2001) has previously been shown to amplify and be Y-linked in some *Sorex* species although it has not been tested specifically in common and pygmy shrews. However, Matsubara *et al*. (2001) reported amplification of a faint product in males when tested on male and female *C. suaveolens*, indicating its potential for cross-amplification in other species. The limitation of using a Y-linked marker is that it will only amplify a product in males and not females. Consequently, it is not possible to verify whether an individual that does not amplify products is truly a female or is merely a sample that failed to amplify.

6.1.7 Aim and objectives

The aim of this part of the study is to estimate numbers of live-trapped water shrews using DNA sampling to identify individuals. The objectives are to:

- Collect DNA samples from live-trapped water shrews using buccal swabs

- Evaluate buccal swab sampling as a method for obtaining DNA from water shrews
- Identify individual water shrews using genetic profiling
- Estimate water shrew abundance using genetic profiling

6.2 Methods

6.2.1 Live trapping

During the live trapping surveys at Ash Spinney Pond, Hockerton Pond, Twenty-Acre Piece and Sheepwalks Pond (see Chapter 5) all caught water shrews had buccal swabs taken to identify individuals through genomic DNA profiling techniques. Additionally, swab samples were acquired from individuals at a fifth site (Whatton Brook) to ensure sufficient individuals were genotyped in order to assess if loci exhibited Hardy-Weinberg equilibrium and to estimate null allele frequencies. Trapping was undertaken during a 7 day trapping session using methods previously described (see Chapter 5). Whatton Brook was selected on the basis of having water shrew presence and convenience of the location.

6.2.2 Microsatellite library creation

A microsatellite-enriched genomic water shrew library was created by the Natural Environment Research Council (NERC) Bimolecular Analysis Facility (NBAF) – Sheffield, The University of Sheffield. The method used was that of Armour *et al*. (1994) but without the pre-enrichment PCR and utilising magnetic beads during the enrichment (Glenn and Schable, 2005). The library was created from one female water shrew found dead at the Hockerton study site which had been stored in a freezer at -80^{o}C. Upon dissection, the presence of a uterus confirmed the corpse was female (dissection was performed by a vet) Genomic DNA was extracted from the brain tissue using an ammonium acetate protocol (Nicholls *et al.,* 2000) and digested with the restriction enzyme *Mbo*I (Promega) overnight at 37°C.

The linkers (*Sau*-L-A and *Sau*-L-B; Royle *et al.,* 1992) were annealed together and ligated to the DNA fragments that had been size-selected (250-750 bp) on a 1.5% agarose gel stained with ethidium bromide. The restriction fragments were enriched for the following di- and tetra-nucleotide microsatellite motifs separately and their complements: $(GT)_n$, $(CT)_n$, $(GTAA)_n$, $(CTAA)_n$, $(TTTC)_n$ and $(GATA)_n$; which had been bound to magnetic beads (following Glenn and Schable, 2005). Enriched DNA was amplified via PCR using the *Sau*-L-A linker as the primer. Amplified DNA was then cloned using the TOPO TA Cloning Kit (Invitrogen) according to the manufacturer's instructions. Clones were sequenced in both directions (using ABI BigDye v3.1 and analysed on an ABI 3730 DNA analyser) at the NBAF – Edinburgh, The University of Edinburgh. A consensus sequence was created from which the primer sets were designed. The water shrew primers were designed using PRIMER3 software (Rozen and Skaletsky, 2000) at NBAF – Sheffield. Whenever possible conserved primer sets were developed which were designed to be a consensus between the water and common shrew. This was performed in order to enhance cross-species utility (method modified from that of Dawson *et al.* 2010; Dawson, D.A. unpublished data). Sequences suitable for the design of conserved primer sets were identified based on their sequence similarity to those of the common shrew (Dawson, D.A. unpublished data). These sequences were aligned against their homologous common shrew sequence (obtained from the ENSEMBL common shrew assembly sequence; ENSEMBL, 2010) and primer sequences were designed to be as consensus as possible between the two shrew species (Dawson, D.A. unpublished data).

6.2.3 Sample collection and storage

Buccal cells were collected from each water shrew using a new cotton-tipped swab (Technical Service Consultants Mini Tip Plain Swab) by gently scraping the inner cheeks for approximately 10 seconds (see Figure 6.2). Swabs from assumed different individuals were labelled numerically and those swabs from what were thought to be the same individual were also alphabetised (e.g. 1a,b,c etc.). The water shrews aggressively bit the swabs

during sampling which made inserting the swab and obtaining the buccal cells straightforward as their mouths were already open. Buccal swabs collected during autumn/winter 2007 live-trapping were air dried for 5 minutes in the field and then replaced in their individual plastic collection tubes and stored at room temperature for approximately two years until extraction. Buccal swabs collected in subsequent live-trapping sessions had their tips removed, using scissors cleaned with a 10% bleach solution, and while still moist placed in rubber-sealed screw-topped microfuge tubes containing 1.5ml of absolute ethanol (Analytical Reagent grade) immediately following collection and stored at room temperature until extraction.

Figure 6.2 A water shrew having a buccal swab sample taken.

6.2.4 Assessment of DNA extracted from buccal swabs

Before genomic DNA was extracted from the water shrew buccal swabs, a number of extraction techniques were tested using additional swabs from a range of other species (mouse *Mus mus*, zebra finch *Taeniopygia guttata,* ferret *Mustela putorius furo,* dog *Canis lupus familiaris* and human *Homo sapiens*). These were stored in various ways, to assess which storage method resulted in the best quality and quantity of DNA following

extraction. Mice were used as they have a similar mouth size to water shrews and therefore a similar number of buccal cells were expected to be collected, zebra finches, dogs and ferrets were used as positive controls, as DNA has already been successfully extracted from mouth swabs of these species (G.J.Horsburgh pers. comm; Chang *et al*., 2007; Cain *et al*., 2011, respectively), and humans were used which included those sampling the water shrews and assisting the lab work to provide an additional check for sample contamination (see Table 6.1 for details of samples and storage methods).

Table 6.1 Details of buccal swab samples and storage methods.

Sample	Species	Sex	Storage method
1	Ferret	Female	Air
2	Dog	Female	Moist
3	Ferret	Female	Moist
4	Ferret	Female	Air
5	Zebra finch chick	Unknown	Moist
6	Zebra finch chick	Unknown	Air
7	Zebra finch chick	Unknown	Air
8	Mouse	Male	Air
9	Mouse	Female	Air
10	Mouse	Female	Moist
11	Mouse	Female	Moist
12	Mouse	Female	Moist
13	Human	Female	70% Ethanol
14	Human	Female	99% Ethanol
15	Mouse	Male	99% Ethanol
16	Mouse	Unknown	70% Ethanol

The three storage methods used were moist (whereby the swab was replaced immediately back into its plastic collection tube whilst moist without any buffer), an air dried method (whereby the swab was air dried for 5 minutes in the field and then replaced back into its plastic collection tube without any buffer) and an ethanol preservation method (whereby the swab was air dried for approximately 5 minutes before its tip was removed, using clean scissors, and transferred to a rubber-sealed screw-topped microfuge tube containing either 70% or absolute ethanol (analytical reagent grade)).

The three extraction methods tested were the QIAamp DNA Mini Kit (DNA Purification from Buccal Swabs Spin Protocol, February 2003) a sodium chloride extraction method (Mitrečić *et al*., 2008) and a technique which involved incubating the swabs at 100°C in 70 µl ddH_20 for five minutes (to identify if boiling the cells would simply release the DNA by breaking open the cells and denaturing any DNAses, thereby allowing the DNA to be amplified).

Firstly, genomic DNA was checked for amplification, via PCR techniques, by extracting DNA via the QIAamp DNA Mini Kit from the buccal samples. PCRs were undertaken using five different primer sets to ascertain if products were amplified from the samples. The primer sets used were the markers *Z002A* (nuclear; Dawson, 2007), *SRY* (nuclear sex-typing; Matsubara *et al*., 2001) and *LL, CR* and *ND* (mitochondrial), and contained 1µl, 2µl and 5µls of diluted DNA per reaction (see Table 6.2 for PCR conditions used). High quality DNA at a PCR concentration of approximately 10ng/µl from blood (mink) and tissue (water shrew) were used as positive controls and a sterile H_20 sample was used as a negative control. Mitochondrial DNA markers were used to assess if any DNA at all was present since mitochondrial DNA occurs at very high copy numbers compared to nuclear DNA, much lower concentrations of DNA in a PCR will amplify.

Table 6.2 PCR programmes used to amplify products from buccal swabs.

Z002A	***SRY***	***LL, CR* and *ND***
1. 94°C for 3 minutes	1. 94°C for 3 minutes	1. 94°C for 3 minutes
2. 94°C for 30 seconds	2. 94°C for 30 seconds	2. 94°C for 30 seconds
3. 56°C for 30 seconds	3. 60°C for 30 seconds	3. 49°C for 30 seconds
4. 72°c for 30 seconds	4. 72°c for 30 seconds	4. 72°c for 30 seconds
5. Cycle to Step 2 for 34 more times	5. Cycle to Step 2 for 29 more times	5. Cycle to Step 2 for 34 more times
6. 72°C for 10 minutes	6. 72°C for 30 minutes	6. 72°C for 10 minutes
		7. Incubate at 10°C for 10 seconds

Secondly, ten further samples were checked for amplification by extracting DNA from swabs via the sodium chloride and boiling methods (see Table 6.3 for details). PCRs were again undertaken, but only using two of the five primer sets (*Z002A* and *LL*), and again contained 1µl, 2µl and 5µls of DNA per reaction, thereby allowing an assessment to be made of the different extraction techniques, storage methods and amounts of DNA. The same positive and negative controls were used.

A volume of 10ul of each PCR reaction was loaded onto a 0.8% agarose gel and run at 110 V for 1 hour. Following Frantz *et al*. (2003), amplifications were deemed successful if a PCR product was present, even if the genotype may not have been reliable.

Table 6.3 Details of buccal swab samples, storage and extraction methods.

Sample	Species	Sex	Storage method	Extraction method
5E	Mouse	Female	Air	Boiling
7E	Mouse	Unknown	70% Ethanol	Boiling
9E	Mouse	Male	Absolute Ethanol	Boiling
1E	Human	Female	70% Ethanol	Boiling
2E	Human	Female	Absolute Ethanol	Boiling
6E	Mouse	Female	Control	Sodium Chloride
8E	Mouse	Unknown	70% Ethanol	Sodium Chloride
10E	Mouse	Male	Absolute Ethanol	Sodium Chloride
3E	Human	Female	70% Ethanol	Sodium Chloride
4E	Human	Female	Absolute Ethanol	Sodium Chloride

6.2.5 Assessment of primer sets

All of the water shrew primer sets were initially checked for amplification using 21 tissue samples from common, pygmy and water shrews (extracted using an ammonium acetate protocol; Nicholls *et al.,* 2000; see Table 6.4 for details). DNA amounts were quantified on a BMG – Fluostar Optima fluorometer and ranged from 0.82-14.3 µg at concentrations of 5.49 to 95.59 ng/µl (mean ± SE = 56.86 ± 10.90). PCRs were undertaken using 2µl of DNA with 25 primer sets (see Table 6.5) and a touchdown PCR program was used for all loci (see Table 6.6).

Table 6.4 Details of tissue samples used for optimisation of the new microsatellite primer sets.

ID code	Species	Sample type	Sex (dissection)	Collection location	Sample provided by
DJR22	Common shrew	Liver	Male	Grindleford, Derbys.	Douglas Ross
DJR22	Common shrew	Kidney	Male	Grindleford, Derbys.	Douglas Ross
DJR25	Pygmy shrew	Liver	Female	Grindleford, Derbys.	Douglas Ross
DJR26	Pygmy shrew	Liver	Male	Grindleford, Derbys.	Douglas Ross
DJR21	Pygmy shrew	Kidney	Male	Grindleford, Derbys.	Douglas Ross
DJR21	Pygmy shrew	Liver	Male	Grindleford, Derbys.	Douglas Ross
DJR20	Common shrew	Liver	Female	Grindleford, Derbys.	Douglas Ross
DJR23	Pygmy shrew	Kidney	Male	Grindleford, Derbys.	Douglas Ross
DJR24	Pygmy shrew	Liver	Male	Grindleford, Derbys.	Douglas Ross
WS1T	Water shrew	Tail	No body available	Pembrokeshire	Jeremy Searle
WS1M	Water shrew	Muscle	No body available	Anglesey	Jeremy Searle
WS1MT	Water shrew	Muscle	No body available	Unknown	Unknown
WS2T	Water shrew	Tail	No body available	Sweden	Jeremy Searle
PS4L	Pygmy shrew	Liver	Not sexed	Unknown	Unknown
PS1i	Pygmy shrew	Unknown	Male	Unknown	Anna Bone
PS2i	Pygmy shrew	Unknown	Male	Unknown	Anna Bone
CS1i	Common shrew	Unknown	Female	Unknown	Anna Bone
CS2i	Common shrew	Unknown	Female	Unknown	Anna Bone
WS43	Water shrew	Unknown	No body available	Unknown	Anna Bone
WS45	Water shrew	Unknown	No body available	Unknown	Anna Bone
WSBF1	Water shrew	Tail	Female	Kegworth, Derbys.	Anna Champneys

Table 6.5 Details of 24 microsatellite loci and a Y-linked locus assessed for the genotyping of three shrew species.

Locus	Source Species	Clone name	EMBL accession number	Primer set name	Homology of primer sequences between water and common shrew	Repeat motif in source species	Selected to genotype water shrew swab samples	Locus reference
NFo010	*N. fodiens*	WS39B10	FM957164	Nfo010-sorex	Part	$(TG)_{22}$	Yes	This study
NFo016	*N. fodiens*	WS39D06b	FM957170	Nfo016-sorex	Part	$(AG)_{5}$	Yes	This study
NFo026	*N. fodiens*	WS39F06	FM957180	NFo026	Low/none	$(TTCT)_{33}$	No	This study
NFo030	*N. fodiens*	WS39G06	FM957184	Nfo030-sorex	Part	$(TG)_{9}$	Yes	This study
NFo031	*N. fodiens*	WS39G07	FM957185	Nfo031	Low/none	$(TAGA)_{14}$	Yes	This study
NFo037	*N. fodiens*	WS39H08	FM957191	NFo037	Low/none	$(GT)_{19}$	No	This study
NFo041	*N. fodiens*	WS40A05	FM957195	Nfo041-sorex	Part	$(TG)_{14}$	Yes	This study
NFo043	*N. fodiens*	WS40A08	FM957197	Nfo043-sorex	Part	$(TG)_{22}$	Yes	This study
NFo045	*N. fodiens*	WS40A10	FM957199	Nfo045-sorex100	100%	$(GT)_{11}$	Yes	This study
NFo046	*N. fodiens*	WS40A11	FM957200	Nfo046-sorex100	100%	$(AG)_{20}$	Yes	This study
NFo047	*N. fodiens*	WS40A12	FM957201	Nfo047-sorex100	100%	$(CT)_{5}$	Yes	This study
NFo055**	*N. fodiens*	WS40C02	FM957209	Nfo055-sorexdd	Part	$(TG)_{26}$	Yes	This study
NFo055**	*N. fodiens*	WS40C02	FM957209	Nfo055	Low/none	$(TG)_{26}$	Yes	This study
NFo068	*N. fodiens*	WS40E02	FM957222	Nfo068-sorex	Part	$(CA)_{21}$	Yes	This study
NFo070	*N. fodiens*	WS40E06	FM957224	Nfo070-sorex100	100%	$(GT)_{25}$	Yes	This study
NFo072	*N. fodiens*	WS40E12	FM957226	Nfo072-sorex	Part	$(AG)_{14}$	Yes	This study
NFo073	*N. fodiens*	WS40F01	FM957227	Nfo073-sorex	Part	$(TG)_{18}$	Yes	This study
NFo074	*N. fodiens*	WS40F03	FM957228	Nfo074	Low/none	$(GA)_{26}$	Yes	This study
NFo086	*N. fodiens*	WS41B05	FM957240	Nfo086	Low/none	$(GA)_{15}$	Yes	This study
NFo098	*N. fodiens*	WS41D05	FM957252	NFo098-sorex	Part	$(AG)_{19}$	No	This study
NFo120	*N. fodiens*	WS41H12	FM957274	Nfo0120	Low/none	$(AG)_{10}$	Yes	This study
L9	*S.araneus*	-	U82711	L9	Unknown	$(CA)_{13}$	Yes	Wyttenbach *et al*. 1997
L67	*S.araneus*	-	U82716	L67	Unknown	$(GT)_{17}$	Yes	Wyttenbach *et al*. 1997
L68	*S.araneus*	-	AF032913	L68	Unknown	$(CA)_{11}$	Yes	Balloux, *et al*. 1998
SRY (Y-Linked)	*S. unguiculatus*	-	AB055219	SRY	Unknown	-	Yes	Matsubara *et al*. 2001

**Note: for locus *Nfo055* two different primer sets were tested.

Table 6.6 Touchdown PCR programme used to amplify products in common, pygmy and water shrew DNA.

1. 95°C for 15 minutes
2. 94°C for 30 seconds
3. 65°C for 90 seconds

Decrease by 1°C every cycle

4. 72°C for 60 seconds
5. Cycle to Step 2 for 10 more times
6. 94°C for 30 seconds
7. 55°C for 90 seconds
8. 72°C for 60 seconds
9. Cycle to Step 6 for 25 more times
10. 72°C for 10 minutes

6.2.6 Buccal swab DNA profiling

Following optimisation of the primers, genomic DNA was extracted from 36 water shrew buccal swabs (13 stored in absolute ethanol and 23 stored air-dried with no buffer) using the QIAamp DNA Mini Kit. DNA amounts were very low when quantified on a BMG – Fluostar Optima fluorometer and ranged from 59-119ng at concentrations of 0.39 to 0.79 ng/µl (mean = 0.48 ± 0.01). As well as the water shrew buccal swab samples, a number of controls were used; water shrew tissue (from the UK and Sweden) pygmy shrew tissue, mouse buccal swab samples, and common shrew tissue and buccal samples (positive controls), human buccal swab samples (to check for human contamination), a field vole tissue sample (to check if any of the primers had high cross-species amplification potential), and sterile H_20 (negative control) (see Table 6.7 for details). DNA concentrations of the control samples when used in the PCR reaction ranged from 700-4661ng at concentrations of 4.67 to 31.07 ng/µl (mean = 19.94 ± 4.09).

PCRs were undertaken for all samples using 23 selected primer sets (Table 6.8). For the buccal samples 3µl volumes of DNA were used and for the tissue samples 1µl was used. Each 2µl PCR contained a maximum of 3ng (swabs) to 31ng (controls) of lyophilised genomic DNA, 0.2µM of each

primer and 1µl QIAGEN multiplex PCR mix (QIAGEN Inc.; Kenta *et al.*, 2008). PCR amplification was performed using a DNA Engine Tetrad 2 thermal cycler (MJ Research, Bio-Rad, Hemel Hempstead, Herts., UK) with the previously stated touchdown program. Nine loci were selected which displayed the best amplification across the swab samples (see Table 6.8) and the PCRs of these loci repeated to obtain a consensus genotype for individual identification. This repeat PCR data will also be used to quantify estimated null allele frequencies, alleleic dropout and genotyping errors caused by scoring errors.

Amplified products were loaded on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, California, USA) and allele sizes were assigned using GENEMAPPER v3.7 (Applied Biosystems, California, USA).

6.2.7 Data analysis

Observed and expected heterozygosities, estimates of allelic diversity and estimated null allele frequencies were calculated using CERVUS v3.0.3 (Marshall *et al*., 1998; Kalinowski *et al*., 2007). Tests for departures from Hardy-Weinberg equilibrium were conducted using a Markov-chain method implemented in GENEPOP v4.0.10 (Rousset, 2008). Genotyping errors (mean allelic dropouts and false alleles) were estimated at 0.005 using PEDANT v1.0 (Johnson and Haydon, 2007) with 10 000 search steps.

Individual identities were conducted using the CERVUS v3.0.3 (Marshall *et al*., 1998) identity analysis tool. Since the DNA obtained was of a low concentration, alleleic dropout was high (Table 6.12). Therefore, the over-estimation of individual numbers was avoided as much as possible by selecting the "fuzzy alleles" option on CERVUS and allowing a minimum of three identical loci per genotype, although all mismatches were checked by eye. Differences between homozygote individuals were disregarded and assumed to be a result of alleleic dropout. Unique individuals were identified using an exclusion-based approach. Individuals were only assigned as

Table 6.7 Details of water shrew buccal swab samples plus associated controls.

Sample	Species	Date	Site sampled	Storage method	Comments
AS1	Water shrew	23/09/2007	Ash Spinney Pond	Air	
AS2	Water shrew	23/09/2007	Ash Spinney Pond	Air	
AS2A	Water shrew	27/09/2007	Ash Spinney Pond	Air	
AS2B	Water shrew	27/09/2007	Ash Spinney Pond	Air	
H3	Water shrew	24/10/2007	Hockerton Pond	Air	
H3A	Water shrew	25/10/2007	Hockerton Pond	Air	Took 2 swabs H3A=H3B
H3B	Water shrew	25/10/2007	Hockerton Pond	Air	Took 2 swabs H3A=H3B
H3C	Water shrew	25/10/2007	Hockerton Pond	Air	
H3D	Water shrew	27/10/2007	Hockerton Pond	Air	
H4	Water shrew	03/06/2008	Hockerton Pond	Absolute Ethanol	Looked pregnant
H4A	Water shrew	04/06/2008	Hockerton Pond	Absolute Ethanol	
H4B	Water shrew	05/06/2008	Hockerton Pond	Absolute Ethanol	Nipple patches
H4C	Water shrew	08/06/2008	Hockerton Pond	Absolute Ethanol	Took 2 swabs H4C=H4D
H4D	Water shrew	08/06/2008	Hockerton Pond	Absolute Ethanol	Took 2 swabs H4C=H4D
H5	Water shrew	03/06/2008	Hockerton Pond	Absolute Ethanol	
H5A	Water shrew	04/06/2008	Hockerton Pond	Absolute Ethanol	
H6	Water shrew	19/10/2008	Hockerton Pond	Absolute Ethanol	
H6A	Water shrew	21/10/2008	Hockerton Pond	Absolute Ethanol	
H7	Water shrew	15/05/2009	Hockerton Pond	Absolute Ethanol	Looked pregnant
SP8	Water shrew	04/12/2007	Sheepwalks Pond	Air	
SP9	Water shrew	06/12/2007	Sheepwalks Pond	Air	
SP10	Water shrew	28/05/2008	Sheepwalks Pond	Absolute Ethanol	Took 2 swabs SP10=SP10A
SP10A	Water shrew	28/05/2008	Sheepwalks Pond	Absolute Ethanol	Took 2 swabs SP10=SP10A
SP10B	Water shrew	29/05/2008	Sheepwalks Pond	Absolute Ethanol	
TAP11	Water shrew	14/11/2007	Twenty-Acre Piece	Air	
TAP11A	Water shrew	15/11/2007	Twenty-Acre Piece	Air	
TAP11B	Water shrew	16/11/2007	Twenty-Acre Piece	Air	
TAP11C	Water shrew	19/11/2007	Twenty-Acre Piece	Air	
WB12	Water shrew	23/09/2007	Whatton Brook	Air	
WB13	Water shrew	07/10/2007	Whatton Brook	Air	
WB13A	Water shrew	08/10/2007	Whatton Brook	Air	
WB13B	Water shrew	11/10/2007	Whatton Brook	Air	
WB14	Water shrew	07/10/2007	Whatton Brook	Air	
WB14A	Water shrew	11/10/2007	Whatton Brook	Air	
WB15	Water shrew	08/10/2007	Whatton Brook	Air	
WB15A	Water shrew	11/10/2007	Whatton Brook	Air	
CSA	Common shrew	19/11/2007	-	Air	
CSE	Common shrew	29/05/2008	-	Absolute Ethanol	
M70	Mouse	29/06/1905	-	70% Ethanol	
M100	Mouse	29/06/1905	-	Absolute Ethanol	
Anna	Human	02/07/1905	-	Absolute Ethanol	
WSTC1	Water shrew		Hockerton		Tissue sample
WSTC2	Water shrew		Hockerton		Tissue sample
WSL1	Water shrew		-		Tissue sample
WS92	Water shrew		Sweden		Tissue sample
CS3i	Common shrew		Grindleford		Tissue sample
PS3i	Pygmy shrew		Grindleford		Tissue sample
VTC1	Field vole		Grindleford		Tissue sample

Table 6.8 Optimised primer details used for individual identification.

Locus	Clone name	EMBL accession number	Primer set name	Repeat motif in source species	Locus reference
NFo010	WS39B10	FM957164	Nfo010-sorex	$(TG)_{22}$	This study
NFo016	WS39D06b	FM957170	Nfo016-sorex	$(AG)_{5}$	This study
NFo030	*WS39G06*	*FM957184*	*Nfo030-sorex*	*$(TG)_{9}$*	*This study*
NFo031	WS39G07	FM957185	Nfo031	$(TAGA)_{14}$	This study
NFo041	*WS40A05*	*FM957195*	*Nfo041-sorex*	*$(TG)_{14}$*	*This study*
NFo043	*WS40A08*	*FM957197*	*Nfo043-sorex*	*$(TG)_{22}$*	*This study*
NFo045	WS40A10	FM957199	Nfo045-sorex100	$(GT)_{11}$	This study
NFo046	WS40A11	FM957200	Nfo046-sorex100	$(AG)_{20}$	This study
NFo047	WS40A12	FM957201	Nfo047-sorex100	$(CT)_{5}$	This study
*NFo055***	*WS40C02*	*FM957209*	*Nfo055-sorexdd*	*$(TG)_{26}$*	*This study*
NFo055**	WS40C02	FM957209	Nfo055	$(TG)_{26}$	This study
NFo068	*WS40E02*	*FM957222*	*Nfo068-sorex*	*$(CA)_{21}$*	*This study*
NFo070	WS40E06	FM957224	Nfo070-sorex100	$(GT)_{25}$	This study
NFo072	WS40E12	FM957226	Nfo072-sorex	$(AG)_{14}$	This study
NFo073	*WS40F01*	*FM957227*	*Nfo073-sorex*	*$(TG)_{18}$*	*This study*
NFo074	WS40F03	FM957228	Nfo074	$(GA)_{26}$	This study
NFo086	*WS41B05*	*FM957240*	*Nfo086*	*$(GA)_{15}$*	*This study*
NFo120	*WS41H12*	*FM957274*	*Nfo0120*	*$(AG)_{10}$*	*This study*
L9	-	U82711	L9	$(CA)_{13}$	Wyttenbach *et al*. 1997
L67	-	U82716	L67	$(GT)_{17}$	Wyttenbach *et al*. 1997
L68	-	AF032913	L68	$(CA)_{11}$	Balloux, *et al*. 1998
SRY (Y-Linked)	-	AB055219	SRY	-	Matsubara et al. 2001
Z-002A	confidential	confidential	confidential	confidential	Dawson, 2007

**Note: for locus *Nfo055* two different primer sets were tested.
Primer sets that were used to re-genotype individuals are indicated by italicised font

unique when at least one loci displayed different heterozygotes in two different individuals. In these cases the two individuals were regarded as different unique individuals even when only one allele was different. The "microsatellite toolbox" add-on option of excel (Park, 2001) was also used to assist the identification of unique individuals.

The maximum number of water shrews estimated through live-trapping was determined by the total number of individuals (as determined by fur-clipping) caught during all trapping sessions. The minimum number was determined by calculating the maximum number of water shrews caught per trapping round at a site i.e. only those individuals which were caught at the same time so were undoubtedly different animals.

6.3 Results

6.3.1 Comparison of the methods for extraction of DNA from buccal swabs

PCR products were amplified from all species swabbed, regardless of the size of the species sampled, indicating that obtaining water shrew DNA from buccal swabs was possible.

The 2µl quantity of DNA amplified more PCR products than the 1µl or 5µl quantities. In addition, the 2µl quantity of DNA amplified products for all primers apart from the *SRY* sex-typing primer (which did not amplify any products at all) whereas the 1µl and 5µl quantities of DNA only amplified products for some primers. Unfortunately, the concentrations of DNA used for genotyping were too small to be quantified.

There was a tendency for more products to be amplified from the ethanol and less from the air storage methods. There was also a tendency for more products from the absolute ethanol storage method. In addition, there was a tendency for more products to be amplified from the QIAamp extraction method and less from the sodium chloride extraction method.

6.3.2 Optimisation of primer sets

Twenty two of the twenty five primer sets tested on a range of shrew tissue samples amplified products in at least one species (Table 6.9). All of these primers were found to be polymorphic and displayed between two and sixteen alleles in each species. The Y-linked primer set amplified only homozygotes. Nineteen of the twenty two primers developed specifically for water shrews amplified products. Furthermore, fourteen of the water shrew primers cross-amplified in common and pygmy shrews, and the *Sorex* primer sets *L67* and *SRY*, cross-amplified in water shrews (Table 6.9). The *SRY* Y-linked primer set amplified products in the two *Sorex* species tested (common shrew and pygmy shrew) but not the water shrew. Only males

Table 6.9 Assessment of primer sets optimised in common (CS), pygmy (PS) and water shrews (WS).

Locus	Primer set name	No. samples	% samples amplified	Species amplified in	Observed allele size range (bp)	Expected allele size (bp) based on sequence/species cloned	No. alleles observed in all species	% homozygotes in all species
NFo010	Nfo010-sorex	20	70.00	All	386-408	416	11	14.29
NFo016	Nfo016-sorex	20	70.00	All	345-482	355	6	50.00
NFo026	NFo026	22	0.00	None	-	299	-	-
NFo030	Nfo030-sorex	60	75.00	All	218-244	236	7	11.11
NFo031	Nfo031	12	41.67	WS only	347-365	352	5	0.00
NFo037	NFo037	20	0.00	None	-	320	-	-
NFo041	Nfo041-sorex	20	85.00	All	163-193	178	8	64.71
NFo043	Nfo043-sorex	20	20.00	WS only	247-262	252	5	50.00
NFo045	Nfo045-sorex100	20	80.00	All	357-369	337	6	87.50
NFo046	Nfo046-sorex100	60	65.00	All	252-341	258	16	69.23
NFo047	Nfo047-sorex100	20	70.00	All	240-359	240	9	50.00
NFo055**	Nfo055-sorexdd	20	35.00	All	286-340	299	9	28.57
NFo055**	Nfo055	22	22.73	WS only	212-224	221	5	60.00
NFo068	Nfo068-sorex	20	80.00	All	188-209	191	8	56.25
NFo070	Nfo070-sorex100	40	75.00	All	358-419	365	13	76.67
NFo072	Nfo072-sorex	20	55.00	All	490-502	472	3	90.91
NFo073	Nfo073-sorex	60	75.00	All	107-181	136	14	80.00
NFo074	Nfo074	22	18.18	WS only	220-252	232	7	0.00
NFo086	Nfo086	20	30.00	All	210-256	223	6	50.00
NFo098	NFo098-sorex	20	0.00	None	-	155	-	-
NFo120	Nfo0120	20	20.00	WS only	144-179	179	3	0.00
L9	L9	40	65.00	CS + PS only	128-181-	160	9	30.77
L67	L67	40	65.00	All	72-102	108	5	96.15
L68	L68	40	20.00	All	82-111	88-107	5	75.00
SRY[+]	SRY	9 males 3 females 9 unknown	100.00 33.33 66.67	CS+PS PS WS + PS	153 159 153	155	1 1 1	100.00 100.00 100.00

**Note: for locus *Nfo055* two different primer sets were tested
[+]Note: For locus SRY products were amplified for water shrew samples but they were of unknown sex

and no female common shrews amplified (Table 6.9) supporting the published Y-linked status of this locus in common shrew. However, female (XX) as well as male (XY) pygmy shrews amplified (Table 6.9) indicating that either sample or a primer set mix-up had occurred or that this locus was not sex-linked in pygmy shrews but was autosomal or that the primer set was amplifying a X chromosome amplicon in addition to or instead of the Y chromosome amplicon.

6.3.3 Buccal swab DNA profiling

Nineteen primer sets produced products from the water shrew buccal samples (see Table 6.10). However, *NFo074*, *L9*, *L67* and *L68* failed to amplify although *L67* amplified a product from the water shrew tissue. Only one optimised primer set (*NFo074*) from the water shrew library failed to amplify PCR products, whereas only one of the published common shrew primer sets (*SRY*) amplified. Eighteen of the primers were found to be polymorphic in water shrews and displayed between two and fourteen alleles in a minimum of four individuals (see Tables 6.10 and 6.11). Only one loci did not deviate significantly from Hardy-Weinberg proportions, and many had a high estimate of null allele frequency. Genotyping errors were relatively high (Table 6.12) with estimates of allelic dropout being greater than false alleles.

Individual identification of water shrews was based on nineteen polymorphic loci. However, there was limited amplification for a number of loci (see Tables 6.10 and 6.14) creating incomplete genotypes for most individuals. The number of individuals determined by DNA profiling was seventeen and ranged between one and six individuals at each site, with many individuals being swabbed more than once (Table 6.13).

Table 6.10 Characterisation of 23 water shrew microsatellite loci using DNA extracted from buccal swabs.

Locus	Primer set name	No. samples (no. repeats)	% samples amplified	Observed allele size range (bp)	Expected allele size (bp)	No. alleles observed	Ho	He	P_{HW}	Estimated null allele frequency
NFo010	Nfo010-sorex	36	19.44	409-421	416	4	0.286	0.659	0.032	-
NFo016	Nfo016-sorex	36	38.89	311-350	355	3	0.071	0.204	0.036	0.456
NFo030	Nfo030-sorex	36(2)	88.89	232-246	236	7	0.281	0.695	0.000	0.394
NFo031	Nfo031	36	27.78	346-357	352	4	0.100	0.732	0.000	0.756
NFo041	Nfo041-sorex	36(2)	88.89	168-195	178	11	0.438	0.804	0.000	0.307
NFo043	Nfo043-sorex	36(2)	63.89	239-264	252	9	0.696	0.844	0.000	0.072
NFo045	Nfo045-sorex100	36	19.44	339-369	337	7	0.429	0.813	0.030	-
NFo046	Nfo046-sorex100	36	66.67	246-275	258	14	0.542	0.902	0.000	0.241
NFo047	Nfo047-sorex100	36	61.11	240-242	240	2	0.136	0.333	0.018	0.409
NFo055**	Nfo055-sorexdd	36(2)	55.56	278-298	299	8	0.550	0.819	0.000	0.170
NFo055**	Nfo055	36	61.11	205-226	221	7	0.409	0.801	0.000	0.313
NFo068	Nfo068-sorex	36(2)	77.78	171-202	191	10	0.750	0.791	0.000	-0.022
NFo070	Nfo070-sorex100	36	19.44	356-370	365	5	0.571	0.802	0.024	-
NFo072	Nfo072-sorex	36	11.11	472-474	472	2	0.000	0.571	0.086	-
NFo073	Nfo073-sorex	36(2)	91.67	124-142	136	10	0.909	0.882	0.000	-0.028
NFo074	Nfo074	36	0.00	-	232	-	-	-	-	-
NFo086	Nfo086	36(2)	69.44	186-233	223	10	0.400	0.846	0.000	0.368
NFo120	Nfo0120	36(2)	94.44	175-181	179	4	0.471	0.462	0.461	-0.007
L9	L9	36	0.00	-	160	-	-	-	-	-
L67	L67	36	0.00	-	108	-	-	-	-	-
L68	L68	36	0.00	-	88-107	-	-	-	-	-
SRY	SRY	36(2)	6.94	153	155	1	0.000	0.000	0.000	-
Z-002A	Z-002A	36	80.56	229-231	-	2	0.000	0.290	0.000	0.978

**Note: for locus *Nfo055* two different primer sets have been tested.

Table 6.11 Consensus genotype data from 19 loci obtained from water shrew buccal swabs.

Sample	NFo010		NFo016		NFo030		NFo031		NFo041		NFo043		NFo045		NFo046		NFo047		NFo055** (Primer set Nfo055-sorexdd)	
AS1									172	172										
AS2			350	350					174	195	241	241			254	259	240	240		
AS2A			311	311	236	236			174	174	241	241			254	254				
AS2B					240	240			174	185							240	240		
H3					242	242			185	185	243	256			263	263	240	240	288	292
H3A	409	409	350	350	236	242	357	357	185	185	243	256			261	263			288	292
H3B					236	236			181	185					261	263			288	288
H3C	409	409	350	350	236	242			185	185	243	256	363	365	261	263	240	240	292	292
H3D									185	185	256	256			261	263				
H4	421	421	350	350	236	242	356	356	185	185	243	256	365	365	263	263	240	240	288	292
H4A	409	421	350	350	236	242	357	357	185	185	243	256	355	365	263	263	240	240	288	292
H4B					236	242	357	357	185	185	243	256			261	263	240	240	288	288
H4C			350	350	236	242	357	357	185	185	243	256			263	263			288	292
H4D					236	236			185	185							240	240	288	288
H5	421	421	350	350	236	236			185	185	243	256			248	259	242	242	298	298
H5A	421	421	346	350	236	242	357	357	185	185	243	256			247	258	240	242	288	298
H6	411	419	350	350	234	234	347	351	179	189	243	260	339	339	256	256	240	240	278	296
H6A					234	234	347	347	179	189	243	260	341	341	256	257	240	240	278	296
H7					238	238			172	183	239	239			248	248	240	240	290	292
SP8					238	238														
SP9									183	183					267	267				
SP10					236	236	346	346	183	183			365	365	273	275	240	240	286	288
SP10A					236	236														
SP10B					232	232														
TAP11			350	350	236	236			174	174	241	245			250	252			294	294
TAP11A					238	238	349	349	174	181	245	245	367	369	251	252	240	240	294	294
TAP11B					236	236			174	174	245	245			247	248	240	240		
TAP11C					236	238			174	181	241	245			247	247	242	242		
WB12					236	236			181	187										
WB13					246	246			170	181	260	262							296	296
WB13A					234	234			170	181	245	245							288	288
WB13B					236	236														
WB14					236	236			181	181	247	264							286	286
WB14A					246	246			170	181	260	262								
WB15					234	236			168	181										
WB15A					236	236			168	181										

Table 6.11 Consensus genotype table continued.

Sample	NFo055** (Primer set Nfo055)		NFo068		NFo070		NFo072		NFo073		NFo086		NFo120		Z-002A		SRY		% of loci amplifying per sample
AS1									136	138	186	186			231	231	153	153	13.89
AS2			171	190					134	140			177	177	229	229			25.00
AS2A			171	190									175	177	231	231			22.22
AS2B	222	222	190	190					134	140	224	224	177	177	229	229			25.00
H3	215	215	190	196					126	142	222	222	177	179	231	231			33.33
H3A	215	220	190	196	366	366			126	142	222	222	177	179	231	231			41.67
H3B			190	196					126	142	222	222	177	179	231	231			25.00
H3C	215	220	190	196					126	142	222	222	177	179	231	231			41.67
H3D	215	215							126	142			177	179	231	231			19.44
H4	220	220	190	196	366	368			126	142	222	222	177	179	231	231			47.22
H4A	215	220	190	196	366	368	474	474	126	142	222	222	177	179	231	231			50.00
H4B	220	220	190	196					126	142	222	222	177	179	231	231			36.11
H4C	215	220	190	196					126	142	222	222	177	179	231	231			36.11
H4D	220	220							126	142			177	177	231	231			22.22
H5	226	226	190	190					130	134			175	177					33.33
H5A	215	226	186	190			474	474	130	134	186	186	175	177	231	231			44.44
H6	205	224	186	190	368	370	472	472	130	138	186	216	177	177	231	231			50.00
H6A	205	224	186	190	366	368	472	472	130	138	186	216	177	177	229	229			44.44
H7	218	220	194	194					124	140	186	224	177	179	231	231			33.33
SP8									124	136	204	207	177	177	229	229			13.89
SP9									124	132			179	179					11.11
SP10			188	200					124	136	204	207	177	177	231	231			33.33
SP10A	220	220	200	200					124	136			177	177	231	231			16.67
SP10B	220	220							124	124	204	207							11.11
TAP11	218	222	190	192	356	356			126	130	210	224	177	177	231	231			36.11
TAP11A			190	192					126	130	224	224	177	177	231	231			36.11
TAP11B	218	218	190	192					126	130	210	224	177	177	231	231	153	153	33.33
TAP11C	218	218	190	192					126	130	224	224	177	177	231	231			30.56
WB12			190	202					128	138	218	218	179	181	231	231			19.44
WB13			198	200					128	136	229	233	177	177	231	231	153	153	27.78
WB13A			200	200							233	233	177	177					19.44
WB13B													177	177	229	229			8.33
WB14			190	202					128	138	211	217	179	181					22.22
WB14A									136	136			177	177	231	231			16.67
WB15			194	194					126	128			177	177			153	153	16.67
WB15A			194	194					128	128			177	181					13.89

**Note: for locus *Nfo055* two different primer sets have been tested

Table 6.12 Estimates of allelic dropout and false allele likelihood error rates for water shrew genotype data obtained from swab samples.

Locus	Allelic dropout rate per allele	False allele rate per allele	Allelic dropout rate per genotype	False allele rate per genotype
NFo030	0.337	0.000	0.504	0.000
NFo041	0.404	0.074	0.575	0.103
NFo043	0.216	0.030	0.355	0.048
NFo055 (Primer set NFo055-sorexdd)	0.279	0.097	0.436	0.146
NFo068	0.182	0.011	0.309	0.180
NFo073	0.094	0.030	0.171	0.054
NFo086	0.264	0.024	0.417	0.038
NFo120	0.169	0.000	0.289	0.000

Table 6.13 Identities of unique individuals as suggested by DNA profiling of swab samples at each site[ł]

Site	Unique individuals
Ash Spinney Pond	AS1
	AS2=AS2A=AS2B
Hockerton Pond	H3=H3A*=H3B*=H3C=H3D
	H4=H4A=H4B=H4C=H4D*
	H5=H5A
	H6=H6a
	H7
Sheepwalks Pond	SP8
	SP9
	SP10*=SP10A*=SP10B
Twenty Acre Piece	TAP11=TAP11A=TAP11B=TAP11C
Whatton Brook	WB12
	WB13=WB14A
	WB13A
	WB13B
	WB14
	WB15=WB15A

ł, Individuals were assigned as different only if they each were heterozygous at the same locus and one (or both) allele size was different between individuals. Differences between the sizes of alleles of the same locus when found to be homozygous in two individuals were ignored and assumed to be attributable to allelic dropout.

*Two swabs taken from the same individual corresponding to H3A=H3B and H4C=H4D and SP10=SP10A.

Table 6.14 Consensus genotype data from the unique individuals obtained from water shrew buccal swabs.

Sample	NFo010		NFo016		NFo030		NFo031		NFo041		NFo043		NFo045		NFo046		NFo047		NFo055**	
AS1									172	172										
AS2=AS2A=AS2B			311	350	236	240			174	185	241	241			254	259	240	240		
H3=H3A=H3B=H3C=H3D	409	409	350	350	236	242	357	357	181	185	243	256	363	365	261	263	240	240	288	292
H4=H4A=H4B=H4C=H4D	409	421	350	350	236	242	357	357	185	185	243	256	355	365	261	263	240	240	288	292
H5=H5A	421	421	346	350	236	242	357	357	185	185	243	256			248	259	240	242	288	298
H6=H6A	411	419	350	350	234	234	347	351	179	189	243	260	339	341	256	256	240	240	278	296
H7					238	238			172	183	239	239			248	248	240	240	290	292
SP8					238	238														
SP9									183	183					267	267				
SP10=SP10A=SP10B					232	236	346	346	183	183			365	365	273	275	240	240	286	288
TAP11=TAP11A=TAP11B=TAP11C			350	350	236	238	349	349	174	184	241	245	367	369	247	252	240	242	294	294
WB12					236	236			181	187										
WB13=WB14A					246	246			170	181	260	262							296	296
WB13A					234	234			170	181	245	245							288	288
WB13B					236	236														
WB14					236	236			181	181	247	264							286	286
WB15=WB15A					234	236			168	181										

Sample	NFo055**		NFo068		NFo070		NFo072		NFo073		NFo086		NFo120		Z-002A		SRY	
AS1									136	138	186	186			231	231	153	153
AS2=AS2A=AS2B	222	222	171	190					134	140	224	224	175	177	229	231		
H3=H3A=H3B=H3C=H3D	215	220	190	196	366	366			126	142	222	222	177	179	231	231		
H4=H4A=H4B=H4C=H4D	215	220	190	196	366	368	474	474	126	142	222	222	177	179	231	231		
H5=H5A	215	226	186	190			474	474	130	134	186	186	175	177	231	231		
H6=H6A	205	224	186	190	368	370	472	472	130	138	186	216	177	177	229	231		
H7	218	220	194	194					124	140	186	224	177	179	231	231		
SP8									124	136	204	207	177	177	229	229		
SP9									124	132			179	179				
SP10=SP10A=SP10B	220	220	188	200					124	136	204	207	177	177	231	231		
TAP11=TAP11A=TAP11B=TAP11C	218	222	190	192	356	356			126	130	210	224	177	177	231	231	153	153
WB12			190	202					128	138	218	218	179	181	231	231		
WB13=WB14A			198	200					128	136	229	233	177	177	231	231	153	153
WB13A			200	200							233	233	177	177				
WB13B													177	177	229	229		
WB14			190	202					128	138	211	217	179	181				
WB15=WB15A			194	194					126	128			177	181			153	153

Maximum numbers of water shrews estimated through live-trapping (total numbers over all trapping sessions) were similar to the number of individuals identified through DNA profiling (not including the extra Whatton Brook site) (see Table 6.15). However, the minimum number of water shrews estimated through live-trapping (total numbers for each trapping session), were less than the actual numbers identified through DNA profiling.

The numbers of individual water shrews sampled (n=17) was too few to enable analysis to be undertaken to establish relatedness either within or between populations.

Table 6.15 Comparison of the numbers of unique individual water shrews as estimated by live-trapping and DNA profiling.

Site	Number of swabs taken for DNA profiling	Number identified from DNA profiling	Minimum number identified from fur-clips	Maximum number identified from fur-clips
Ash Spinney Pond	4	2	2	2
Hockerton Pond	15	5	2	5
Sheepwalks Pond	4	3	2	3
Twenty Acre Piece	5	1	1	1
Whatton Brook	8	6	-	-
Total	**36**	**17**	**7**	**11**

6.4 Discussion

6.4.1 Comparison of the methods for extraction of DNA from buccal swabs

DNA amplification was greater when 2µl of DNA was used in each reaction when compared to 1 µl and 5 µl regardless of storage or extraction method. It is presumed that a volume of 1 µl contained too little DNA for PCR amplification and 5 µl contained too many PCR inhibiting contaminants or,

less likely, too much DNA. However, as the concentration of DNA was not quantified this finding is of little use. Nevertheless, DNA was successfully extracted from the swabs in sufficient quantities to allow PCR amplification to be undertaken.

DNA amplification was greater when the swabs were stored in absolute ethanol compared to 70% ethanol or air dried and stored at room temperature but due to the small sample sizes involved for each treatment these observations are inconclusive. Previous studies utilising buccal swabs have reported good DNA yields from both air dried samples (Handel *et al*., 2006; Broquet *et al*., 2007; Yannic *et al*., 2011) and those stored in ethanol (Seki, 2003; Poschadel and Möller, 2004; Miller, 2006). However, Taberlet *et al*., (1999) note that swabs should be kept perfectly dry to avoid moisture development that could irreversibly degrade DNA (which may not be the case for the swabs which were simply replaced in their plastic container tubes whilst still moist). Indeed, the only sample not to produce any PCR products in this study was the one that was moist when placed for storage at room temperature (see Table 6.9). Although no study has actually compared yields from these different storage methods of swab samples, Seki (2003) reported lower DNA yields when the swab was stored in absolute ethanol than when stored in a preservation buffer, yet Miller (2006) found that although swabs collected into a DNA lysis buffer appeared of similar quality/quantity to those stored in ethanol, PCR amplification was not successful due to a possible PCR inhibitor from the sample stick.

DNA amplification was greater when extracted using a QIAamp DNA Mini Kit but again due to the small sample sizes for each extraction methods used, these observations are not conclusive. Other studies utilising buccal swabs have reported good yields from DNA extracted both using a kit (Poschadel and Möller, 2004; Handel *et al*., 2006; Miller, 2006; Broquet *et al*., 2007; Yannic *et al*., 2011) and via a sodium chloride DNA extraction method (Handel *et al*., 2006; Mitrečić *et al*., 2008). Handel *et al*. (2006) found yields of DNA isolated to be similar whichever extraction method was used

and Miller (2006) reported greater yields for DNA extracted using a phenol/chloroform method rather than a kit.

In summary, PCR was amplified from a proportion of individuals from all swabs, indicating that obtaining water shrew DNA from buccal swabs is possible. There is some evidence that buccal swabs stored in absolute ethanol and extracted using a QIAamp DNA Mini Kit isolated higher quantity DNA but this needs to be investigated further using a larger sample size for each extraction method. PCR using a volume of 2µl of extracted DNA amplified in the highest proportion of individuals regardless of storage media or extraction method.

6.4.2 Optimisation of primer sets

The majority of the newly developed primer sets amplified in all shrew species tested and were found to be polymorphic in at least one shrew species (Table 6.9). A total of 22 loci were amplified and used to identifying individuals from the water shrew buccal swab samples. Three loci were not used (*Nfo26*, *Nfo37 and Nfo98*) because they amplified in a lower proportion of individuals.

The design of conserved primer sequences that were consensus between the water shrew and common shrew for some loci enabled the majority of those conserved primer sets to amplify in all three shrew species tested comprising two different genera: *Neomys* (water shrew) and *Sorex* (common and pygmy shrews; Table 6.9). These conserved shrew markers are expected to be of utility not only in genetic studies of these three species tested, but for the majority of other *Neomys* and *Sorex* shrew species. Furthermore, as they amplified across species representing two genera, it is possible that some of the conserved markers might amplify in other insectivores.

The *L67* and *L68* primer sets designed from *S. araneus* sequences (Wyttenbach *et al*. 1997; Balloux *et al*. 1998) amplified in all three shrew

species tested including water shrew. This is interesting as both Wyttenbach *et al*. (1997) and Balloux *et al*. (1998) found these primer sets to amplify in all *Sorex* species tested but not in any other insectivorous mammals including *Neomys* species (*N. anomalus* and *N. fodiens* were tested). These results suggest that these two *S. araneus* primer sets have higher cross-species amplification than previously thought.

Finally, the Y-linked sex marker (Matsubara *et al*., 2001) amplified products in all three shrew species (see Table 6.9). Products amplified in all common shrew males (n=4) but not females (n=1). However, products were amplified in both male (N=5) and female (n=1) pygmy shrew samples (although they were of a different size) indicating that either an error had occurred or that they were not Y-linked in the species. Nevertheless, the difference in size of the product might be useful as an indicator of sex although further testing is necessary as only one known female was tested. The *SRY* primer has previously been shown to work in all Sorex species tested (n=6) although it has not been tested specifically in common and pygmy shrews. In addition, Matsubara *et al*. (2001) reported a faint product when tested on *C. suaveolens* (a white-toothed shrew) males (no product when tested on females) indicating its potential for cross-amplification in other species. Indeed, products were amplified in this study (Table 6.9) in four out of six water shrews of unknown sex and no product was amplified for a known female sample providing some evidence of the utility of a Y-linked marker in water shrews and further indication of cross-amplification in non-Sorex species.

6.4.3 Buccal swab DNA profiling

Individual identification of water shrews was based on the genotyping data of nineteen polymorphic loci (Table 6.11). This number of polymorphic loci gives a greater degree of certainty when identifying individuals than studies that have relied on fewer (e.g. Frantz *et al*., 2003; Moran *et al*., 2008). Thus, there are now a number of polymorphic loci that have been identified

as of utility in the water shrew which can be used for further genetic studies of this species.

Individual identification revealed a total of seventeen water shrews (Table 6.13). However, genotyping error in this study was relatively high (Table 6.12) with mean allelic dropout and false allele rates per genotype of 0.382 ± 0.045 and 0.071 ± 0.023, respectively. In comparison, Yannic *et al*. (2011) utilised buccal swabs and found mean allelic dropout and false allele rates per genotype of 0.0038 ± 0.0022 and 0.0005 ± 0.00005, respectively. The high genotyping error in this study is likely due to the small amount of DNA extracted from the swabs in this study (59-119 ng/extraction with mean concentrations of 0.48 ± 0.01ng/µl) which was low in comparison to others (e.g. ~2µg/extraction with mean concentrations of 11.76 ± 18.10ng/µl, Yannic *et al*., 2011). Consequently, the amplification success in this study was low and inconsistent which has also contributed to genotyping errors.

Previous studies utilising buccal swabs have found little evidence of allelic dropout (Miller, 2006; Broquet *et al*., 2007; Yannic *et al*., 2011) and little or no evidence of false alleles (Broquet *et al*., 2007; Yannic *et al*., 2011), so have consequently required only two to three repetitions for 100% genotyping accuracy (Broquet *et al*., 2007; Yannic *et al*., 2011). Unfortunately, due to a lack of DNA extracted from the buccal swabs in this study, only eight loci were genotyped twice and with yields as low as found, more repetitions would be needed to increase genotyping accuracy. In this case, the number of repetitions should be increased at the expense of the number of loci amplified,

Waits and Leberg (2000) have shown that genotyping errors in noninvasive mark-recapture studies can result in severe overestimates of population size and miscalculation of population sizes have been reported previously (e.g. Gagneux *et al*., 1997b, 2001; Vigilant *et al*., 2001). Indeed, it is likely that the number of water shrews in this study has been overestimated. For example, samples SP8 and SP10 show similarity and only differ at two loci

(*NFo030* and *Z-002A*) due to the presence of different heterozygotes, which may be a result of allelic dropout or contamination. Indeed, allelic dropout rate for the *NFo030* loci is high, estimated at 0.337 (Table 6.12), and there is a lack of amplification for sample SP8 so it is likely that the samples have come from the same individual. Likewise, the lack of amplification success for sample WB13B is likely to have led to this sample being identified as a unique individual.

6.4.3.1 Estimating number of water shrews

The number of individual water shrews identified at each of the sites through DNA profiling was identical to the maximum numbers estimated through live-trapping (not including the Whatton Brook site), but more than the minimum (see Table 6.15). For example, it was estimated through fur-clipping that a minimum of two water shrews were present at the Hockerton site yet maximum numbers estimated through fur-clipping and through DNA profiling identified five (Table 6.15). The minimum number of individual water shrews estimated through fur-clipping at Hockerton was less than half of the number of individuals that were subsequently identified through DNA profiling. However, the maximum number of individual water shrews estimated through fur-clipping was identical to that estimated through DNA profiling for every site. Despite the low sample size, DNA profiling using buccal swabs of live-trapped water shrews appears to be a more accurate method of estimating population numbers than estimates of minimum number of individuals through fur-clipping and is the only certain method for determining individuals.

6.4.3.2 Potential sex-typing in water shrews

The ability to identify individual water shrews and determine their gender has wide ranging applications for their conservation. However, the *SRY* sex-typing marker used in this study was Y-linked in *S. unguiculatus* (Matsubara *et al*., 2001) and therefore only amplified products for males (XY). Consequently, when no amplification was observed with this marker it was not certain whether an individual was actually female or whether the primer had just failed to amplify. Obviously, this is not ideal and the development

of a marker which amplifies in both sexes and shows different allele sizes for males and females would be more definitive. Four of the water shrew swab samples included in this study amplified a product using the *SRY* primer (see Table 6.11) further confirming that the marker does amplify in the species. However, according to the unique individuals suggested by the DNA profiling (Table 6.13) three of these four 'male' individuals were swabbed on several occasions so should have consistently amplified products several times. Therefore, due to the lack of consistency in amplifications and ambiguity of determining females, the marker cannot at present be relied upon for accurately identifying sex in water shrews.

6.4.3.3 Other sources of non-invasive sampling

Genotyping DNA from water shrew faecal and hair samples has the potential to be undertaken alongside bait tube surveying, as remote methods of monitoring shrew species, although as previously mentioned, hair sampling from water shrews might be problematic. However, genotyping DNA from the faecal samples collected during a bait tube survey would be possible. Discriminating water shrew faeces from other small mammal faeces (see Chapter 2, Section 2.1.3.1) could be undertaken prior to the genotyping to avoid the cross-amplification of water shrew microsatellites in other species. However, many studies report low success rates of extracting DNA from faeces (e.g. Wilson *et al*., 2003), especially when more than a day old (Frantz *et al*., 2003). In addition, DNA extracts are often of poor quality and repeated amplifications are required to obtain reliable profiles (Taberlet *et al*., 1996; Frantz *et al*., 2003). Therefore, a comparative study into the quantity and quality of DNA extracted from water shrew faecal and buccal swab samples would enable the best method of genotyping individuals to be determined.

Buccal swab sampling is ideal for monitoring water shrews in conjunction with live-trapping, where animals are already in the hand and clear marking, and therefore identification, of individuals via fur-clipping can be difficult. In addition, when live-trapping surveys are undertaken seasonally, buccal swabs allow the identification of individuals from previous seasons

unlike fur-clips which grow out in a few weeks, thereby giving accurate information about water shrew population densities and dynamics across seasons.

Buccal swab sampling is a minimally invasive and effective way of collecting sufficient amounts of DNA for identifying individual water shrews. For example, Mitrečić *et al*. (2008) found that buccal swabs from laboratory mice yielded approximately the same amount of DNA as isolated from a section of tail tissue. The technique does not rely on having to pluck hair samples, which could be painful, and because water shrews (and common and pygmy shrews) readily open their mouths to bite the swabs, DNA collection is simple. In addition, water shrew DNA acquired from buccal swabs can be easily and quickly extracted. Storing swabs in absolute ethanol was found to produce better results than those air-dried, amplifying products in a greater number of samples.

6.4.4 Conclusion

Collecting and storing water shrew buccal swabs in screw-topped rubber-sealed microfuge tubes already containing absolute ethanol, was an efficient method of preserving DNA, for later extraction and profiling, and easily achieved in the field. Furthermore, the buccal swabs yielded sufficient DNA to enable single-plex genotyping at multiple loci and allow individual water shrews to be identified. In addition, this technique may be used for the collection of samples for further population genetic studies.

A set of seventeen microsatellite markers has been successfully isolated and characterised from the water shrew. DNA was able to be extracted from mouth swabs, and those swabs stored in absolute ethanol and extracted using the QIAamp kit amplified most products. Using the seventeen new markers plus two published markers, unique water shrew individuals were able to be identified by genotyping the DNA extracted from mouth swabs. Due to the low yields of DNA extracted from the buccal swabs genotyping errors were high and are likely to have caused an overestimation of the population of water shrews studied.

The number of individual water shrews sampled during this study was too few to undertake analysis of population structure and parentage. However, sufficient polymorphic markers have been identified to enable further genetic studies. Future studies could utilise these markers to perform investigations of genetic relatedness, both within and between populations, reveal water shrew behavioural ecology via kinship studies, enable the investigation of population structure and establish whether historical genetic bottlenecks have occurred.

Chapter 7
General Discussion and Conclusions

The water shrew has been identified as a 'species of concern' under the UK Biodiversity Action Plan due to the threat to its population through its dependence on freshwater environments and the destruction of suitable bankside habitat. The main aim of this study was to establish the most important habitat features for predicting water shrew presence at a given site by developing successful habitat suitability indices (HSIs). This would allow a more rapid and thorough assessment of the occurrence of water shrews at various sites and assist decision making when designing conservation measures for this species and other aquatic mammals. Additional aims of this study were to focus on a subset of sites and investigate the association between water shrew presence and numbers and diversity of potential prey, the association between abundance of water shrews and other small mammal species and to develop and test a new minimally invasive method of identifying individual water shrews via genetic profiling. The following four questions were designed to meet the aims of the study.

7.1 What are the important features for predicting water shrew presence?

In order to determine which habitat features are most important for predicting water shrews, their presence was established by undertaking bait tube surveys at 32 freshwater sites in a variety of habitats in central England. Evidence of water shrews was found at approximately half of the sites surveyed and, unlike previous studies (e.g. Carter and Churchfield, 2006a), no preference was found for a particular habitat type. This may be a result of the considerably smaller sample size in the current study, so if more sites were surveyed a preference for a particular habitat type may become apparent. Conversely, it may have been because the sites surveyed were all lowland riparian habitats whereas the National Water Shrew Survey encompassed a much broader range of habitat types. However, like Carter

and Churchfield (2006a), water shrews appeared unaffected by close proximity of humans, occurring at sites close to urban areas as well as more remote sites.

Water shrews were also present at similar numbers of lentic and lotic sites suggesting no preference for either habitat type. However, a subsequent power analysis revealed that there was an insufficient number of sites sampled to indicate a preference. Nonetheless, the findings are similar to more recent studies which also found no evidence that water shrews have a particular preference of freshwater habitat being present at a wide variety of lentic or lotic sites (e.g. Greenwood *et al*., 2002; Carter and Churchfield, 2006a). This is contrary to the traditional association of water shrews and fast-flowing streams and rivers (e.g. Churchfield, 1990; Macdonald and Tattersall, 2001; French *et al.*, 2001). However, the findings from these studies could merely be a reflection of the habitats surveyed rather than the actual habitat preferences of water shrews.

One of the main aims of the current study was to develop a HSI model to elucidate the most important variables for predicting water shrew presence. Although HSI models have previously been attempted for water shrews (French *et al*., 2001; Greenwood *et al*., 2002 and Carter and Churchfield, 2006a), their success varied. In this study habitat surveys were undertaken and in combination with the bait tube survey used to develop HSIs. Thirty-two variables were measured at each site such as habitat, physical and chemical characteristics of the waterbody, vegetation and environmental impacts. Several variables were identified as being important predictors of water shrew presence but management intensity, dissolved oxygen and water depth were found to be the most important. There was a positive association between water shrew presence and occasional or frequent bankside management, low levels of dissolved oxygen and low water depth. Positive associations were also found between water shrew presence and bank heights above one metre, low levels of phosphates, overhanging vegetation and absence of dense, floating vegetation.

A number of these variables which were identified as important for water shrew presence were also found to be key features in previous studies (e.g. French *et al.*, 2001; Greenwood *et al*., 2002; Carter and Churchfield, 2006a). However, contrary to Carter and Churchfield (2006a) who found dense tree cover to have a negative effect on water shrew occurrence, in the current study water shrews were more likely to occur at sites where vegetation was overhanging at least three-quarters of the waterbody.

All of the ANN training models developed during the study performed well and had high predictive ability. Management intensity was identified as the most important predictor of water shrew presence in all but one of the models and, when combined with dissolved oxygen and water depth, created the highest performing training and validation models with area under the curve values of 0.88 and 0.85 respectively. The probability of water shrew presence was greatest when bankside management was occasional, and dissolved oxygen and water depth were low.

Of all the factors investigated during this study that potentially influence water shrew occurrence, bankside management was the most important for predicting presence. This suggests that factors such as adjacent habitat, type of waterbody, proximity to human habitation and water quality are less important factors for water shrews in habitats with sufficient bankside ground cover.

In common with other ecological studies (e.g. Mastrorillo *et al*., 1997; Manel *et al*., 1999; Dedecker *et al*., 2004, 2005; Goethals *et al*., 2007; Ozesmi *et al*., 2006; Tirelli *et al*., 2009) the ANN training models developed during the study performed well and had high predictive ability. This is further evidence to support the ability of ANNs to make predictions from complex and non-linear data sets and makes them an ideal tool in species conservation. However, like any statistical method, ANNs are limited by the quality and reliability of the data from which the models are created.

Obtaining precise data on species presence or absence within a given area is practically impossible as a species which is present at a site may go

undetected even after lengthy searching (Kéry, 2002; Hirzel *et al*., 2002; MacKenzie *et al*., 2004; Durso, 2011). However, the problem of imperfect detection can be dealt with by estimating the probability of detecting a species during a given survey through multiple visits, in time or space, to a survey site (MacKenzie *et al*., 2002; MacKenzie *et al*., 2006). Obtaining site occupancy rates corrected for detection probability improves the reliability of inferences made about species and habitat associations (Jeffress *et al*., 2011). However, due to resource limitations, the bait tube survey in the current study was only undertaken. Therefore, it is likely that water shrews were detected imperfectly, leading to false absences i.e. where water shrews were not detected but were in fact present. These false absences may have resulted in misrepresentation of habitat preferences (MacKenzie and Nichols, 2004; MacKenzie and Royle, 2005; Pagano and Arnold, 2009; Jeffress *et al*., 2011) which would explain the misclassification by the ANN models of some of the sites. However, a retrospective estimation of detection probabilities was undertaken by treating each bait tube survey within a 10 km square as an independent survey of a population, with the assumption that water shrews have uniform presence over that scale. The corrected estimate of occupancy was very similar to the naive occupancy which suggests that even a single bait tube survey is a relatively accurate method of detecting water shrews (assuming that the estimated detection probability is representative of water shrews on a smaller spatial scale). Furthermore, the detection probability of water shrews was relatively high compared with other mammal species (Gu and Swihart, 2004; O'Connell *et al*., 2006; Gibson, 2011). However, MacKenzie *et al*. (2002) recommend a minimum of two surveys at a given site to provide a 'reasonable' estimate of occupancy when occupancy is greater than 0.7 and estimated detection probability greater than 0.3 (as in the current study). Therefore, inferences regarding habitat selection must be treated with caution.

7.2 Is water shrew presence associated with numbers and diversity of prey?

In order to assess water shrew prey availability, a subset of four sites with known water shrew presence plus an additional four sites where water shrews were not detected, was selected for investigation. Lentic habitats were used because ponds and lakes have been underrepresented in previous water shrew studies (e.g. DuPasquier and Cantoni, 1992; Castien, 1995; French *et al*., 2001; Greenwood *et al*., 2002).

Considering that water shrews do not contribute much to the diet of any of their predators (Southern, 1955; Buckley and Goldsmith, 1975; Churchfield, 1990) it is unlikely that their distribution and abundance is limited by top down control. Bottom-up control, of which competition for resource availability is the key process (Power, 1992), is more likely to be the limiting factor. Food availability is one of the main bottom-up factors determining the distribution and abundance of populations (Getz, 1961; Cassini and Krebs, 1994; Gurnell, 1996; Strong and Sherry, 2000; Kager and Fietz, 2009). Consequently, certain types of prey have been found to have an impact on shrew distribution (French *et al.* 2001) and seasonal or annual declines in prey availability may be a limiting factor affecting water shrew numbers and occurrence at particular sites (Churchfield, 1997b). Previous studies investigating the feeding habits of water shrews have often concentrated purely on aquatic invertebrates (e.g. DuPasquier and Cantoni, 1992; Castien, 1995). As water shrews exploit both aquatic and terrestrial prey, in the current work both types of invertebrate were surveyed in order to get a complete and accurate assessment of potential sources of food.

Overall, there was no significant difference between the total numbers of terrestrial and aquatic invertebrate individuals, either combined or separately, at sites with known and unknown water shrew presence. However, fewer numbers of terrestrial and greater numbers of aquatic invertebrates were found at sites with known water shrew presence. For example, similar or greater numbers of terrestrial species of Coleoptera,

Hemiptera, Araneae and Opiliones were caught at sites with unknown water shrew presence. However, greater numbers of adult aquatic Hemiptera and Diptera larvae individuals were caught at sites with known water shrew presence and although adult aquatic Hemiptera are not major prey items for water shrews, aquatic Diptera larvae are known to be an important food source (Churchfield, 1984b; 1985; Carter and Churchfield, 2006b). Coleoptera were caught in similar numbers at sites with known and unknown water shrew presence further supporting the evidence that they are not a particularly important food source (Churchfield, 1984b; 1985; Carter and Churchfield, 2006b).

Water shrews are generalist feeders eating a wide range of terrestrial and aquatic prey (Wolk, 1976; Churchfield, 1984b, DuPasquier and Cantoni, 1992; Castien, 1995). The high energy requirement of shrews (Crowcroft, 1957; Hawkins and Jewell, 1962; Churchfield, 1990) means that a more or less constant supply of food is crucial to their survival. This vulnerability to temporal variation in food availability is overcome by their opportunistic nature, having a diverse diet and switching the emphasis to alternative prey when necessary (DuPasquier and Cantoni, 1992; Churchfield, 1993; French *et al.* 2001). Therefore, unlike specialist feeders (e.g. Wickramasinghe *et al*., 2004) they may not be particularly affected by the lack of availability of any one prey type, which may explain their wide distribution (Churchfield, 2008). In support of this, DuPasquier and Cantoni (1992) found no evidence to suggest that annual reductions in water shrew populations during winter were a result of lack of aquatic invertebrate prey.

Woodlands tend to have greater terrestrial invertebrate species richness than grasslands, especially improved grassland (as used in this study), because of the diverse woodland microhabitats and niches for species to exploit (Harris and Harris, 1997). This was evident in the current study with greater numbers of terrestrial invertebrates caught at the woodland sites, suggesting woodlands have greater prey availability for water shrews. However, the presence of water shrews at both woodland and grassland sites demonstrates their capacity to exploit the available prey in different aquatic habitats.

Although species diversity is a good measure of habitat quality, findings from this study suggest that it is not the habitat quality that determines site suitability but rather the presence of a range of species that water shrews are known to eat. For example, a site with low species diversity but high numbers of one or two species may be more preferable to water shrews, if those two species are their favoured prey, than a site with high diversity but fewer numbers of favoured prey. The lack of a significant difference in the BMWP scores at sites with and without water shrews would suggest that they are not necessarily as sensitive to water quality as previously thought (e.g. French *et al*., 2001; Carter and Churchfield, 2006a). This is supported by evidence from a recent study (Scott *et al*., 2012) which found no relationship between water shrew occurrence and physical, chemical or biological water quality.

Despite studies to the contrary (e.g. French *et al*., 2001) prey availability was not found to be associated with water shrew occurrence in the current study. Therefore, other factors such as habitat structure, water and temperature may play a more important role. For example, Churchfield *et al*. (1997) found evidence to suggest that habitat structure, rather than invertebrate prey availability, may have the greater influence over shrew distribution in the central Siberian taiga.

A significant limitation in the experimental design of the prey availability investigation was its reliance on the results of the bait tube survey. As the issue of imperfect detection was not addressed it is likely that there were a number of sites where water shrews were not detected but were in fact present. It is not known whether any of the four sites where water shrews were not detected were actually false absences. Consequently, it is not possible to conclude definitively whether there is an association between water shrew occurrence and numbers and diversity of invertebrate prey, or whether food availability is an important bottom-up regulator of water shrew occurrence. Therefore, it is recommended that for future work any such investigation is based on presence data which has taken into account detection probability and has thus been obtained with a higher degree of certainty. Furthermore, samples of aquatic invertebrates taken from each

pond were pooled prior to analyses which meant analysis of within site variation to obtain information on the distribution of prey was not possible. Therefore, for future studies of prey availability it is recommended that samples from within a single waterbody are analysed separately.

7.3 Is there an association between the relative abundance of water shrews and other small mammal species?

The estimation of abundance plays an important role in ecology (Loreau, 1992; He and Gaston, 2000; Nichols and Mackenzie, 2004; Conn *et al*., 2006; Wiewel *et al*., 2009) particularly with respect to rare or vulnerable species (Rosenberg *et al*., 1995; Gregory and Gaston, 2000; Magurran and Henderson, 2003). However, assessing numbers of rare species can be particularly problematic by the very nature of their scarcity (Mackenzie *et al*., 2005; Williams and Thomas, 2009) as sample sizes may be too low for accurate estimates (Gaston, 1994; Mills *et al*., 2000). The decline in numbers of many mammal species due to abiotic factors (e.g. climate change and habitat loss) makes assessing and monitoring populations essential for their conservation and management (Dirzo and Raven, 2003; Pimm *et al*., 2006; Isaac, 2009; Morris, 2011). However, biotic factors such as interspecific competition also influence species abundance and are therefore an important factor in the structure of small mammal communities (Munger and Brown, 1981; Heske *et al*., 1994; Eccard and Ylonen, 2003; Liesenjohann *et al*., 2011; Zhang and Zhang, 2012). In order to estimate the abundance of water shrews and other small mammal species and to investigate any apparent relationships, live-trapping was undertaken at the four sites with water shrew presence previously used in the prey availability investigation.

Bank voles and wood mice were the most frequently caught species overall followed by common shrews, whereas water shrews, pygmy shrews and harvest mice were caught in much fewer numbers, reflecting their

comparatively lower population sizes (Harris and Yalden, 2008). Capture probabilities varied between species due to a number of factors such as time, behavioural response and individual heterogeneity (Menkens and Anderson, 1988). Water shrews had a relatively low capture probability and were often not caught until several days into, and sometimes not until the final day of, the trapping session. This could suggest that they were trap-shy (Hammond and Anthony, 2006), which is unlikely considering the inquisitive nature of shrews particularly with regard to novel objects (Churchfield, 2000), or possibly that they were not resident at the site but just passing through. Standard live-trapping methodology usually recommends trapping for three days as after this time numbers of new captures tails off (Gurnell and Flowerdew, 2006). When studying water shrews it may be beneficial to trap over a longer period because unlike more common species which can occur in very high numbers (e.g. wood mice and bank voles; Harris *et al*., 1995), there may be a water shrew population of only one or two individuals at a site (Carter and Churchfield, 2006b)."

Despite evidence to suggest that minimum number alive significantly underestimates species abundance (e.g. Macdonald *et al*., 1998; Bryja *et al*., 2001; Conn *et al*., 2006), in common with various studies (e.g. Hanley and Barnard, 1999; Ruscoe *et al*., 2001; Pryde *et al*., 2005; Grenier *et al*., 2009) POPAN abundance estimates reflected the minimum number alive. Therefore, for studies of water shrews when numbers are too low for more complex analysis, minimum number alive may not be as negatively biased as previously thought.

Although, water shrews were caught at both woodland and grassland sites, abundance estimates at the grassland sites were higher. A grassland pond was the only site where water shrews were caught during every trapping session, indicating the site to be a consistently optimal habitat for water shrews. Water shrews were only caught at woodland ponds during the first trapping session despite these ponds having the highest overall abundance of species through large numbers of wood mice and bank voles being caught in their favoured habitats (Flowerdew *et al*., 2004; Flowerdew and

Tattersall, 2008; Shore and Hare, 2008). This suggests that the woodland sites were sub-optimal and that either the water shrews were only visitors or that they may have died, either through old age or predation, between trapping sessions. This apparent preference for grassland sites may be due to the availability of prey in woodland ponds. For example, although woodlands may have a higher diversity and abundance of terrestrial invertebrates (Harris and Harris, 1997), heavily shaded woodland ponds have less vegetation due to the lack of sunlight, and therefore lack food and habitat for aquatic and marginal invertebrates (Williams *et al.*, 1999). This finding appears to be in contrast to Carter and Churchfield (2006a) who found water shrews to occur most commonly in freshwater habitats adjacent to arable (25.3%) followed by woodland (19.6%) and grassland habitats (17.3%). However, although water shrews may occur more frequently at habitats adjacent to arable land it does not necessarily mean abundance is greatest at these sites. With such a small sample size it is not possible to draw any definitive conclusions regarding preferences for a particular habitat type based on the live-trapping surveys.

As expected, overall species abundance was higher during the autumn/winter trapping session because populations are at their largest following the summer breeding season (Flowerdew and Tattersall, 2008). Water shrew abundance decreased over the trapping sessions which may either be a result of their transient nature (Churchfield, 1990) and the animals had simply moved on, or due to the deaths of the individuals caught. However, as abundance was so low any real patterns are hard to detect. Common shrews comprised the greatest proportion of shrew abundance during all sessions, whereas the proportion of water shrews and pygmy shrews appeared to alternate, suggesting some form of seasonal competition between the two species. Differential seasonal changes in the activity patterns of shrew species have been documented (Churchfield, 1984a; Voesenek and Van Bemmel, 1984) and may reduce competition at a time when overall activity and population are at their highest.

Negative relationships were found between total abundance per site of water shrews and both pygmy and common shrews although these

relationships were not significant. Competition between shrew species has been well documented (e.g. Michielsen, 1966; Churchfield, 1980; Voesenek and Bemmel, 1984; Sheftel, 1989; Hanski and Kaikusalo, 1989; Sheftel and Hanski, 2002) and may be the cause of this negative relationship. However, despite co-existence of related shrew species in similar environments (Crowcroft, 1957; Buckner, 1966; Dieterlen and Heim de Balsac, 1979; Churchfield and Brown, 1987; Dokuchaev, 1989; Neet and Hausser, 1990; Churchfield *et al*., 1997), habitat use varies both spatially and temporally which maintains some level of segregation (Michielsen (1966; Yalden *et al*., 1973; Churchfield, 1990; Rychlik and Ramalhinho, 2005). All three British mainland shrew species have a fairly large niche overlap (Churchfield, 1984b) and particularly in times of poor aquatic prey availability, the smaller terrestrial shrews are likely to be competing directly with the larger water shrew for terrestrial invertebrate prey. Conversely, competition for prey may not be the reason for the negative relationships between water shrews and terrestrial shrews seen in the current study, with other factors such as habitat suitability potentially playing a role. However, it must be stressed that in this survey the area of trapping was relatively small and it is possible that the negative relationships found could be due to sampling within the home ranges of either common, pygmy or water shrews. To further elucidate the relationships between the shrew species it is recommended that live-trapping is carried out over a larger area at each site and to survey more sites. However, the negative relationship between overall abundance per site of water shrews and common and pygmy shrews, and the lack of a relationship between these species across all sites and sessions, suggest that habitat suitability, rather than direct competition, is the cause.

7.4 Can buccal swabs be used as a minimally-invasive method of genetic identification of water shrews?

The identification of individuals within a species is important for estimating population sizes (Wilson *et al*., 2003; Frantz *et al*., 2004; Miller *et al*., 2005). During the live-trapping surveys buccal swabs were taken of all caught

water shrews in an attempt to identify individuals via genetic profiling in order to more precisely estimate abundance.

Typical methods of obtaining DNA from vertebrates (e.g. tail, toe or ear clipping; Mitrečić *et al.*, 2008) have ethical considerations and the most commonly used minimally-invasive sampling techniques currently used in wild animals for DNA profiling (i.e. hair and faecal sampling; e.g. Moran *et al.*, 2008) are not ideal for water shrews. Buccal swabs are an alternative, minimally invasive and reliable method of genetically sampling individuals (Seki, 2003; Broquet, 2007; Yannic *et al*., 2011) and have been used in a range of species (e.g. Hashimoto *et al*., 1996; Pidancier *et al.*, 2003; Poschadel and Möller, 2004; Miller, 2006; Handel *et al.*, 2006; Mitrečić *et al.*, 2008) although the method had not been used in wild small mammals before this study. DNA was successfully extracted, amplified and profiled from buccal swabs taken from the live-trapped water shrews. In addition, a set of seventeen microsatellite markers was successfully isolated and characterised from the water shrew. However, the small quantities of DNA extracted from the buccal swabs, in common with other minimally invasive techniques (e.g. Gerloff *et al*., 1995; Taberlet *et al*., 1996; Gagneux *et al*., 1997a; Bayes *et al*., 2000; Constable *et al*., 2001; Miller *et al*., 2002) meant genotyping errors (e.g. lack of amplification for some individuals and allelic dropout) were high which may have caused an overestimation of the water shrew populations studied.

Despite the low sample size, DNA profiling using buccal swabs of live-trapped water shrews appears to be an accurate method of estimating population numbers. In addition, when live-trapping surveys are undertaken seasonally, buccal swabs allow the identification of individuals from previous seasons, unlike fur-clips which grow out in a few weeks, thereby giving accurate information about temporal water shrew population densities and dynamics. In addition, this technique may be used for the collection of samples for further population genetic studies. The number of individual water shrews sampled during this study was too few to undertake analysis of population structure and parentage. However, sufficient polymorphic markers have been identified to enable further genetic studies. Future

studies could utilise these markers to perform investigations of relatedness, both within and between populations, reveal water shrew behavioural ecology via kinship studies, enable the investigation of population structure and establish whether historical genetic bottlenecks have occurred.

7.5 Conclusion

Water shrews are known to have an elusive nature (Churchfield *et al.*, 2000; Carter and Churchfield, 2006a) and therefore historical records of the species are relatively scarce (Aybes and Sargent, 1997). This, and the fact that they occur only at low population densities (Churchfield, 1984a; Churchfield, 1990; Cantoni, 1993; Greenwood *et al*., 2002), has previously made them a difficult species to study. However, since the development of the bait tube method (Churchfield *et al*., 2000), water shrews have been surveyed on a much wider scale than before (French *et al*., 2002; Greenwood *et al*., 2002; Carter and Churchfield, 2006a; Scott *et al*., 2012). These recent studies have revealed that although water shrews may have much lower population densities than the terrestrial shrew species (Aulak, 1970; Yalden, 1973; Sheftel, 1989; Cantoni, 1993; Churchfield, 1998; Rychlik and Ramalhinho, 2005) they are nonetheless widespread and ubiquitous in freshwater habitats (Carter and Churchfield, 2006a).

As previously discussed (see Chapter 1, Section 1.2) rarity is a natural state and in most communities only a few species are common while most others are more or less rare (Loreau, 1992; de Lange and Norton, 1998; Hartley and Kunin, 2003; Magurran and Henderson, 2003). Therefore, rarity in itself does not necessarily mean a species is under threat of extinction (de Lange and Norton, 1998; Robbirt *et al*., 2006; Mace *et al*., 2008). However, rare species are more vulnerable than common species and theoretical models show that small populations can have relatively high extinction risks (Mace *et al*., 2008). Rabinowitz's (1981) seven forms of rarity utilises three characteristics to determine whether a species is rare (i) the species distribution area, (ii) the variety of habitats occupied by a species and (iii) the local population density. Water shrews are evidently widespread

(Churchfield, 1998; Carter and Churchfield, 2006a) so cannot be considered rare on that characteristic. However, on the basis of their dependence on freshwater habitats (Churchfield, 1997b) and low population densities (e.g. Churchfield, 1984a; Churchfield, 1990; Cantoni, 1993; Greenwood *et al*., 2002) they can be deemed, at the least, a relatively rare species.

Water shrews are an interesting species in their apparent contradictions. The fact that they exploit both aquatic and terrestrial habitats makes it surprising that they are not more locally abundant. In addition, their large body size should make them competitively superior when it comes to interference competition (Dickman, 1988; Churchfield, 1998; Churchfield, 2002; Rychlik and Zwolak, 2006). However, they are usually outnumbered by terrestrial shrews, even in their favoured habitats (Churchfield, 1998; Rychlik and Ramalhinho, 2005). Furthermore, although they are generalists in terms of freshwater environments, occurring at a wide variety of lentic and lotic habitats (e.g. French *et al.*, 2001; Greenwood *et al*., 2002; Carter and Churchfield, 2006a; Scott *et al*., 2012), on the wider habitat scale they are specialists. It is this reliance on freshwater habitats and potential vulnerability to habitat loss and degradation which has resulted in the water shrew being on Natural England's 'conservation action priority' list (Wynne *et al*., 1995; Greenwood *et al*., 2002) and deemed a 'species of conservation concern' under the UK Biodiversity Action Plan (Churchfield, 1997a). The National Water Shrew Survey was the first wide-scale survey of the species and has provided baseline data on its distribution in the UK. However, whether water shrew populations are in decline will only become apparent with long-term monitoring.

7.6 Limitations

7.6.1 Bait tube surveys

The main limitation of this study was that both the HSI models and prey availability investigation was based on the results of the bait tube survey which was undertaken only once and therefore did not address the issue of

imperfect detection. This was likely to have resulted in a number of false absences which has implications for the inferences that were made regarding habitat suitability. The number of sites surveyed was limited by the length of time required to visit all of the sites, undertake habitat surveys and analyse the contents of the bait tubes. However, considering the importance of obtaining accurate presence and absence data for a study on habitat suitability, undertaking repeated surveys should be a priority for future work.

The 100m sampling unit used in the bait tube survey is greater than most similar studies of water shrews (e.g. 30m by French *et al*., 2001; 50m by Greenwood *et al*., 2002; and 40-80m by Carter and Churchfield, 2006a). However, it is acknowledged that a 100m length may fall within a single linear home range of a water shrew. Therefore, it is recommended that for future work a longer sampling unit is used or several transects at one site are undertaken.

7.6.2 Sample size

The relatively small sample of water shrews, which was a product of the naturally small population sizes, was another limitation of the study. The water shrew is an elusive species known to exist at low population densities and, accordingly, was caught in very low numbers. This meant that the number of individual water shrews sampled during the study was too few to undertake more detailed genetic analysis, although sufficient polymorphic markers were identified which will enable further genetic studies.

In addition, the investigation into the relationships between water shrews and other small mammals was limited by the small number of sites used for live-trapping. However, the number of sites was constrained by the labour intensive nature of live-trapping which meant a great deal of effort was expended for minimum return. Nevertheless, live-trapping is currently the only method which allows the simultaneous surveying of multiple small mammal species, thereby giving information on species interactions and the collection of biometric data. Furthermore, live-trapping water shrews

allowed the collection of buccal swab samples which were used to genetically profile individuals.

7.6.3 Prey availability

A significant limitation in the experimental design of the prey availability investigation was its reliance on the results of the bait tube survey. Therefore, as previously discussed, the issue of imperfect detection was not addressed. Consequently, it is not known whether any of the four sites where water shrews were not detected were in fact false absences, affecting any subsequent inferences regarding habitat selection. It is recommended that for future work any such investigation is based on presence data which has taken into account detection probability and therefore been obtained with a higher degree of certainty.

A further limitation of the prey availability investigation was the pooling of aquatic invertebrate samples prior to analysis. This meant information on the distribution of prey and within site variability was unable to be obtained. Therefore, for future studies of prey availability it is recommended that samples from within a single waterbody are analysed separately.

7.6.4 HSI

Another limitation of the study was that the HSI models were developed and tested only in lowland riparian habitats in central England. Caution should therefore be exercised in trying to generalise to habitats and countries where conditions are different. Counter to this argument, the area was identified by the National Water Shrew Survey as having a high concentration of water shrews, suggesting that the region is typical water shrew habitat. Furthermore, many of the variables identified in this study as being important predictors of water shrew presence were also identified in other studies from other parts of the country.

7.7 Major contributions

Despite the above limitations, this thesis is the first in-depth study of factors affecting the occurrence and habitat selection of water shrews in central England and the current work has made some important contributions to the understanding of habitat analysis and species identification. A HSI model developed for water shrews using ANNs performed extremely well in predicting presence at a range of freshwater sites within the study region. This model will allow the rapid assessment of sites for likely water shrew presence without the need for labour intensive and costly techniques such as live-trapping. In addition, the minimally-invasive method of collecting DNA samples from water shrews using buccal swabs was deemed successful and therefore could potentially be applied to further wild mammal species. A number of primer sets have been developed, which will allow researchers to accurately identify individuals and obtain more information about population structure and dynamics. Furthermore, these have been found to cross-amplify in other shrew species. Both the HSI model and the new method of identifying water shrews will contribute to the conservation of this much understudied species.

7.8 Further work

The findings from this thesis offer scope for further work. During the study a successful HSI model was developed for water shrews using ANNs which is able to predict presence at sites within central England with a high degree of accuracy. Initially, surveys could be repeated at all of the study sites in order to quantify detectability of water shrews using bait tubes and obtain occupancy estimates. This would give more accurate data on presence and absence at sites which would better inform the HSI model. Further assessment of the performance of the model is recommended by testing it in similar lowland riparian habitats in different regions of the UK and Europe. In addition, further development of the model to increase applicability to a wider range of aquatic habitats is also suggested. The

model was developed using data from a relatively small range of freshwater sites over a limited geographical area. In order to increase the generalisability of the model and apply it to a wider range of sites further development is necessary. It is proposed that further surveys are undertaken covering a larger geographical area encompassing a wider variety of riparian habitats in uplands as well as lowlands. This would allow habitat data to be collected from aquatic environments which include a wide range of flow conditions, substrate types, thermal regimes, channel dimensions and water quality. The data could be analysed using ANNs and the best predictors of water shrew presence used to create a model which could be applied to a range of freshwater habitats both in the UK and other countries.

The ability to identify individuals is crucial in wildlife population studies. However, many historic methods of identifying individual small mammals such as toe clipping and ear punching are no longer considered appropriate because of the animal welfare implications. Current minimally invasive methods of DNA sampling of small mammals include the use of hair samples which are often plucked from the animal (e.g. dormice, Naim *et al*., 2009). In species such as water shrews with short, dense fur, plucking the required 25 hairs to allow sufficient DNA extraction to counter genotyping errors (Henry *et al*., 2011) would be both difficult and also likely to cause discomfort. Buccal swabs are a minimally invasive technique of obtaining genetic material from small mammals, as well as a wide range of other species, and could be used as a more humane alternative to hair samples. The ability to determine the sex of water shrews has wide ranging applications for their conservation. The SRY sex marker (Matsubara *et al*., 2001) which was used in this study was Y-linked which made it difficult to ascertain whether an individual water shrew was female or whether the primer had failed to amplify. Therefore, the development of a primer which shows different allele sizes for males and females would allow gender to be determined definitively. This would give important information on the population structure and dynamics of the species. Due to the low numbers of water shrews caught during this study insufficient DNA samples were obtained to enable detailed analysis of genetic structure and relatedness

within or between populations. Further intensive live-trapping surveys in areas identified through bait tube surveys as having water shrews present would allow more in-depth analyses. Such information would facilitate the long term monitoring of populations as well as the identification of populations at risk of reduced genetic diversity, such as those which are isolated or fragmented. A genetic database for water shrews could be created to provide detailed information about the genetic structure and dynamics of water shrew populations. County mammal recorders, students or organisations, such as the Mammal Society, that are regularly involved in small mammal trapping surveys, could routinely take buccal swab samples from water shrews. These samples could be sent to a central point and genotyped. In addition, samples could be collected from common and pygmy shrews and genotyped using the primers developed in this study for water shrews which cross-amplified in those species. Furthermore, future studies could utilise the water shrew primers developed for this study in other species of Soricomorpha.

Previous studies of water shrews have tended to concentrate on lotic habitats such as streams and rivers. Ponds are valuable freshwater habitats contributing more to biodiversity regionally than rivers, streams or ditches (Williams *et al*., 2003; Davies *et al*., 2008) and therefore, they may be an important resource for water shrews. However, pond numbers in Europe are at an all-time low (Hull, 1997; Keeble *et al*., 2009). Despite this, the term 'pond' is not included in the Water Framework Directive, (EC legislation designed to improve and integrate the management of water bodies throughout Europe and improve their chemical and ecological status). As a result, unlike rivers and streams, which both have monitoring programmes, surveillance of ponds is unlikely to be undertaken (Davies *et al.*, 2008). Only a small sample of ponds were investigated in the current study therefore further work could be undertaken investigating more extensively the value of ponds as habitat for water shrews.

This study offers fresh insight, techniques and opportunities for those interested in investigating and conserving this elusive species.

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Ash Spinney Pond (SK476240 – SK476240)


Whatton Brook (Mill House) (SK492232 – SK492233)


Whatton Brook (Mill Lane Bridge) (SK484235 – SK485235)


Brinsley Flash (SK446501 – SK446501)


American Adventure Pond (SK457438– SK452438)


Shipley Country Park Stream (SK429441 – SK429440)


American Adventure Stream (SK448433 – SK448432)


Fairham Brook (Road End) (SK561338 – SK561337)


Fairham Brook (School End) (SK558333 – SK558334)


Rushcliffe Country Park Lake (SK573321 – SK573320)


Rushcliffe Country Park Pond (SK570324 – SK570324)


Harlow Wood Pond (SK562566 – SK562566)


Harlow Wood Stream (SK555564 – SK554564)


Newstead Park Pond (SK543534 – SK543534)


River Leen, Newstead Park (SK542530 – SK542531)


Clock Farm Stream (SK620222 – SK620221)


Wymeswold Meadows (SK611231 – SK611231)


Twenty-Acre Piece (SK639211 – SK639211)


Ella's Pond (SK636235 – SK636235)


Shelford Manor (River Pond) (SK670435 – SK671435)


River Trent (Shelford End) (SK664431 – SK665432)


Shelford Manor (Wood Pond) (SK675427 – SK675427)


River Trent (Gunthorpe Bridge) (SK679436 – SK680436)


Whatton Manor (Mink End) (SK750382 – SK749381)


Whatton Manor (Road End) (SK742372 – SK743373)


Washdyke Farm (Railway Pond) (SK761320 – SK761321)


Washdyke Farm (Secret Pond) (SK757308 – SK758309)


Kelham Hall (SK774556 – SK775556)


Hockerton Pond (SK718560 – SK718560)

Hockerton Stream (SK716562 – SK716561)

Sheepwalks Pond (SK758552 – SK758552)

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List of Invertebrate Species

Numbers of terrestrial invertebrate individuals caught at sites with known and unknown water shrew presence.

Terrestrial invertebrate taxa	Sites with known water shrew presence				Sites with unknown water shrew presence			
	AS	**HP**	**SWP**	**TAP**	**NP**	**RCP**	**SM**	**WF**
COLEOPTERA								
Cantharidae								
Cantharidae larvae	0	0	0	1	0	0	0	0
Carabidae								
Bembidion biguttatum	0	0	0	0	0	1	1	1
Bembidion guttula	0	0	0	1	0	0	0	0
Bembidion obtusum	0	0	1	0	0	0	1	0
Bembidion quadripustulatum	0	0	0	0	0	1	0	0
Carabus	0	0	0	0	1	0	0	0
Carabus nemoralis	0	0	0	0	0	1	0	0
Harpalus sp.	0	0	0	0	0	5	0	0
Leistus ferruginosus	0	0	0	1	0	0	0	0
Leistus rufomarginatus	0	0	0	0	0	0	0	1
Loricera pilicornis	3	0	0	2	0	1	2	1
Nebria brevicollis	0	1	0	0	5	5	25	1
Notiophilus buguttatus	0	0	0	0	0	0	6	1
Pterostichus madidus	0	1	0	0	1	0	0	1
Pterostichus niger	1	0	0	0	0	0	0	0
Pterostichus strenuus	0	0	0	0	0	0	6	0
Stomis pumicatus	0	0	0	0	0	0	5	0
Trechus obtusus	0	0	0	0	0	0	2	1
Hydrophilidae								
Megasternum sp.	0	1	0	0	0	0	0	0
Leiodidae								
Choleva sp.	1	0	0	0	0	0	0	0
Nargus velox	0	0	0	1	0	0	0	5
Staphylinidae								
Aleochara sp.	1	0	0	0	0	0	0	0
Conosoma pubescens	0	0	0	0	0	0	0	1
Philonthus laminates	1	0	0	0	0	0	0	0
Quedius fuliginosus	1	0	0	2	0	0	0	0
Quedius sp.	0	0	0	0	0	0	1	0
Staphylinidae	0	0	0	0	0	0	2	0
Staphylinus acritona	0	0	0	0	1	0	0	0
ADULT HEMIPTERA								
Hydrometridae								
Hydrometridae	0	0	0	0	0	1	0	0
Ochteridae								
Ochteridae	1	0	0	0	0	0	0	0
DIPTERA LARVAE								
Stratiomyidae								
Stratiomyidae	3	0	0	0	0	0	0	0
LEPIDOPTERA								
Noctuidae larvae								
Scoliopteryx libatrix	0	0	0	0	0	0	0	1
Unknown Lepidopteran larvae								
Caterpillar (black)	0	0	0	0	0	1	0	0
HYMENOPTERA								
Formicidae								
Myrmica rubra	0	0	0	0	0	1	0	1

Terrestrial invertebrate taxa	Sites with known water shrew presence				Sites with unknown water shrew presence			
	AS	HP	SWP	TAP	NP	RCP	SM	WF
Ichneumonidae								
Ichneumonidae	0	2	0	0	0	0	0	0
COLLEMBOLA								
Entomobryidae								
Orchesella sp.	7	0	0	55	5	0	0	5
Tomoceridae								
Tomocerus sp.	0	1	0	0	0	0	0	0
ARANEAE								
Araeidae								
Agriope sp.	0	0	1	0	0	0	0	0
Gnaphosidae								
Gnaphosidae	1	0	0	0	0	0	0	0
Linyphiidae								
Erigone dentipalps	0	0	0	0	1	2	0	0
Hypomma bituberculatum	0	0	0	0	1	0	0	0
Lepthyphantes sp.	0	0	0	0	0	0	2	5
Linyphiidae sp.	4	0	1	0	0	0	0	0
Lycosidae								
Alopecosa sp.	0	3	0	0	0	0	0	0
Lycosa lycosa	0	3	0	0	0	0	0	0
Lycosa sp.	0	0	0	0	4	1	0	0
Pardosa hortensis	0	0	0	0	1	0	0	0
Trochosa sp.	0	0	0	0	0	1	0	0
Tetragnathidae								
Pachygnatha sp.	0	0	0	0	0	0	0	1
OPILIONES								
Nemastomatidae								
Nemastoma bimaculatum	0	0	1	2	0	0	0	5
ISOPODA								
Oniscidae								
Oniscus asellus	0	0	0	2	0	0	0	0
Porcellionidae								
Porcellio scaber	0	0	0	1	0	0	0	0
CHILOPODA								
Lithobiidae								
Lithobius forficatus	1	0	0	0	0	0	0	0
Lithobius sp.	0	1	0	0	0	0	1	0
DIPLOPODA								
Blaniulidae								
Blaniulus guttulatus	0	0	0	1	0	0	0	0
Julidae								
Cylindroiulus sp.	0	1	0	0	1	0	0	0
Polydesmidae								
Polydesmus gallicus	0	1	2	0	0	0	0	0
Polydesmus sp.	6	0	0	1	0	0	5	0
GASTROPODA								
Clausiliidae								
Clausilia sp.	1	0	0	0	0	0	0	0
Hygromiidae								
Trichia hispida	1	0	0	1	0	0	0	0
Limacidae								
Limax sp.	0	0	0	0	0	0	1	0
LUMBRICIDAE								
Lumbricus rubellus	0	0	0	0	0	0	1	0
Lumbricus sp.	1	0	0	0	0	0	1	0
Total	**34**	**15**	**6**	**71**	**21**	**21**	**62**	**31**

Numbers of aquatic invertebrate individuals caught at sites with known and unknown water shrew presence (* indicates BMWP score).

Aquatic invertebrate taxa	Sites with known water shrew presence				Sites with unknown water shrew presence			
	AS	HP	SWP	TAP	NP	RCP	SM	WF
COLEOPTERA								
Dytiscidae								
Dytiscidae **5***	0	0	0	0	1	7	0	0
Hydroporus sp. **5***	0	1	0	0	0	0	0	0
Haliplidae								
Haliplus ruficollis **5***	0	0	3	0	0	0	0	0
Haliplus sp. 1	0	0	1	0	0	0	0	0
Haliplus sp. 2	0	0	1	0	0	0	0	0
ADULT HEMIPTERA								
Corixidae								
Corixa sp. **5***	0	0	10	7	0	0	0	0
Micronecta sp. **5***	0	100	100	0	0	7	0	0
Gerridae								
Gerridae **5***	0	3	1	0	0	0	0	0
Hydrometridae								
Hydromecta stagnorum **5***	0	0	0	1	0	0	0	0
DIPTERA LARVAE								
Ceratopogonidae larvae								
Ceratopogonidae	0	1	0	0	0	0	0	0
Chironomidae								
Charborus sp.	0	0	0	200	0	0	0	0
Chironomidae **2***	0	0	5	55	0	0	7	0
Tanypus sp.	0	0	0	55	0	0	0	0
Culicidae larvae								
Anopheles sp.	0	2	0	0	0	0	0	0
Culex sp.	0	0	0	200	0	0	0	0
Tipulidae								
Tipulidae **5***	0	1	0	0	0	0	0	0
ODONATA LARVAE								
Aeshnidae								
Aeshna sp. **8***	0	0	0	0	0	7	0	0
Agriidae								
Agriidae sp. 8*	0	9	3	0	0	55	0	0
Agriidae sp. 8*	0	4	0	0	0	0	0	0
Agrion sp. **8***	0	0	0	0	55	0	0	0
Libellulidae								
Libellula **8***	0	0	0	0	7	7	0	0
EPHEMEROPTERA LARVAE								
Baetidae								
Baetidae **4***	0	3	6	0	0	0	0	0
TRICHOPTERA 5*	0	0	0	7	0	0	0	0
AMPHIPODA								
Asellidae								
Asellus sp. **3***	0	0	1	55	0	7	7	0
Crangonyctidae								
Crangonyx pseudogracilis	0	0	2	0	0	0	0	0
Crangonyx sp.	0	0	0	0	0	55	0	0
HAPLOTAXIDA								
Erpobdellidae								
Erpobdella **3***	0	0	0	0	1	0	0	0
Leeches **3***	0	0	0	0	0	3	0	0
Naididae								
Naididae **1***	0	1	0	0	0	0	0	0

Aquatic invertebrate taxa	Sites with known water shrew presence				Sites with unknown water shrew presence			
	AS	**HP**	**SWP**	**TAP**	**NP**	**RCP**	**SM**	**WF**
GASTROPODA								
Lymnaeidae								
Lymnaea peregra **3***	0	0	0	0	0	7	0	0
Lymnaea sp. **3***	0	0	6	0	55	0	0	0
Neritidae								
Theodoxus sp.	0	1	0	0	0	0	0	0
Physidae								
Physidae sp. **3***	0	0	0	0	0	7	0	0
Planorbidae								
Hippeutis complanatus **3***	0	2	0	0	0	0	0	0
Planorbis carinatus **3***	0	0	9	0	0	0	0	0
Succineidae								
Snails (tiny)	0	0	0	0	0	55	0	0
Succinea sp.	0	0	1	0	0	0	0	0
TURBELLARIA								
Dugesiidae								
Dugesia tigrina **5***	0	6	0	0	0	0	0	0
Total	**0**	**134**	**149**	**580**	**119**	**217**	**14**	**0**

The minimum number alive of species caught at each site during the four trapping sessions.

Site	Date	Common shrew	Pygmy shrew	Water shrew	Bank vole	Field vole	Harvest mouse	Wood mouse	Total
Ash Spinney Pond	21/09/2007	17	3	2	42	0	0	28	92
	03/05/2008	0	0	0	5	0	0	18	23
	08/12/2008	7	0	0	28	0	0	30	65
	30/04/2009	9	2	0	27	0	0	27	65
Hockerton Pond	22/10/2007	1	0	1	3	1	0	8	14
	01/06/2008	0	0	3	0	0	0	3	6
	16/10/2008	5	1	1	0	1	8	10	26
	08/05/2009	3	0	1	0	0	0	0	4
Sheepwalks Pond	01/12/2007	8	0	2	15	12	0	11	48
	22/05/2008	5	0	1	0	0	0	1	7
	23/10/2008	2	0	0	3	3	8	8	24
	02/04/2009	4	3	0	0	3	0	1	11
Twenty-Acre Piece	12/11/2007	7	1	1	51	5	0	16	81
	24/06/2008	7	0	0	15	0	0	2	24
	31/10/2008	7	0	0	21	0	0	24	52
	20/04/2009	13	6	0	27	0	0	26	72
Total		**95**	**16**	**12**	**237**	**25**	**16**	**213**	**614**