INVESTIGATING THE CAUSES AND CONSEQUENCES OF INDIVIDUAL NICHE VARIATION IN GROUP LIVING BADGERS

Submitted by:

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I certify that all materials in this thesis that are not my own work have been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

ABSTRACT

Individual niche variation is increasingly being demonstrated in animal populations in a wide variety of species and taxa. Niche variation among individuals has important implications for the ecology, evolution and management of animal populations and is a subject of increasing interest. However, despite its widespread occurrence the causes and consequences of individual niche variation remain poorly understood. In this thesis I use the European badger (*Meles meles*), a well studied species of high ecological interest, as a model system to investigate individual niche variation. In order to achieve this I combine information on individual foraging niches derived via stable isotope analysis (SIA) of badger vibrissae with detailed life history and ecological data from a long-term study population to investigate the incidence, cause and consequence of individual niche variation within badger social groups. First I use the biomarker Rhodamine B to investigate vibrissae growth rates and patterns in badgers and demonstrate that the isotopic composition of a single vibrissa likely reflects diet over several months (Chapter 2). Next I explore the use of SIA as a tool to investigate badger diet, by comparing isotopic patterns to seasonal changes in diet measured using faecal analysis (Chapter 3). My results provide validation that SIA is powerful tool for investigating foraging variation in this species, and suggest that within badger populations substantial dietary variation may occur among individuals. Further investigation of isotopic variation Indicates that individuals within social groups differ markedly and consistently in their isotopic signature, independent of age and sex effects and that in some instances these differences are remarkably consistent across year (Chapter 4).This suggesting long term individual specialisation (Chapter 4). I find that the degree of this individual specialisation, and the relationship between specialisation and body condition is influenced by competition for resources (Chapter 5). Social groups with higher levels of competition exhibit greater specialisation and specialised individuals within these highly competitive environments are in better condition. Finally, I discuss the implications of these results for individual niche variation, for the application of SIA to study this behaviour and for badger ecology generally (Chapter 6). I also outline future directions for further research.

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thank Matt Perkins who during that trip very politely woke me from my tent in time for our first close encounter with a grizzly bear.

Ethical Statement

All work in this thesis involving the capture and sampling of live badgers was carried out by the Food and Environment Agency under English Nature and UK Home Office licences, in accordance with the Animals (Scientific Procedures) Act 1986 and was subject to an internal ethical review process.

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AUTHOR'S DECLARATIONS

During the research contributing to this thesis Andrew Robertson (AR) was supported by a studentship from the ESF. All the chapters presented in this thesis were written by AR with comments and editing from Stuart Bearhop (SB), Robbie McDonald (RM) and further comments from Richard Delahay (RD) and Simon Kelly (SK). The Woodchester Park study population used in all chapters (2 to 5) is funded by DEFRA.

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CHAPTER 1: General introduction

1.1 Introduction

Each of the data chapters within this thesis (Chapters 2,3,4 & 5) was written as a stand-alone piece of research with the aim of being published as separate scientific papers. This has resulted in a certain degree of repetition, particularly with regards to the methods section within each chapter. In this rest of this introductory chapter introduction I shall introduce the main ideas and themes underlying the work, and go on to outline the structure of the thesis itself.

1.2 Ecological niche variation

It has long been recognised that individuals within species may differ in their morphology, behaviour or physiology and that such differences might result in variations in the ability to use differing resources of habitats (Darwin 1859). This variation between individuals is central to the process of natural selection (Darwin 1859). Adaptation by natural selection towards differing resources or differing 'niches' is therefore a key factor in evolutionary theory and understanding diversity within the natural world.

The concept of the 'niche' was first applied to the field of ecology in the early 20th century as a means to describe the environmental requirements of a species, as well as its diet and foraging style (Grinell 1917). Later concepts described the niche as encompassing the ecological role or 'recess' occupied by a species, or group of species, within a community (Elton 1927). These earlier ideas were then further developed into the most widely applied concept of the ecological niche by Hutchinson (1957). Hutchinson described the ecological niche as an n-dimension hyper volume with axes that represent different biotic and abiotic variables which determine the conditions which a species requires to survive and reproduce (Hutchinson 1957). Hutchinson (1957) also made the distinction between the *fundamental* and *realised* niche. The fundamental niche is the total range of conditions which permit the existence of the species. In contrast, the realised niche is the niche which is actually occupied in reality, and may be smaller than the fundamental niche due to competition with other species, which constrain niche width (Hutchinson 1957).

Traditionally the concept of the ecological niche has been applied at the species level, with the aim of investigating the factors which determine species coexistence and divergence, and ultimately what factors influence the structure, composition and diversity within ecological communities (Macarthur and Levin's 1967, MacArthur and Wilson 1967, May and MacArthur 1972, Levine & HilleRisLambers 2009). As a consequence it was generally assumed that niche variation primarily occurred among species, such that individuals within a species may be effectively characterised by average species level values. However, it has also long been acknowledged that populations are heterogeneous with regards to a variety of traits, and that ultimately this could result in variation in the ability of individuals to utilise resources within a population (Darwin 1859).

Early work by Van Valens (1965) acknowledged the potential importance of niche variation within populations. Van Valens identified that island populations of several passerine species occupied a wider niche and exhibited greater phenotypic variation than those on the mainland. He suggested that the reduction in intraspecific competition on islands facilitated niche expansion at the population level and that this was achieved via greater niche variation between individuals, rather than all individuals expanding their niche accordingly. This is the basis for the 'Niche expansion hypothesis' that generalised populations are also more heterogeneous (Van Valen 1965). Further studies of Anolis lizards by Roughgarden (1972, 1974) provided a quantitative framework for measuring niche variation within populations. Roughgarden (1972) stated that the total niche width (TNW) of a population along a given continuous niche axis (such as prey size), can be described as the range that the population obtains most (e.g. 95%) of its resources (Roughgarden 1972). Specialised species or populations therefore have narrower TNW than those of generalists, as they consume a narrow specialised range of resources. Roughgarden (1974) also suggested that a population's TNW can be divided into two distinct components; the 'within individual component' (WIC, which reflects the range of resources exploited by an individual) and the 'between individual component' (BIC, reflecting the differences between individuals) which together combine to equal TNW (TNW=WIC+BIC, Roughgarden 1974). Generalist populations with a broad TNW can therefore exist either due to all individuals consuming a large range of resources (high WIC

+ low BIC= low individual niche variation) or due to individuals consuming a narrow range of resources, but being highly variable in the niche that they occupy (low WIC + high BIC= high individual niche variation, Roughgarden 1974; Grant & Grant 1976).

Such intrapopulation niche variation has historically been attributed to differences among distinct classes or subsets of the population (Schoener 1986). For example, males and females may differ in their resource or habitat use as a function of physiological differences or energetic requirements, which is termed 'ecological sex dimorphism'. Age classes may also differ in the resources they use, due to phenotypic or behavioural changes throughout the course of development and growth resulting in 'ontogenic niche shifts'. Some species may also contain 'resource polymorphisms', with discrete morphological classes which occupy distinct niches (Smith & Skúlason 1996).
Until relatively recently individual niche variation independent of such factors was believed to be rare and ecologically unimportant. However, Bolnick *et al.* (2003) challenged this view by reviewing the literature and finding evidence of individual niche variation unrelated to age, sex or morphological reasons in over 100 species in a wide range of taxa. For example sea otters *Enhydra lutris* are generalist predators at the population level consuming a variety of marine prey, but within populations individual otters specialise on consuming a narrow range of prey types and this is unrelated to age or sex (Estes *et al.* 2003). Bolnick *et al.* (2003) term this 'Individual specialisation' and suggest that this behaviour is not only widespread but also has several important ecological and evolutionary implications (Bolnick *et al.* 2003).

Since the review by Bolnick *et al.* (2003) there has been increased interest in individual niche variation, with evidence of individual specialisation now recorded in close to 200 species (189 species in a recent review, Araújo, Bolnick, & Layman 2011). There is also growing recognition that individual niche variation has important implications for ecological and evolutionary processes, both at the population and the community level (Bolnick *et al.* 2011; Violle *et al.* 2012; Sih *et al.* 2012; Dall *et al.* 2012). As a consequence researchers are becoming increasingly interested investigating in the causes and consequences that drive and maintain individual specialisation within populations (Araújo *et al.* 2011).

In some species such as sea otters the causes of individual specialisation are relatively well studied. For example, studies have shown that the degree of individual specialisation increases with competition for resources (Tinker, Bentall & Estes 2008; Tinker *et al.* 2012) and that individual foraging preferences may be passed down matrilines via social learning (Estes *et al.* 2003; Tinker, Mangel & Estes 2009). Individual differences in habitat or prey utilisation in sea otters also results in fitness consequences, as certain resources result in a higher risk of pathogen exposure (Johnson *et al.* 2009).

Although studies are increasingly beginning to investigate individual specialisation in more detail, the causes and consequences of this behaviour remain unknown in the majority of cases where it occurs (Araújo, Bolnick & Layman 2011). For example it is often not known whether specialisation is due to differences in the realised niches (e.g. due to competition) or fundamental niches (e.g. due to resource preference or heritable differences) of individuals. Furthermore, it is often unclear if niche variation results in corresponding variations in fitness (Bolnick *et al.* 2003; Woo *et al.* 2008). Investigating individual specialisation requires data on long-term resource use, combined with detailed individual life history and ecological data which is challenging to obtain. It is therefore not surprising that relatively few studies can approach these questions. However, given the widespread occurrence and the potential implications of individual specialisation, further studies are required in order to further understand this complex and important behaviour.

1.3 Stable isotope analysis as a tool to study niche variation

Stable isotopes are naturally occurring stable variants of chemical elements which differ from each other in the number of neutrons in their nucleus, resulting in corresponding differences in atomic mass. For example, carbon atoms can exist in two stable forms, either with six protons and six neutrons, resulting in an atomic mass of 12 (^{12}C), or six protons and seven neutrons, resulting in a atomic mass of 13 (^{13}C). Due to differences in their physical properties, molecules containing different stable isotopes react at different rates. For example, water molecules containing heavier stable isotopes of hydrogen or oxygen evaporate and precipitate at differing rates to those with lighter isotopes, a process termed ‘fractionation’. This fractionation results in isotopic gradients within the

natural world at a range of scales, which are aligned to chemical and biological processes (Ben-David *et al.*2012).

A consumer's tissues are synthesised using the molecular components from their diet and as a consequence the isotopic ratios within consumer's tissues reflect that that of their diet over the period of tissue synthesis (Deniro & Epstein 1978, 1981). Isotopic ratios are measured relative to international standards and expressed as δ values in parts per mil or parts per thousand ‰. With respect to foraging studies the most commonly measured isotope ratios are that of ^{13}C to ^{12}C (expressed as $\delta^{13}C$) and ^{15}N to ^{14}N (expressed as $\delta^{15}N$) as these isotopes fractionate within ecosystems due to biological processes which make them particularly ecologically informative. Within food webs, $\delta^{15}N$ fractionates with trophic level, as isotopically light nitrogen is preferentially lost in nitrogenous waste such that $\delta^{15}N$ increases by approximately 2-5‰ with each trophic step (Deniro & Epstein 1981; Post 2002). In comparison $\delta^{13}C$ varies slightly by approximately 1‰ with each trophic step, but instead varies predominantly across the base of the food web as different producers differ in their ^{13}C fractionation when utilising CO_2 from the atmosphere (Deniro & Epstein 1978). This results in $\delta^{13}C$ differences between plants in marine, terrestrial and freshwater environments and between those using C_4 and C_3 photosynthetic pathways (Smith & Epstein 1971). The $\delta^{15}N$ and $\delta^{13}C$ values of a consumer's tissue therefore reflect an amalgamation of the trophic levels and habitats that the consumer has utilised over the period of tissue growth (Deniro & Epstein 1978, 1981). This isotopic information can then be used to infer differences in foraging behaviour or diet. For example, by measuring the $\delta^{13}C$ of arctic fox (*Aloplex lagopus*) fur it is possible to differentiate foxes consuming seabirds and shellfish differ from those consuming small mammals and game birds (Angerbjörn *et al.* 1994; Dalerum *et al.* 2012). Similarly by measuring the $\delta^{15}N$ in brown bear claws it is possible to identify individual bears consuming either plant or animal prey (Edwards *et al.* 2011).

SIA is particularly well suited to studies of individual niche variation and is increasingly being applied to this subject (Bolnick *et al.* 2003; Araújo, Bolnick & Layman 2011). Studies of individual niche variation require data on individual resource use over long temporal scales in order to disentangle resource preference from the actions of

foraging randomly from among patchily distributed resources (Bolnick *et al.* 2002, 2003). This is difficult to obtain using traditional methods such as faecal or gut content analysis which reflect diet over hours or days and therefore require extensive sample collection to characterise individual diets (Prugh 2005). Using SIA it is possible to get long term measures of individual resource use, either by serially sub sampling from inert tissues which are grown over extended periods , such as vibrissae or teeth (e.g Newsome *et al.* 2009; Lowther & Goldsworthy 2011), by simultaneously sampling several tissue types which reflect differing time scales, such as blood and feathers (e.g Bearhop *et al.* 2006), or by repeatedly sampling individuals at differing time periods (e.g Votier *et al.* 2010).

As the $\delta^{15}N$ and $\delta^{13}C$ of consumers tissues reflect the habitats and resources utilised, the isotopic variance within an individual (among multiple samples analysed) or within a population (among different individuals) therefore reflect the diversity of resources or prey types utilised (Bearhop *et al.* 2004). Some authors have suggested the use of the term 'isotopic niche' (Newsome *et al.* 2007) as isotopic axes are comparable to axes traditionally used to describe a species niche. By combining isotopic measures of niche width with traditional metrics used to quantify niche variation, it is therefore possible to quantify the degree of 'isotopic niche specialisation' within populations. For example the isotopic variation within and among individuals within a population is comparable to the within individual component (WIC) and between individual component (BIC) of niche width (Roughgarden 1974; Newsome *et al.* 2009).

Although isotopic differences among individuals may arise due to differences in resource use, physiological differences may also result in isotopic heterogeneity within a population (Mathews and Mazumder 2004). For example, even in captive conditions with an identical diet, isotopic differences may occur between individuals, although such differences are typically far less than 1‰ (Deniro and Epstein 1978, Roth and Hobson 2000, Hilderbrand *et al.* 1996). In some instances high levels of nutritional stress may also generate isotopic differences independent of diet, as stressed individuals catabolise their own muscle tissues resulting in elevated N^{15} values (Cherel *et al.* 2005). Physiological processes such as growth, pregnancy and lactation may also potentially result in isotopic differences independent of diet, although relatively few studies have explored these

relationships (Newsom *et al.* 2010). Researchers therefore need to be cautious in their interpretation of isotopic differences; particularly where the magnitude of such variation is small and where pronounced physiological differences between individuals are likely. The use of Bayesian isotopic mixing models also makes it possible to further investigate individual differences in diet or resource use. Isotopic mixing models compare the isotopic composition of consumers to that of their potential prey in order to calculate the estimated combination of these sources which combine to produce the consumer signature observed (Phillips 2012). The values obtained from these models can therefore be used to investigate the consumption of a specific resources consumed, or turned into several metrics of individual niche variation (Bolnick *et al.* 2002; Newsome *et al.* 2012). SIA is therefore a powerful method for quantifying and investigating individual niche variation within wild populations.

1.4 Badgers as a model to study niche variation

The European or Eurasian badger *Meles meles* is a medium sized mustelid (Figure 1.1) with a wide geographical distribution from Ireland in the west, to the Volga river in Russia to the East, and from Scandinavia in the north to Spain, Crete and Afghanistan in the south (Figure 1.2). Badgers are commonly described as generalist omnivores occupying a diverse array of habitat types and consuming a wide range of animal and plant prey (Roper 1994, Roper 2010). Depending on the environment occupied, potential dietary items may include insects (adults and larvae), earthworms, gastropods, small mammals, lagomorphs, carrion, birds, reptiles, amphibians, fruits, nuts, roots, cereals and human refuse (Roper 2010). In some instances badgers occupy a narrow 'specialised' niche where certain prey sources are highly abundant. For example, in areas of southern Spain, badgers primarily consume young rabbits *Oryctolagus cuniculus* (Martín, Rodríguez & Delibes 1995). In the UK, several studies have shown that badgers primarily consume earthworms, which lead to the hypothesis that badgers were in fact earthworm specialists (Kruuk *et al.* 1979; Kruuk & Parish 1981), although this has largely been discredited (Roper 1994).

European badgers live in social groups occupying a burrow system or 'sett' located centrally within a defended communal territory. Social group size varies across their

range, with small groups of two to three individuals in southern and central Europe, and larger groups of on average five, but occasionally up to 20 or more individuals in the UK (Johnson, Jetz, & Macdonald 2002, Roper 2010). Small groups are generally composed of reproductive pairs and their offspring, while larger groups are composed of several adult individuals of both sexes (Kruuk 1978a). Within social groups, individual badgers potentially compete with one another for mates and food resources. Aggressive encounters between individuals resulting in bite wounds are not uncommon, particularly between males (Delahay *et al.* 2006b). Compared to other social carnivores badges have a relatively loose social structure without a strict dominance hierarchy (Hewitt, Macdonald & Dugdale 2009) . Badgers have a polygnandrous mating system, with several individuals within a group potentially reproducing, resulting in multiple paternity in some instances (Dugdale *et al.* 2007). However, females will compete for reproductive status resulting in a female social hierarchy in some cases (Woodroffe & MacDonald 1995), but not others (Hewitt, Macdonald & Dugdale 2009).

Badgers are an excellent candidate for studying niche variation. Studies at the social group level have demonstrated that significant niche variation may exist within badger populations, although primarily due to variations in territory habitat composition (Hofer 1988). Within social groups niche variation could potentially occur between individual badgers for a variety of reasons. Firstly badgers are sexually dimorphic with differences in size and skull morphology between males and females (Johnson & Macdonald 2001). Differences in morphology between sexes correlates with differences in resource use in other mustelid species such as stoats *Mustela erminea* (McDonald *et al.* 2002) and American mink *Neovision vision* (Birks & Dunstone 1985). Secondly, despite being social animals badgers forage predominantly on their own away from the rest of the group (Kruuk 1978b; Kowalczyk & Zalewski 2006), therefore potentially utilising different resources. The broad range of potential resources available to badgers may mean that it is difficult for individuals to effectively utilise all prey types and it may be more efficient to specialise (Bolnick *et al.* 2003). Social interactions between individuals within groups of social mammals may also potentially result in individual niche variation via competition (Darimont, Paquet & Reimchen 2009) or vertical or horizontal transmission of learned

specialised behaviours (Estes *et al.* 2003; Sargeant *et al.* 2006; Sargeant & Mann 2009). However, despite a substantial body of published research on badger foraging ecology individual niche variation within this species has not been explored, largely due to the limitations of the traditional methods used such as gut content and faecal analysis (Kruuk & Parish 1981; Cleary *et al.* 2009). Radio-telemetry and observations using cameras or night vision equipment have also been used to investigate foraging patterns and habitat use, however the sample sizes of such studies are invariably small, due to the expense of these techniques (Kruuk *et al.* 1979; Kowalczyk & Zalewski 2006). As a consequence, age, sex or individual niche variation within groups has not been investigated.

Badgers are also a particularly good candidate species to investigate individual niche variation as their social territorial behaviour results in a situation where all individuals within the same social group share the same foraging environment. This makes it possible to investigate individual differences in foraging where resource availability is effectively controlled. As individual badgers share the same territory and have individual home ranges which overlap almost entirely, such that the social groups territory is a shared resource (Roper 2010). Individual differences in foraging niche within groups are therefore not due to differences in resource availability but due to differences in foraging behaviour or resource preference. As social groups potentially differ from one another in their group or habitat composition it is also possible to compare niche variation within differing groups to investigate how varying ecological factors influence patterns of niche variation.

Individual niche variation in badgers also has potentially important implications. In some locations badgers are also agricultural pests, consuming significant amounts of cereal and fruit crops (Roper *et al.* 1995; Moore *et al.* 1999). Badgers are also a reservoir for *Myobacterium bovis* the causative agent of bovine tuberculosis (Krebs 1997), a disease which has increased in prevalence in the UK in recent decades and is currently of intense management focus (Bourne 2007). Badgers forage regularly on cattle pastures where worm density is high (Kruuk *et al.* 1979) and within farm buildings where they will consume animal feed (Garnett, Delahay & Roper 2002; Tolhurst *et al.* 2009). Both these activities potentially bring badgers into close contact with cattle, resulting in the

possibility of disease transmission either via direct or indirect (e.g via faeces or contaminated food) mechanisms (Tolhurst *et al.* 2009). Individual variations in foraging behaviour may therefore have important implications for the management of the disease in cattle.

1.5 The Woodchester park study system

All of the research in the current thesis was carried out using the long-term study population of badgers at Woodchester Park Gloucestershire, UK (2°16' E, 51° 43 'N,). This population has been the location of a long-term mark recapture study of badgers since 1976, carried out by the food and environment research agency (FERA). The primary aim of this research has been to investigate the disease dynamics of bovine tuberculosis for the purposes of informing disease management. The Woodchester Park study area is approximately 7km^2 and consists of a central wooded valley comprising mixed deciduous (mainly beech *Fagus sylvatica,* hazel *Corylus avellana* and ash *Fraxinus excelsior*) and coniferous (Douglas fir *Pseudotsuga menziesii*, Norway spruce *Picea abies* and larch *Larix* spp.) woodland and surrounded by farmland (Delahay *et al.* 2006a). This habitat supports a population of around 200 individuals in approximately 20 social groups making this one of the highest density badger populations known, with a density of >20 adults per km^2 (Roper 2010).

Each year badger social group territories within Woodchester Park are mapped in the spring using a bait marking technique (Delahay *et al.* 2000, Figure 1.3). This involves feeding bait (peanuts and syrup) containing a different colour of indigestible plastic beads at each active main sett (and then surveying the study area to record the presence of the beads at latrines which are particularly concentrated along territory borders (Delahay *et al.* 2000). Cage trapping of badgers is carried out at all active setts within territories four times per year, roughly coinciding with each season. Once captured, individual badgers are anaesthetised by an intramuscular injection of a combination of ketamine hydrochloride (Vetalar™, Pharmacia and Upjohn, Crawley, UK), medetomine hydrochloride (Domitor®, Pfizer, Sandwich, UK) and butorphanol tartate (Torbugesic®, Fort Dodge Animal Health ltd, Southampton, UK) (de Leeuw *et al.*, 2004). Individuals are

then permanently marked with a unique id tattoo , samples of blood, saliva and urine are taken for analysis and a range of variables are recorded, including sex, weight, body condition, length, reproductive status and tooth wear (Cheeseman & Harris, 1982)

Working with this study population provides an ideal situation to investigate individual niche variation. The regular mark-recapture protocol means that we can potentially obtain samples for SIA repeatedly from a large number of individuals. Crucially working with this long-term study also provides detailed population level ecological data, along with individual life history data which can be matched to individual foraging data.

1.6 Thesis outline

The main aim of thesis is to use stable isotope analysis of badger vibrissae in combination with the detailed long-term study population at Woodchester Park in order to use badgers as a model system to investigate individual niche variation. Specifically I aim to use applications of stable isotope technology to test i) to what extent individual niche variation is driven by resource availability at the group level or individual variation within groups independent of these effects; ii) whether individual variation is the product of age or sex differences or due to individual specialisation; iii) whether the degree of individual niche variation varies with competition or resource availability and v) whether individual niche variation has fitness consequences for individuals.

However prior to addressing these questions I also aim to investigate several question fundamental to the application of a stable isotope approach to my chosen study system, namely, i) what is the growth rate and growth pattern of badger vibrissae?; and ii) how does SIA compare to traditional methods used to investigate badger diet?

Throughout my research I use badger vibrissae (whiskers) as my principle tissue for stable isotope analysis in order to quantify individual patterns of resource use. To date, very few studies have investigated vibrissa growth in mammals and none in badgers. In chapter 2 I investigate the growth of badger vibrissae to determine the rate of growth and potential patterns of shedding and retention. This information is crucial for informing further research using this tissue and provides me with a temporal scale for which to match further isotopic data obtained via SIA (Chapters 3, 4 & 5).

In chapter 3 I then use SIA of badger vibrissae along with faecal analysis in order to investigate the diet of individual badgers in spring and autumn at Woodchester to Park. By analysing isotopic patterns in badgers and their prey, I aim to compare methods and determine how isotopic patterns reflect seasonal changes in diet. I also determine the extent that isotopic gradients within a potentially diverse prey base can be utilised to disentangle specific resource use. This is key to understanding how isotopic patterns between individual may reflect variation in prey or habitat utilisation.

In chapter 4, I then investigate individual niche variation within social groups to determine if individuals within groups with the same territory, and therefore resource availability, differ in their foraging niche. I also investigate whether this variation is due to age, sex or individual specialisation.

In chapter 5, I further investigate the causes and consequences of individual niche variation within groups. By using several stable isotope derived metrics of individual specialisation I test the hypotheses that i) the degree of individual specialisation varies with competition and resource limitation, and ii) that niche variation correlates with measures of fitness.

Finally, in chapter 6, I discuss the overall findings from the thesis and outline potential directions for future research both in the areas individual specialisation and badger ecology.

Figure 1.1 An adult European badger (*Meles meles*).

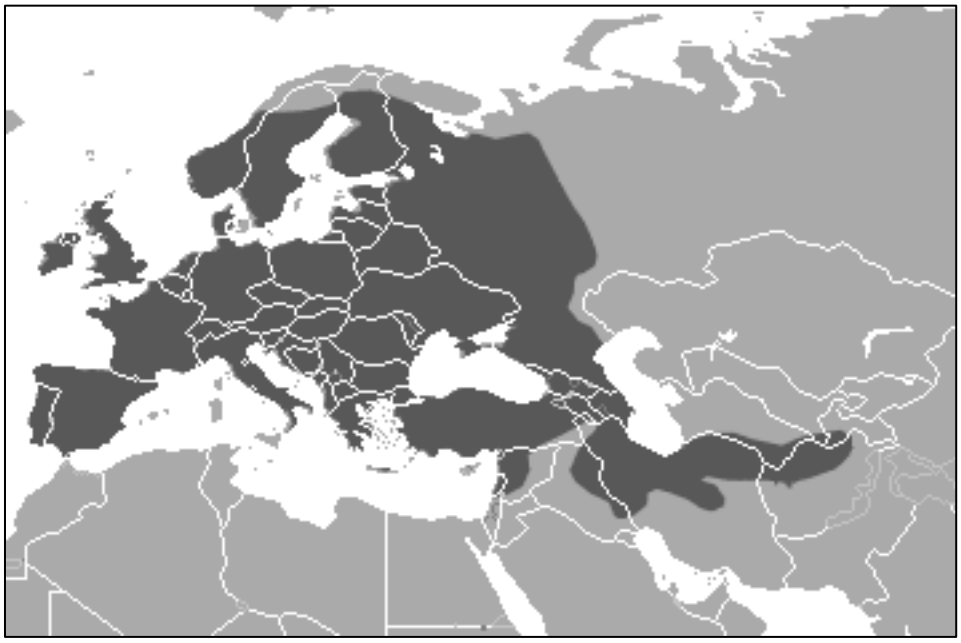


Figure 1.2 – The distribution of the European or Eurasian badger *Meles meles* (source of map - http://www.iucnredlist.org)

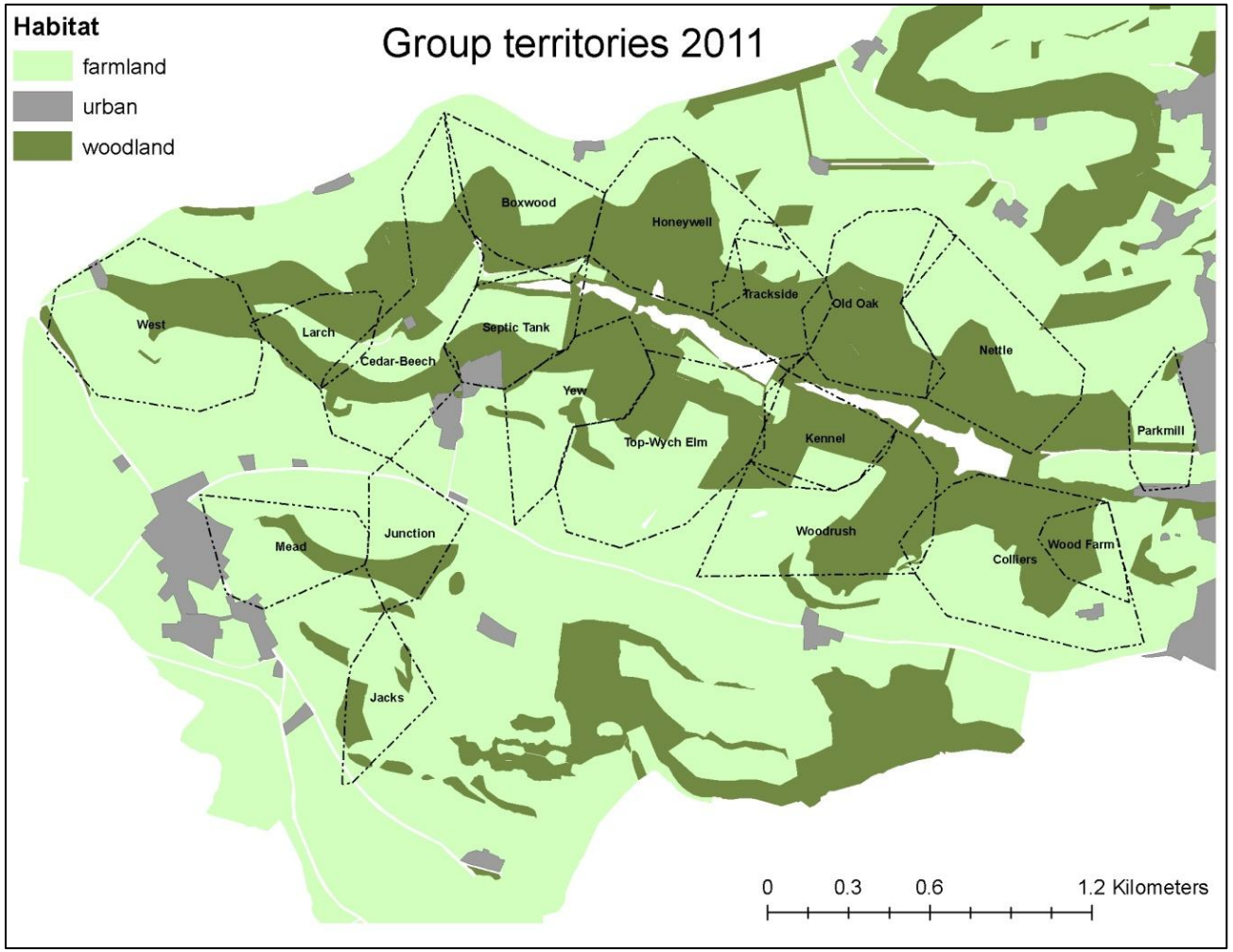


Figure 1.3 – Map of badger social group territories at Woodchester Park in 2011. Dashed lines represent territory borders labelled by social group ID. White areas in the centre of the map are open water. Data provided by FERA.

CHAPTER 2: Whisker growth in wild European badgers *Meles meles*: implications for stable isotope and bait marking studies

2.1Abstract

The use of biomarkers such as stable isotopes to study the foraging ecology and movement of animals is a rapidly expanding area of research. With respect to mammals, the analysis of inert keratinous tissue such as whiskers (vibrissae) is particularly attractive as they can be sequentially sampled to provide a long-term time series of individual movement or diet. However, in order to interpret data from such tissues researchers require details of growth rates and patterns, and also how these vary within populations. In this study we use the fluorescent biomarker Rhodamine B to measure vibrissa growth rate and patterns in a wild population of European badgers. In addition, we compare stable isotope ratio values of blood and vibrissae in order to test whether vibrissae are retained for long periods following growth. We found that badger vibrissae grow at an average rate of 0.43 mm day^{-1} (range 0.23-0.83) such that single vibrissae sampled for stable isotope analysis contain an average of 104 days of ecological data. Age, sex and body condition did not affect growth rate, and there was no evidence of consistent individual differences in growth rate or long term retention of vibrissae following growth. However, variation in growth rate within the population suggest that the temporal scales reflected in vibrissae may vary both between and within individuals, such that results are not always directly, temporally comparable. This research provides useful information for any future research using vibrissae in combination with biomarkers to study mammalian ecology.

2.2 Introduction

Stable isotope analysis SIA is becoming an increasingly important and widespread technique for investigating the foraging ecology and movement of animals (Kelly 2000; Crawford, McDonald, and Bearhop 2008; Inger and Bearhop 2008; Newsome, Clementz, and Koch 2010a). SIA works on the premise that the stable isotope ratios in a consumer's proteinaceous tissues reflect that of their diet (Deniro and Epstein 1978, 1981; Hobson

and Clark 1992). The technique utilises the natural isotopic variation that exists among habitats and different prey items. These isotopic heterogeneities among habitats and prey are then incorporated into consumer tissues in a predictable manner such that the isotope ratios of the consumer reflect those of the prey sources and habitats they have utilised. The temporal scale of diet and habitat selection reflected by the isotope ratios of consumer's tissue depends on the length of time over which that tissue was synthesized (Hobson et al. 1996; Bearhop et al. 2002; MacAvoy, Arneson, and Bassett 2006). In metabolically active tissues this is determined by the tissue turnover time. For example, the isotope ratios of plasma and red blood cells reflect dietary information over timescales of days and weeks respectively (Klaassen, Thums, and Hume 2005; Tieszen et al. 1983; MacAvoy, Macko, and Arneson 2005; MacAvoy et al. 2006; Kurle 2009). Similarly, the isotope ratios of inert keratinous tissues reflect foraging and movement patterns over the period of tissue synthesis, but this information is stored indefinitely after formation (Hobson et al., 1996), because the tissue ceases to be metabolically active once growth is complete. Inert tissues that have commonly been analysed when studying mammals include claw (Edwards et al. 2011; Hénaux et al. 2011), fur (Mowat and Heard 2006; Darimont, Paquet, and Reimchen 2007), whiskers / vibrissae (Bodey et al. 2010, Newsome et al. 2010b) and baleen (Best and Schell 1996; Caraveo-Patiño, Hobson, and Soto 2007). Inert tissues are advantageous as they can be serially sub-sampled to provide a time series of long-term and seasonal changes in resource use or movement (Hobson et al. 1996). For example, temporal shifts in the habitat preferences of individual American mink *Neovison vison* and long term individual niche specialisation in sea otters *Enhydra lutris* have been investigated using serial sub-samples of vibrissae (Bodey et al. 2010; Newsome et al. 2009).

Although stable isotope measurements of metabolically inert tissues can provide major insights into the long-term dietary and habitat preferences of individual animals, in order to interpret the patterns observed, researchers require information on the chronology of inert tissue synthesis. Only then is it possible to match isotopic information obtained to relevant temporal or spatial scales. Researchers therefore require information

on growth rates, growth patterns (how this varies with time/length) and patterns of shedding and moult.

Vibrissae have great potential in mammal research as a tissue of choice for isotopic investigations of diets. Unlike hairs that often grow in only short periods during moult and replacement, vibrissae are grown continuously (Fisher 1999). To date vibrissae growth rates have been established for several pinniped species (Hirons, Schell, and St. Aubin 2001; Zhao and Schell 2004; Greaves et al. 2004; Hall-Aspland, Rogers, and Canfield 2005; Cherel et al. 2009) and also sea otters (Tyrrell et al. in press), but only a small number of terrestrial species; laboratory rats *Rattus norvegicus* (Ibrahim and Wright 1975), laboratory mice *Mus domesticus* (Su et al. 1999, Ibrahim and Wright 1975) and stoats *Mustela erminea* (Spurr 2002). Patterns of vibrissae growth vary markedly between studies, with species exhibiting linear or non-linear growth patterns followed by varying levels of vibrissae retention and shedding.

As well as varying between species, vibrissa growth characteristics may also vary within populations (Ibrahim and Wright 1975). For example, age and food availability affects vibrissa growth in mice (Wright 1965; Ibrahim and Wright 1975). This is potentially problematic, as investigating individual differences is often the aim of studies using SIA (Bodey et al. 2010; Newland et al. 2011; Newsome et al. 2009,2010b) and variation in vibrissa growth will result in differences in the temporal scale over which foraging or movement is measured. To date, the majority of studies exploring vibrissae growth have been carried out in captivity (but see Cherel et al. 2009 and Hall-Aspland et al. 2005) and have involved small numbers of individuals (e.g Greaves et al. 2004; Hirons et al. 2001). As a consequence, intra-population variation in vibrissa growth in wild populations is yet to be explored.

The European badger *Meles meles* is a terrestrial mustelid whose foraging habits and ecology have been extensively studied (Roper et al 2011). In the UK badgers are implicated in the transmission of bovine tuberculosis to cattle (Donnelly et al. 2006) and previous research has suggested that foraging in cattle sheds and farm buildings by individual badgers may be important in disease transmission (Garnett, Delahay, and Roper 2002). Despite the potential importance of individual foraging variation in this species,

little research has been completed in this area to date largely due to the limitations of traditional methods to determine resource use. For example, it is not possible to assign dietary information from faecal analysis to individual animals, and gut contents offer only a snapshot of resource use. In contrast, SIA of vibrissae can potentially provide long-term individual dietary information (Newsome et al. 2009).

In this study, we aim to measure vibrissa growth rates and patterns in badgers as part of wider research aimed at using SIA of vibrissae to investigate individual foraging variation. In order to calculate vibrissa growth rates we used Rhodamine B (RhB); a fluorescent biomarker which, once ingested, is incorporated into growing keratinous tissues and is visible using fluorescence microscopy (Fisher 1999). RhB is commonly applied to investigate the consumption of toxic or other treated baits for management purposes (Johnston et al. 2007; Palphramand et al.2010; Smyser et al. 2010; Spurr, 2002; Urbano 2010), but it can also be used as a dietary tracer to investigate interspecific competition (Smyser et al. 2010) and movement (Rahelinirina et al. 2009).
Here we apply RhB and SIA to investigate vibrissa growth rate in a large wild population of badgers. This allowed us not only to estimate growth rate itself, but also how this varied within populations due to age, sex, body condition, season and individual differences. This research will add to what is currently a very short list of species with known vibrissa growth rates and establish the extent to which it varies within wild populations. This is valuable information for future research using biomarkers in combination with vibrissae to measure resource use in mammals.

2.3 Methods

Study populations

This study utilizes vibrissa samples taken from wild badgers captured as part of two studies investigating bait consumption and uptake. The first took place in the spring and summer of 2008 (Palphramand et al. 2010, Table 2.1) at Woodchester Park in Gloucestershire, England, where badgers have been intensively studied since 1976. The study area is divided into three zones for the purposes of trapping and two zones (A and B) were used for this study. The second study took place in three other areas of south

west England (near Bath, Cirencester and Langford respectively) in the spring and summer of 2010 (Palphramand et al. unpublished, Table 2.1). In addition, we used vibrissae and blood for stable isotope analysis collected from badgers caught at the Woodchester Park study site as part of routine ongoing mark recapture studies between 11th May and 16th June, 2010. In all study locations the badgers occupy a heterogeneous mixture of farmland and woodland habitats.

RhB bait preparation and deployment

Bait consisted of a mixture of peanuts, golden syrup, and Rhodamine B (Sigma-Aldrich, Dorset, UK) at a concentration of 100mg per 100ml bait. Each badger social group was fed between 8 and 35 100ml baits (depending on group size) placed under paving slabs around sett (burrow) entrances in 2008 (Palphramand et al. 2010), and 30 baits (15 placed down tunnel entrances and 15 under paving slabs on the surface) in 2010. Consumed baits were replaced daily over an 8 day period in 2008 and over 12 days in 2010 (Table 2.1).

Sample collection

Following bait deployment, individual badgers were captured in steel mesh traps baited with peanuts after a period of 24-74 days (depending on location and year, Tables 1). Vibrissae were then collected from anaesthetised badgers by either cutting as close to the skin as possible using steel scissors (2008) or by plucking with steel forceps (2010). In both cases the longest vibrissa was taken from either side of the snout resulting in two samples, although, in some cases only one vibrissae sample was obtained.

Vibrissae and blood samples for SIA were collected from animals trapped as part of regular mark-recapture studies at Woodchester Park in the spring of 2010. Vibrissae were collected from 49 anesthetised animals by cutting them as close to the surface of the skin as possible using steel scissors. In addition approximately 1ml of blood was taken from the jugular vein of each animal using a syringe and a non-heparinised vacutainer. Blood was immediately (prior to clotting) transferred to a centrifuge and spun at 5,000 rpm for 8 minutes to separate samples into plasma and cellular (RBC) components. The two components were then separated using a sterilised syringe and immediately frozen at -20ºC. All work involving the capture and sampling of live badgers was carried under

English Nature and UK Home Office licences, in accordance with the Animals (Scientific Procedures) Act 1986 and was subject to an internal ethical review process.

Vibrissa measurements

Vibrissae were placed on microscope slides and observed at ×4 magnification using a fluorescent microscope with a UV filter (Olympus BX61, with analysis^{D} software, www.olympus.com). Where RhB bands were present four measurements were taken; the distance from the vibrissa base to the start of the Rhodamine band (Figure 2.1, A), the length of the Rhodamine band (Figure 2.1, B), vibrissa tip length (Figure 2.1, C) and the total vibrissa length (Figure 2.1, A+B+C). In plucked vibrissae the root of the vibrissa was also inspected to identify the presence of club roots (Fisher 1998).

Growth rate calculations

If growth rate is linear, segments of equal length represent an approximately similar time interval anywhere along the vibrissa (Hirons et al. 2001). Assuming vibrissae are growing, the growth rate (mm day^{-1}) is therefore the distance from the tip of the Rhodamine band to the vibrissa base (length A + B on Figure 2.1) divided by the length of time between bait consumption and individual capture (Table 2.1). As the bait was available for eight days and it was not known on which day bait was first consumed, there is a range of potential growth periods for each vibrissa (Table 2.1). We therefore calculated eight estimates for growth rate for each vibrissa (one for each day of potential bait consumption), then randomly re-sampled from this range of values 1000 times to calculate a mean and range for each vibrissa. However, measurements of cut vibrissae do not include new growth below the skin surface and will therefore underestimate growth rate. In order to correct this we used analysis of covariance to compare the length of 'new growth' (distance from tip of RhB band to vibrissae base, length A + B on Figure 2.1) of cut vibrissa sampled in summer with those of plucked vibrissae, while controlling for differences in growth period. The mean difference in length was then added as a correction factor to each cut vibrissae. Following analyses were then carried out using the cut vibrissae data. We report mean and 95% interquartile ranges of growth rate estimates in the results to provide a

measure of population level variation as opposed to a measure of uncertainty around the mean such as a 95% confidence interval.

Stable isotope analysis

Individual badger vibrissae were rinsed in distilled water to remove surface contaminants; sub sampled into 0.4-0.5mg sections using a scalpel and then sealed in tin capsules for isotope analysis. Blood samples were dried at 60ºC for 72 hours, homogenised using a pestle and mortar and then approximately 0.8mg of material was sealed in tin capsule for isotope analysis. Measurements were performed using an elemental analyzer EA 1108 (Carlo Erba Instruments, Milan, Italy) coupled to an Isoprime IRMS (GVI, Manchester, UK) configured for simultaneous carbon and nitrogen stable isotope analysis. Isotope ratios are expressed as δ values, which is reported in parts per thousand or per mil (‰) with reference to international standards $\delta X = 1000 [(R_{sample} / R_{standard}) - 1]$. Where R_{sample} is the ratio of heavy to light isotopes ($^{13}C:^{12}C$ or $^{15}N:^{14}N$) and $R_{standard}$ is that of the standard (C = Vienna-Pee Dee Belemnite, N = atmospheric nitrogen). Within-run mean accuracy of a collagen standard was 0.05‰ (standard deviation) for $\delta^{13}C$ and 0.11‰ for $\delta^{15}N$.

Evidence of telogen or long-term vibrissae retention

We compared stable isotope ratios of an individual's red blood cells and plasma to that of their serially sampled vibrissae sections to investigate whether there was evidence of long-term retention following growth. We hypothesised that if vibrissae were growing or had only recently stopped growing, isotope ratios of blood components will be highly correlated with the base of the vibrissae as both will represent the badger's diet over a relatively recent period. The degree of correlation will then decline along the vibrissa length as older tissue is less correlated with contemporary isotope signatures in blood. Alternatively, if vibrissae have ceased growing the blood and vibrissae isotope ratios will be poorly correlated, due to a disparity in the temporal periods represented. As vibrissae analysed varied in the number of sections taken (3-7, mean = 4) we used only the three basal sections (described as 'section 1' or base, 'section 2' and 'section 3' moving from the base along the vibrissae length) so that results were comparable between individuals.
In order to investigate the relationship between vibrissae and blood isotope ratios we carried out four general linear mixed models analyses (one for each isotope and blood

component). Either vibrissa $\delta^{15}N$ or $\delta^{13}C$ were the response variables, with vibrissa section and the corresponding isotope values of either RBC or blood plasma as fixed effects and badger id as a random effect. We then tested for the significance of a two-way interaction between vibrissa section and the isotope values of blood components via model simplification to determine if the degree of correlation decreased along the vibrissa length. In addition we carried out univariate correlation test quantify the degree of correlation between blood components and each vibrissa section.

Relationships between length, time and mass

As samples for SIA are measured in mass rather than length, we measured the relationship between cumulative mass and length in 20 serially sub-sampled vibrissae (mean length 43mm, range 33-52mm) in order to estimate the time period reflected by individual isotope samples along an average length vibrissa used for SIA (45mm). We first compared linear and quadratic models using AIC model comparisons to compare linear and quadratic models to determine if the mass of individual sections along the vibrissa length were of comparable mass or if mass declined from base to tip in a non-linear relationship. We then used estimates of growth rate to calculate the likely time represented by equal mass sections along the length of an individual vibrissa.

Factors explaining within population variation in growth rate

To investigate which factors influence vibrissae growth within the population we used general linear mixed models with standard stepwise removal of non-significant terms. The response variable was growth rate, but as we had a range of growth estimates for each vibrissa, we constructed three models to assess the sensitivity of the results to error in our estimates of growth rate (using either 25^{th} 50^{th} or 75^{th} percentile values). Fixed effects included were; age (adult or cub), sex, body condition and max growth period in days (Table1). Body condition is scored between 1 and 5 (1=very poor, 2=poor, 3=fair, 4=good, 5=very good) based on fat coverage and musculature of shoulder blades, pelvic region, ribs and vertebrae. The majority of individuals sampled were in fair or good condition, with only small numbers in poor (n=2) or very good (n=1) condition, so these were merged with fair and good categories respectively to create a two level factor. Growth period was

included to test for non-linear growth, the assumption being that if growth rate declined with length, then vibrissae sampled after a longer period of time would have lower growth rates. Individual badger was included in the model as a random effect to account for repeated individual measurements. In order to quantify the relationship between mass and length along a single vibrissa we used GLMMs with mass as the response, length as a fixed effect and individual as a random effect. We used backwards stepwise removal of fixed effects and likelihood ratio tests using maximum liklihood simplification to test the significance of individual fixed effects and random effects included in models. All statistics were carried out using R 2.13.1 (cran.r-project.org).

2.4 Results

A total of 190 vibrissae from 97 individuals with evidence of bait consumption (at least one Rhodamine band in one vibrissa) were analyzed; 135 cut vibrissae from 74 individuals collected in 2008 and 46 plucked vibrissae from 23 individuals collected in 2010. In the cut vibrissae data set, 11 animals with two vibrissae samples had fluorescent bands in only one of the vibrissae analyzed, suggesting that the unmarked vibrissa had either stopped growing or emerged after bait consumption. In the plucked vibrissae data set two vibrissae from two individuals appeared broken rather than plucked, so were excluded from these analyses. Club roots (indicating the vibrissae had stopped growing) were noted in 10 (23%) plucked vibrissae. Analysis of covariance indicated that when controlling for differences in growth period (time of bait consumption to sampling), plucked vibrissae were on average 5.6mm (se 1.58) longer than cut vibrissae.

Vibrissa growth rate

Overall mean growth rate was 0.43 mm day^{-1} with a 95% interquartile range of 0.28-0.62 and a standard deviation of 0.1. Variation in growth rate was not explained by individual differences in age, sex or body condition for either of the three estimates of growth rate (25^{th}, 50^{th} and 75^{th} percentiles). All three estimates of growth rate declined significantly with length of growth period (X^2_1=6.8- 13.5, p=0.002-0.013), however, the magnitude of this effect was very small (value = -0.001 - -0.0016, Figure 2.2). Where two vibrissae were

sampled from the same individual, growth rates on either side of the snout were not significantly correlated (R_{58}=0.21, p= 0.09). Removal of the individual random effect from mixed models also did not significantly affect the variance explained (), further suggesting that individuals did not differ consistently in growth rate.

Correlations between blood and vibrissa stable isotope ratios

The $\delta^{15}N$ of badger vibrissa were closely correlated with the $\delta^{15}N$ of blood components and varied significantly with vibrissa section (GLMM, significant two way interaction between vibrissa section × RBC $\delta^{15}N$, X^2_2=21.34, p=<0.001, and between vibrissa section × plasma $\delta^{15}N$, X^2_2=31.74, p=<0.001) with a higher correlation close to the vibrissa base (section1) which declined along the vibrissa length (Figure 2.3). Vibrissa $\delta^{13}C$ values were also correlated with those of RBC (X^2_2=23.54, p=<0.001) and plasma blood components (X^2_2=8.702, p=<0.01), but the strength of the correlations were lower than those of $\delta^{15}N$ and did not significantly vary along the vibrissa length (GLMM, vibrissa section × RBC $\delta^{13}C$, X^2_2=4.20, p=0.12, vibrissa section × plasma $\delta^{13}C$, X^2_2=0.44, p=0.80, Figure 2.4).

Relationships between length, time and mass

The average length of vibrissae sampled for stable isotope analysis was 45mm, with an upper 95th percentile of 62mm. Using the mean growth rate and the 5th and 95th percentiles to calculate a range, a 45mm and 62mm cut vibrissa would represent on average 104 days growth (range 72-160 days) and 144 days growth (range 100-221 days) respectively, with a residual 5.6mm whisker bulb below the surface reflecting 13 days (range 9-20 days).

Cumulative mass and length follows a quadratic relationship (linear model AIC=-104, quadratic model AIC=-309), with the distal tip of the vibrissa contributing little mass to the overall total (Figure 2.5). The average vibrissa of 45mm length was dissected into four sections each weighing ~0.4mg. Using the relationship between cumulative mass and length (Figure 2.5), and the mean growth rate (and 5th and 95th percentiles to calculate a range), we estimate that the three basal 0.4mg sections moving along the vibrissa will represent time periods of 16 days (range 11-24), 17 days (range12 -26) and 20 days (range

14-31) respectively, with the remaining 0.54mg tip representing 52 days (range 36-80, Figure 2.6).

2.5 Discussion

Vibrissa growth rate

We estimate that the mean growth rate for cut vibrissae was 0.42 mm day^{-1} with a range of 0.23-0.83 (5^{th}-95^{th} percentile range (0.28-0.62). This growth rate is much higher than that of most pinniped species (leopard seals 0.08-0.1mm day^{-1}, Stellar sea lions 0.05-0.17 mm day^{-1}, Antarctic fur seals *Arctocephalus gazella* 0.13 mm day^{-1}), slightly higher than that of sea otters (0.21 mm day^{-1}) but within the range of values for laboratory mice (0.3-1.0 mm day^{-1}), rats (0.6 -1.5 mm day^{-1}) and similar to that of stoats (0.60mm day^{-1})(Cherel et al. 2009; Hall-Aspland et al. 2005; Hirons, Schell, and St. Aubin 2001; Ibrahim and Wright 1975; Spurr 2002; Zhao and Schell 2004, Tyrrell et al. in press).

Previous studies on laboratory mice indicate that food deprivation, disease and old age are factors which can reduce vibrissae growth rate (Wright 1965; Ibrahim and Wright 1975; Young and Oliver 1976). In badgers, age and body condition have also been shown to affect the timing of pelage moult, with early moult in juveniles and a later moult in individuals in poor condition (Stewart and Macdonald 1997). However, in the current study we found that vibrissa growth rate dit not differ signifcantly due to sex, age or body condition. Further more, there was no indication of consistent within individual variation in growth rate.

There was a significant decline in estimates of growth rate with growth period length, suggesting growth may be slightly non linear and decline over time. This differs from studies of rats and mice which exhibit linear growth at a relatively constant rate (Wright 1965; Ibrahim and Wright 1975, 1982). Non-linear vibrissa growth has been described in several pinniped species (Zhao and Schell 2004; Greaves et al. 2004; Hall-Aspland et al. 2005; Newland et al. 2011). It has been suggested that this is advantageous as it results in vibrissae rapidly attaining a length which is functional (Newland et al. 2011). However,in the current study, the observed change in growth rate of ~0.1mm day^{-1} over a 100 day period is much less than has been recorded in some pinniped species. For example growth

rate or leopard seal vibrissae may vary from 0.01 to 0.23mm day^{-1} over time depending on vibrissa age and length (Hall-Aspland et al. 2005).

Evidence of telogen or long-term vibrissae retention

The small reduction in growth rate with time in our study may reflect slightly non-linear growth, alternatively this may represent an under estimation of growth rate due to cessation of growth in vibrissae sampled after longer growth periods.

The method used to calculate growth rate in our study assumed that all vibrissae were growing constantly from the point of bait consumption to capture, as we divided vibrissae length (rhodamine band to base, Figure 2.1) by the time between these two periods. Spurr (2002) found that the distance between rhodamine band and vibrissae base was highly variable, though broadly related to the time between bait consumption and capture in stoats. However, as Rhodamine bands were present in only 51 of 91 stoat vibrissae analysed, it was suggested that this method is likely to underestimate true growth rate, as vibrissae are likely to have stopped growing during this period.

In our study, cessation of growth in marked vibrissae may have contributed to an underestimation of growth rate. Indeed, 23% of plucked vibrissae had evidence of club roots indicating they had stopped growing (Fisher 1998). However, out of a total of 89 capture events where two vibrissae were sampled from animals which had consumed marked bait, there were only 11 instances where a band was absent from one of the two vibrissae analysed. This clearly suggests that the majority of vibrissae collected were growing during the period when bait was available. It also indicates that long-term retention of vibrissae following growth is unlikely to occur, at least for periods longer than those between bait consumption and capture in this study (Table 2.1). In addition, the close correlation between RBC and plasma $\delta^{15}N$ with vibrissae $\delta^{15}N$ suggests long-term retention of vibrissae following growth is unlikely. Plasma and RBC have a turnover of several days and one to two months respectively (Hobson and Clark 1992; Hilderbrand et al. 1996; Bearhop et al. 2002; MacAvoy et al. 2005; Dalerum and Angerbjörn 2005) and the close correlation between these tissues and vibrissae base $\delta^{15}N$ values suggests that this tissue was recently synthesised over a similar timescale. The degree of correlation

between plasma $\delta^{15}N$ and vibrissae $\delta^{15}N$ also decreased markedly along the vibrissae length (Figure 2.3). This gives further support to the hypotheses that basal vibrissa tissue is recently synthesised, as older vibrissa tissue in the middle and tip is less closely correlated with current diet. Vibrissa $\delta^{13}C$ values were less strongly correlated with those of RBC or plasma. This is not surprising given the comparatively small amount of $\delta^{13}C$ variation in the population, as $\delta^{13}C$ varies predominantly with habitat (Crawford et al. 2008). Low levels of isotopic dietary variation can potentially make comparisons between tissue types problematic, as all individuals may have similar isotope signatures resulting in weak correlations. Similarly if the degree of isotopic dietary variation is too large this could also potential result in weak correlations due to high within individual variation and increased scatter in the data. Where variation in $\delta^{13}C$ does occur in the current study (for example some high vibrissa tip values), this is likely due to seasonal consumption of C4 plants such as maize which is available to some individuals and is not detected in the blood due to differing time periods reflected.

The above evidence points to it being unlikely that vibrissae are retained for long periods in badgers, as is the case in rats and mice (Wright 1965; Ibrahim and Wright 1975; Young and Oliver 1976). A small proportion of vibrissae sampled during this study may have ceased growing during the 'growth period', lowering estimates of growth rate and this may be more likely when the growth period is longer, resulting in a slight temporal decline with growth rate (Figure 2.2). However, given our large samples size, we believe this is unlikely to have had a marked effect on our overall estimate of growth rate.

Implications for using mammalian vibrissae for biomarker studies

Biochemical analyses of vibrissae has the potential to unlock numerous questions in the field of mammalian ecology (Crawford et al. 2008). However, in order to interpret fully the results of such studies researchers require knowledge of vibrissa growth rates and the factors which influence them (Newsome et al. 2010a). The results of the present study further highlight the potential of vibrissae as long-term markers of individual diet, containing on average 100 days, but potentially up to >200 days of foraging or movement information depending on their mass and length. Serial sampling of vibrissae can therefore provide repeat measurements over long temporal scales. However, our results

indicate that vibrissae subsections of similar mass do not represent equal time periods, with the vibrissa tip representing a much longer time period than sections nearer the base. Age, sex and body condition did not significantly influence vibrissa growth rates, however, the results of this study do highlight that growth rates may vary among individuals within wild mammal populations. The extent of this variation is relatively small and therefore unlikely to have large effects on measures of individual movement or foraging niche. However, researchers must still be cautious in their interpretation of individual patterns derived from vibrissa analysis, as temporal periods represented may not always be directly comparable and may introduce additional variation into the results obtained. In order to further facilitate the use of vibrissae in biomarker studies, future research should focus on determining growth patterns and rates in broader range of taxa, as well as further exploring the influences of other external factors such as season and diet on vibrissa growth. In addition, further studies are required to determine trophic discrimination factors from diet to vibrissae and other mammalian keratinaceous tissues, as this information is available of very few species (but see, Caut et al. 2009; Newsome et al. 2010c; Lecomte et al. 2011).

Table 2.1 Dates of bait feeding, capture and potential growth period (number of days bait availability and capture) for badgers in spring and summer in Woodchester Park in 2008 and Bath, Cirencester and Langford in 2010. Also included is the number of badger individuals sampled in each occasion (with number of vibrissae in brackets) separated into age, sex and body condition categories along with the vibrissa sampling method (plucked or cut) .

	2008		2010			
	Woodchester Park		Bath	Cirencester	Langford	Woodchester Park
	Zone A	Zone B				
Spring						
Feeding period	15-23 February	15-23 February	-	17-28 May	24May -4 June	-
Trapping period	6-7 May	20-21 May	-	22-25 June	28 June-1 July	11May -16 June
Potential growth period	*74-82 days*	*88-96 days*	-	*25-39 days*	*24-38 days*	-
sampling method	cut	cut	-	plucked	plucked	cut
Samples collected			-			
Males	4 (5)	6 (12)	-	1 (2)	2 (4)	25 (25)
Females	9 (15)	10 (16)	-	3 (6)	2 (4)	24 (24)
Adults	12 (19)	15 (26)	-	-	4 (8)	49 (49)
Cubs	1 (1)	1 (2)	-	4 (8)	-	-
Fair condition	5 (5)	5 (7)	-	4 (8)	-	-
Good condition	8 (15)	11 (21)	-	-	4 (8)	-
Total	13 (20)	16 (28)	-	4 (8)	4 (8)	-
Summer						
Feeding period	15-23 June	15-23 June	19-30 July	12-23 July	-	-
Trapping period	22-23 July	5-6 August	23-26 August	16-19 August	-	-
Potential growth period	*30-38 days*	*45-53 days*	*24-38 days*	*24-39 days*	-	-
sampling method	cut	cut	plucked	plucked	-	-
Samples collected					-	-
Males	6 (12)	12 (23)	2 (4)	5 (10)	-	-
Females	20 (39)	7 (13)	2 (4)	6 (12)	-	-
Adults	15 (30)	10 (18)	1 (2)	10 (20)	-	-
Cubs	11 (21)	9 (18)	3 (6)	1 (2)	-	-
Fair condition	9 (18)	8 (16)	4 (8)	2 (4)	-	-
Good condition	17 (33)	11 (20)	-	9 (18)	-	-
Total	26 (51)	19 (36)	4 (8)	11 (22)	-	-

Figure 2.1 Composite image of a badger whisker (vibrissa) as viewed through a fluorescence microscope, indicating vibrissa measurements. A is the base and C is the tip. The bright band in the centre (B) is the fluorescent Rhodamine B band. The rest of the vibrissa is visible due to high exposure for illustrative purposes.

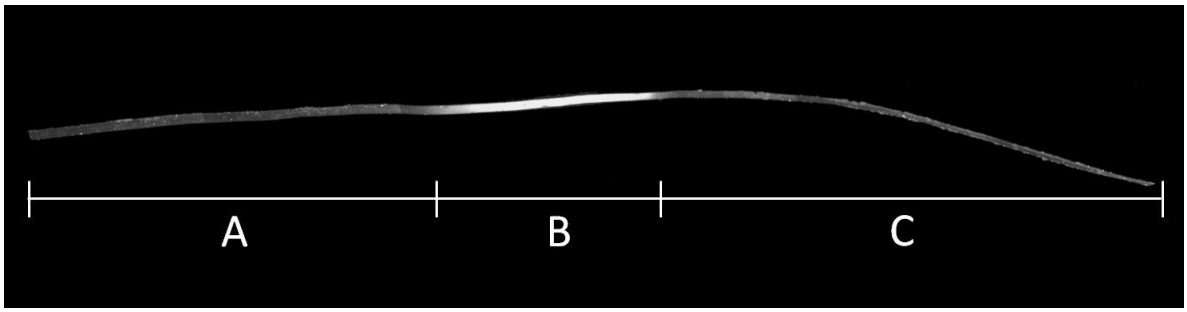


Figure 2.2 Relationship between growth rate and length of growth period for 135 cut badger vibrissae collected from badgers at Woodchester Park in 2008. Values are means, error bars encompass the range of growth rates calculated using the 25th and 75th percentile of growth rate calculated for each vibrissa.

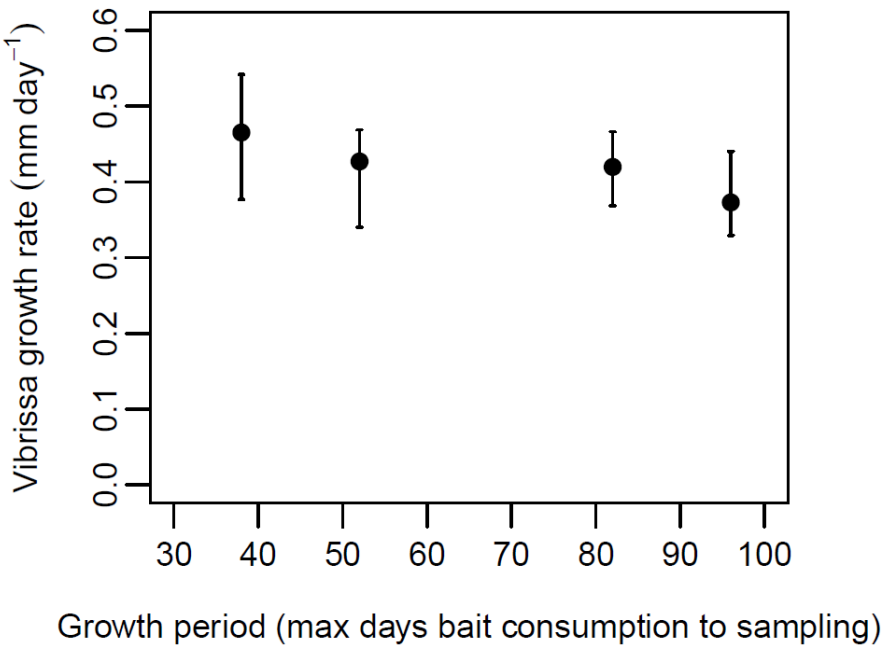


Figure 2.3 Correlations between $\delta^{13}C$ and $\delta^{15}N$ values of badger red blood cells (RBC) and plasma with those of vibrissae sub sampled along their length from base (section 1) towards the tip (sections 2 to3). Values are Pearson r. Data from 49 individuals caught in spring 2010 at Woodchester Park.

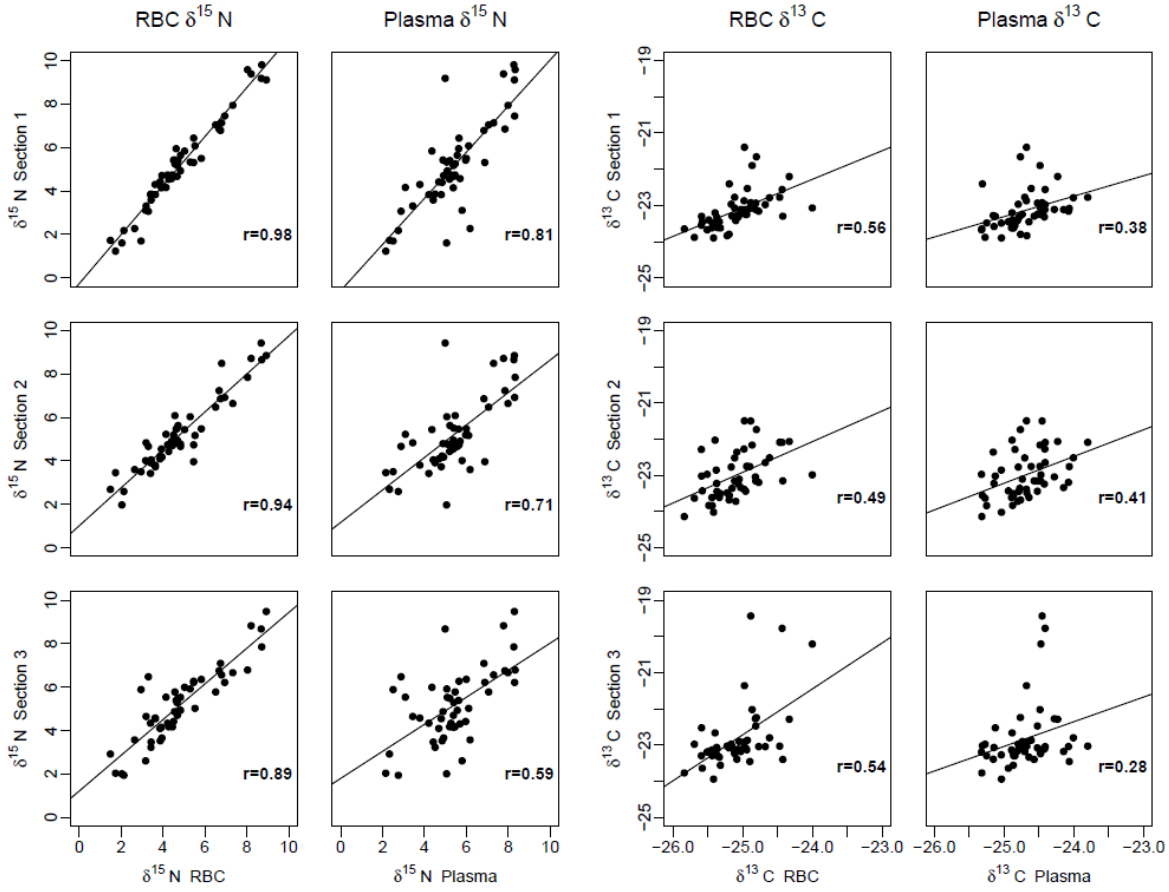


Figure 2.4 Relationship between cumulative mass and cumulative length for 20 badger vibrissae, the black solid line is the average second order polynomial relationship across all 20 vibrissae. The dashed lines are at 0.4mg intervals (0.4,0.8,1.2) and indicate the approximate breaks between vibrissae sections sub-sampled for SIA (Figure 2.5)

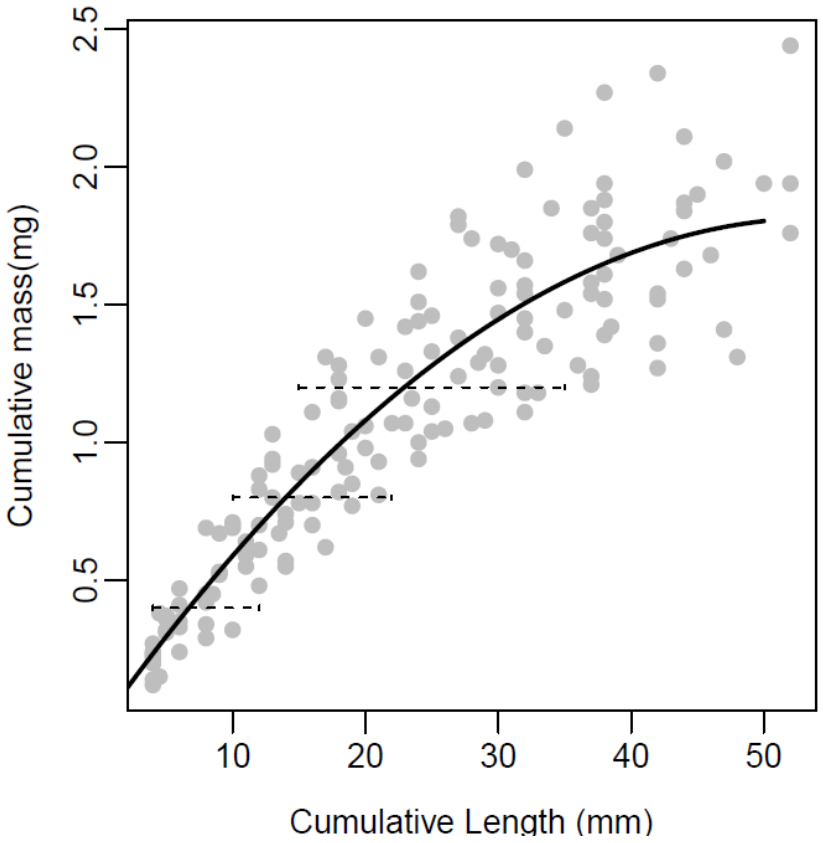
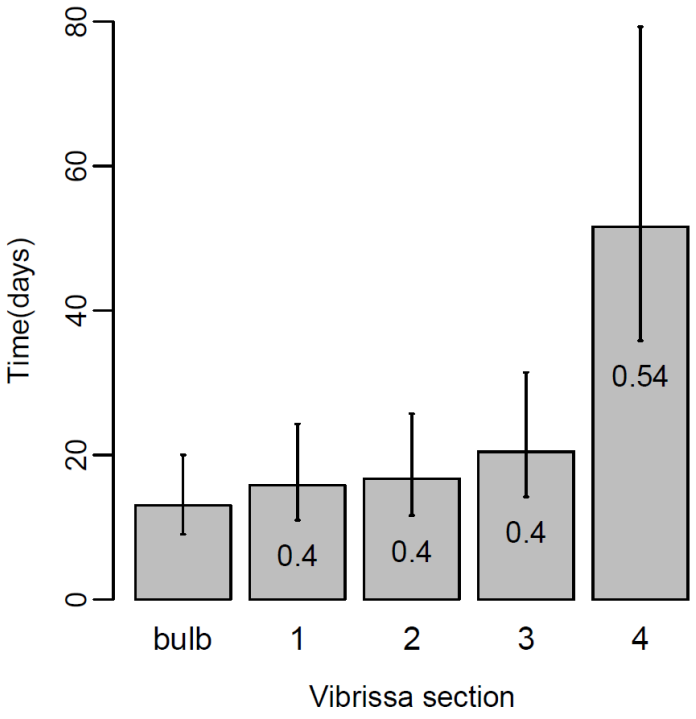


Figure 2.5 Estimated length of time reflected in four ~0.4mg serial subsections (values in mg displayed on bars) along a 45mm badger vibrissa (average length used for SIA) and the vibrissa bulb (length left under the surface when vibrissa cut and removed). Time calculated using the relationship between length and mass (Figure 2.4) to estimate length of each section, then converted to time using growth rates of RhB marked vibrissae. Values are estimated time for mean growth rate (0.43mm day^{-1}) error bars are range of times calculated using the 5^{th}-95 percentiles of growth rate.



CHAPTER 3: The foraging ecology of the European badger (*Meles meles*): an investigation using stable isotope analysis

3.1 Abstract

The foraging ecology of the European badger (*Meles meles*) has been the subject of intense research effort for several decades and to date has primarily been investigated via the analysis of faeces, which is the case in most terrestrial mammals. In this study we investigate temporal changes in badger diet in south west England using faecal analysis (FA) and also by stable isotope analysis (SIA) of $\delta^{13}C$ and $\delta^{15}N$. Using faecal analysis we find that badgers primarily consume invertebrate prey in the spring and shift to increasing amounts of plant prey in summer and autumn. Dietary changes observed via faecal analysis also coincided with isotopic changes in the $\delta^{13}C$ and $\delta^{15}N$ of badger vibrissae (whiskers) collected in spring and autumn. Analysis of isotopic patterns within the badger prey base indicated that several prey sources which are easily identifiable using FA are isotopically similar and therefore difficult to differentiate. However, we also found isotopic differences between some prey, which would be difficult to differentiate using FA, with $\delta^{15}N$ differences between habitat types (farmland/woodland) and $\delta^{13}C$ differences between natural prey and C4 based farm resources. Using the diet mixing model SIAR we found that isotopic estimates of diet were qualitatively similar to those of faecal analysis, although a direct comparison between values obtained via these different method was not possible. Mixing model results also indicated significant dietary variation both at the group and individual level, which has not been demonstrated previously in this species

Our results indicate that SIA may reveal aspects of foraging behaviour, which are not possible to investigate using traditional methods such as faecal analysis. However, in some instances the isotopic complexity of the potential prey base may make it difficult to use this approach alone. Our results demonstrate the strength of a combined approach, using both FA and SIA as that the two methods can potentially determine different aspects of an animal's foraging ecology, while also informing the interpretation of results obtained.

3.2 Introduction

The diet and foraging ecology of the European badger *Meles meles* (hereafter badgers), has been the subject of extensive research for several decades, with more than 200 studies to date (reviewed in Roper 2010). The high number of studies on this subject may seem surprising, however, badgers have several behavioural and ecological characteristics which mean their foraging ecology is of particular interest. For example, badgers are vectors of bovine tuberculosis and it has been suggested that their foraging behaviour may play role in disease transmission, as badgers foraging in pasture fields and farm buildings can come into close proximity to cattle (Kruuk *et al.* 1979; Garnett, Delahay & Roper 2002). In some locations badgers are also agricultural pests, consuming significant amounts of cereal and fruit crops (Roper *et al.* 1995; Moore *et al.* 1999). Badgers are also of particular behavioural interest as they are social carnivores occurring in social groups, but they are non-cooperative. This has resulted in a large number of studies using badgers as a model to understand the origins of sociality, many of which have focused on the role of foraging behaviour and resource distribution (Johnson, Jetz & Macdonald 2002; Macdonald *et al.* 2004; Palphramand, Newton-Cross & White 2007).

Badgers have a wide geographic distribution and occupy a diverse range of habitats, including a variety of forest types, mountains, coastal dunes, scrubland, farmland and urban areas (Roper 2011, Neal & Cheeseman, 1996). This adaptability results in an equally diverse dietary niche, which includes a wide variety of plant, invertebrate and vertebrate prey sources, including fruits, cereals, insects, earthworms, small mammals and birds (Kruuk & Parish 1981; Hounsome & Delahay 2005). For this reason badgers are generally viewed as adaptable omnivores, with their diet primarily determined by local resource availability (Roper 1994; Marassi & Biancardi 2002).

Within temperate seasonal environments, badger foraging niches exhibit marked temporal variation in response to fluctuating resource availability (Fischer, Ferrari & Weber 2005; Palphramand, Newton-cross & White 2007). In the UK, badgers change from a diet primarily composed of invertebrate prey in the spring, to a diet of seasonally

abundant fruits and cereals, with reduced consumption of animal prey in the late summer and autumn (Kruuk & Parish 1981; Hofer 1988; Palphramand, Newton-cross & White 2007). This seasonal niche shift, although driven by changes in resource availability, also coincides with changes in badger behaviour. In late winter and spring badgers devote more time to reproduction and territory defence (Roper, Shepherdson & Davies 1986; Buesching & Macdonald 2004), while in the late summer and autumn badgers focus on foraging in order to gain mass to survive the winter (Roper 2010).

To date, badger dietary studies have primarily been carried out via the analysis of faeces or gut contents, which is the case in the majority of terrestrial mammalian carnivores. Despite their widespread use, these methods have several well established limitations and biases (Putman 1984; Reynolds & Aebischer 1991). For example, results of faecal analysis (FA) may be biased towards prey sources with readily detectable indigestible parts, such as fruit seeds or invertebrate exoskeletons, while other prey such as slugs leave no identifiable remains (Reynolds & Aebischer 1991). Studies of badger diet which quantify the relative volume of prey consumed may use digestibility coefficients (Goszczyński, Jedrzejewska & Jedrzejewski 2000; Rosalino, Loureiro & Macdonald 2003) or in the case of earthworms, researchers use formulae which relates the abundance of chaetae or gizzard rings within the faecal sample to the mass of earthworms ingested (Kruuk & Parish 1981). Estimates of relative volume are potentially subjective and also rely on using average values for the masses of prey items and for their digestibility which may add additional error (Zabala & Zuberogoitia 2003, Roper 2011).

Gut contents can provide more detailed and accurate dietary information (e.g Cleary *et al.* 2009), however, both gut and faecal analysis only offer only a snapshot of resource use and therefore require a considerable number of samples to adequately describe diet and variation in diet, especially over significant temporal or spatial scales. These techniques also make it difficult to quantify dietary variation at the individual level, as gut contents can only be sampled once and it is not usually possible to identify which individual faecal samples are from. Individual level dietary variation is being increasingly recognised as an important component of a populations dietary niche (Bolnick *et al.* 2003). Both gut and faecal analysis also only quantify the ingested or consumed resources,

not the extent to which prey sources are metabolised and utilised by the consumer. As a consequence, the actual energetic importance of a given prey in a badger's diet may differ from the occurrence or mass in their faeces or gut contents.

One technique which does not suffer from these biases and that is particularly powerful when combined with conventional approaches is stable isotope analysis (SIA), which is increasingly been applied to investigate animal foraging ecology (Crawford, McDonald & Bearhop 2008; Newsome, Clementz & Koch 2010). SIA works on the premise that the isotopic composition of a consumer's proteinaceous tissues reflects that of their diet over the period of tissue synthesis (Deniro & Epstein 1978, 1981; Hobson & Clark 1992). SIA differs from gut and faecal analysis in that it provides information on prey source assimilation, not consumption, and therefore does not suffer from biases in detectability or digestion (Crawford, McDonald & Bearhop 2008). Analysis of tissues with long turnover times or periods of growth can also potentially yield long-term dietary information from known individuals in a single sampling event, which is not possible with conventional dietary analyses (Newsome *et al.* 2012).

In this study we combine faecal analysis with SIA of badger vibrissae (whiskers) to investigate temporal variation in the diet of badgers in a long-term study population in south-west England. Given the seasonal changes in resource availability in the UK, we predict that badgers will exhibit a corresponding significant shift in their isotope signature in response to changing diet. By analysing isotopic patterns in badgers and their prey, we aim to test this prediction and also determine the extent that isotopic gradients within a potentially diverse prey base can be utilised to disentangle specific resource use. We also use the Bayesian mixing model SIAR (Parnell *et al.* 2010) to estimate diet composition in both seasons to investigate whether dietary changes are similar to those from conventional analyses.

3.3 Materials and methods

Study area and badger sample collection

This study was carried out on the long-term study population of badgers at Woodchester Park in south west England, UK. The study site is approximately 7km^2 and consists of a central wooded valley surrounded by a matrix of grassland and arable fields (Delahay *et al.*

2006a). The badger population consists of approximately 22 social groups, whose territories are determined annually using a bait marking technique (Delahay *et al.* 2000). Badger vibrissae were collected from live captured individuals in spring in 2010 (11th May – 16th June, n=63), spring 2011 (3rd-25th May, n=61), autumn 2010 (21st September – 5th October, n=31) and autumn 2011(6th September – 5th October, n=20).

Badgers were caught in cage traps baited with peanuts and anesthetised as a part of regular mark recapture operations and following standardised protocols. Vibrissae were collected by cutting the base of the vibrissa as close to the skin as possible using steel scissors. Vibrissae were on average 45mm in length, which reflects on average 104 days (range 72-160 days) of growth (Robertson *et al.* 2012). All work involving the capture and sampling of live badgers was carried under English Nature and UK Home Office licences, in accordance with the Animals (Scientific Procedures) Act 1986 and was subject to an internal ethical review process.

Faecal sampling

Fresh faecal samples were collected from latrines in five sampling periods throughout the study; spring 2010 (April-May), summer 2010 (August), autumn 2010 (November), spring 2011 (April-May) and summer 2011 (August). Approximately 40 samples were collected in each of the five periods (Table 1), with approximately 5 samples collected from separate latrines at each of 8 social groups spread evenly across the study site. Following collection, faecal samples were soaked in >90% ethanol for 24 hours as a precaution against infection and were then analysed following Kruuk (1987). Each sample was broken up and washed through a 1.3mm sieve, with small particles and liquid from the first wash being collected in a 500ml beaker. This was allowed to settle for 10 minutes and a 1.5ml subsample was taken from the bottom of the beaker using a pipette. This liquid was then placed on glass slide and viewed at X40 using a binocular microscope to detect the presence of earthworm chaetae. The remaining solid fraction was then washed thoroughly and examined under water in a large white dish. Undigested prey remains were identified using a reference collection and a variety of commercially available identification guides. Percentage frequency of occurrence (%FO) was recorded for each prey type identified.

Nine prey categories were considered: small mammals, birds, beetles (Coleoptera, few other insects were present), earthworms, snails, wheat, maize, insect larvae and fruit.

Prey collection

Potential prey items were collected for SIA with reference to faecal analysis and by consulting the literature of badger diet in the UK. Large lumbricid worms (*Lumbricus terrestris*, and *L. rubellus*) were collected at night during wet weather and by digging and sifting soil. Only these species were sampled as they have been shown to be those primarily consumed by badgers (Kruuk & Parish 1981). Snails (primarily *Capaea nemoralis, C. hortensis* and *Arianta arbustorum*) and slugs (primarily *Arion ater*) were collected by hand during wet weather. Insect larvae (*Tipulidae* and *Noctuidae*) were collected by digging in grassland habitats. Beetles (predominantly *Pterostichus madus* and *Abax parallelepipedus*) were collected using pitfall traps. A wide range of fruits (*Rubus fruticosus*, *Prunus sp.*, *Pyrus sp., Malus sp., Rosa sp*) and nuts (*Fagus sylvatica, Corylus avellina, Quercus sp*) were also collected by hand. Maize cobs were collected from agricultural fields. Wheat was collected from fields and from pheasant feed hoppers. Cattle feed was also collected as although this was not detected in faecal samples, previous studies at this location have found it to be consumed by badgers (Garnett, Delahay & Roper 2002). Invertebrate prey were sampled in spring of 2010 and 2011, while seasonal fruits and nuts were collected in autumn of both years. Peanuts (used in traps to capture badgers) were also collected. The study area was divided into three locations and all prey types, where possible, were sampled from across the study site and in all habitat types potentially used by badgers. This was carried out in order to accurately assess spatial variation in prey sources and such that prey values were representative of those consumed by badgers across the study area. Once collected prey samples were frozen at -20°C and stored till analysis.

Sample preparation and stable isotope analysis

Prey samples were defrosted and washed in distilled water to remove soil or other detritus. Prey samples were prepared with reference to faecal analysis, and where

necessary indigestible material not digested by badgers was removed. Material removed included the thick exoskeletons of insect larvae, snail shells, and beetle exoskeletons. The gut contents of defrosted earthworms were also removed, as it was assumed that the soil and detritus within the large gut cavity of earthworms were largely indigestible to badgers. Following preparation prey were then dried for 72 hours at 50°C and free lipids were extracted using a Soxhlet apparatus and 2:1 chloroform:methanol solvent. Prey samples were then homogenised using a pestle and mortar and ~0.8mg of material was sealed in a tin capsule for SIA.

Badger vibrissae (one from each individual captured) were thoroughly rinsed in distilled water and scraped with fine forceps to remove surface contaminants and then dried. Cleaned vibrissae were sub-sampled into ~0.4mg sections using a scalpel and sealed in tin capsules for analysis. Each vibrissa was cut into an average of 4 sub-sections (sd=1, range = 3-7).

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios were determined using an elemental analyzer EA 1108 (Carlo Erba Instruments, Milan, Italy) coupled to an Isoprime IRMS (GVI, Manchester, UK) configured for simultaneous carbon and nitrogen stable isotope analysis. Isotope ratios are expressed as δ values, which is reported in parts per mil/thousand (‰) with reference to international standards following the equation $\delta X = 1000\ [(R_{sample} / R_{standard}) - 1]$. Where R_{sample} is the ratio of heavy to light isotopes ($^{13}C:^{12}C$ or $^{15}N:^{14}N$) and $R_{standard}$ is that of the standard for that element (C = Vienna-Pee Dee Belemnite, N = atmospheric nitrogen). Within-run mean accuracy of a collagen standard was 0.05‰ for $\delta^{13}C$ and 0.11‰ for $\delta^{15}N$. Carbon-to-nitrogen ratios (C:N) were also calculated for all prey sources and vibrissae samples.

Statistical analysis

We used MANOVA to investigate how the response variables $\delta^{15}N$ and $\delta^{13}C$ varied within potential invertebrate prey sources, due to the fixed effects: prey type (Slugs, snails, worms, insect larvae, beetles), habitat (woodland, farmland or maize fields) and location within the study area (west, east and core, to account for potential spatial variation in isotope values). Potential plant prey sources, wheat, cattle feed, maize, fruit and nuts

were compared to one another, and to invertebrate prey sources using univariate ANOVA following visual comparisons between prey types.

Due to the high number of potential prey species, prey which were isotopically similar and also ecologically similar (for example, slugs and snails or different types of fruit) were combined into potential prey sources for use in isotopic mixing models (Phillips 2012).

To investigate seasonal and spatial variation in badger $\delta^{15}N$ and $\delta^{13}C$, we carried out two separate linear mixed model (GLMM) analyses using $\delta^{15}N$ or $\delta^{13}C$ as the response variables. Fixed effects were; season of capture (spring or autumn), vibrissa section numbered from base (numbered seven) towards the tip (numbered five to three depending on length) and location within the study area (west, east and core) and Year (2010 or 2011), with individual nested within social group included as random effects. Significance of fixed effects was evaluated by stepwise removal and maximum likelihood ratio tests to produce a minimum adequate model.

The composition of prey sources to individual diets were estimated using the Bayesian mixing model SIAR (Parnell *et al.* 2010). The potential prey sources in models were determined following analysis of prey isotopic values. Trophic discrimination factors of 2.55‰ and 3.05‰ were used for $\delta^{13}C$ and $\delta^{15}N$ respectively and were calculated by averaging across published values for mammalian carnivore hair (Caut, Angulo & Courchamp 2009; Newsome *et al.* 2010a; Lecomte *et al.* 2011). Standard deviations of 0.7‰ and 0.6‰ were used for $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors respectively, based on published values for population variation in sea otter (*Enhydra lutris*) (Newsome *et al.* 2010a). All SIAR models included C:N ratios of potential prey sources to control for concentration dependence (Phillips *et al.* 2012). The results from mixing models were assessed using the diagnostics in SIAR for correlations between prey source estimates ('siarmatrixplot' function in SIAR package). Following mixing model analyses, individual comparisons of the modal (most likely) estimated contributions of prey sources between years or social groups were carried out using ANOVA.

3.4 Results

Faecal analysis

We collected a total of 190 faecal samples, with 35-41 (mean 38) samples collected in each of the five sampling periods (Table 3.1). Badgers exhibited a seasonal dietary change, with a high occurrence of animal prey in the spring and an increasing occurrence of plant prey sources (mainly fruit) in the summer and autumn (Table 3.1).

The most commonly consumed prey items in spring were invertebrates, with earthworms, snails, insect larvae (Tipulidae and Noctuidae) and beetles all having a high % FO of over 50% (Table 3.1). Other prey consumed at low frequencies in spring included mammals (small mammals and one Lagomorph), birds at around 10% (Table 3.1). The occurrence of all invertebrate prey declined from spring to summer (with the exception of earthworms and beetles in 2010) and from summer to autumn in 2010 (Table 3.1). Plant resources were absent from the diet in spring but increased in occurrence in summer and autumn, with particularly high occurrences of fruit in both years and seasons, and a high occurrence of wheat in 2011 (Table 3.1).

Prey source isotope values

Invertebrate prey sources were highly isotopically variable (Table 3.2), with significant isotopic differences between prey types (MANOVA, $Pillai_{4,144}$=1.25, p=<0.001), between habitat types ($Pillai_{2,144}$=1.15, p=<0.001) and between prey within habitat types (habitat × prey type interaction, $Pillai_{3,144}$=0.23, p=<0.001). Invertebrates within woodland habitats had lower $\delta^{15}N$ values than those in farmland habitats (Figure 3.1), possibly reflecting anthropogenic nitrogen input in the latter. Within habitats beetles had higher $\delta^{15}N$ (in accordance with their predatory lifestyle) and $\delta^{13}C$ values than other invertebrate types , although this varied across the study area (Figure 3.1), while insect larvae had lower $\delta^{13}C$ values than other invertebrate types (Figure 3.1). Worms in maize fields had higher $\delta^{13}C$ values than all other invertebrate prey (Figure 3.1). Based on isotopic and ecological differences invertebrate prey were grouped in five prey source categories; 1) beetles, 2) 'OI farmland' (other invertebrates; worms, snails and slugs collected from farmland environments, with the exception of worms collected from maize fields), 3) 'OI woodland'

(other invertebrates; worms, snails and slugs collected from woodland environments), 4) maize worms (worms collected from maize fields) and 5) Insect larvae. As there were significant isotopic differences between prey, some prey sources collected in the three locations ($Pillai_{2,144}$=0.06, p=0.03, figure 3.1), these five invertebrate prey source categories were calculated for each area.

Of potential plant prey resources, fruit and nuts did not significantly differ isotopically ($Pillai_{1,75}$=0.05, p=0.17), or vary across the three sampling locations ($Pillai_{2}$=0.1, p=0.09), but there were slight habitat differences, with higher values in farmland habitats ($Pillai_{1,75}$=0.09, p=0.03). Fruits and nuts had low $\delta^{15}N$ values, but were not significantly different to snails, slugs and worms from woodland habitats (ANOVA, $F_{1,159}$=2.01, p=0.15, Figure 3.1). Wheat was located towards the centre of the bivariate isotope prey space (Figure 3.1), but was significantly isotopically different from farmland worms, slugs and snails in both $\delta^{15}N$ ($F_{1,23}$=20.45, p=<0.001) and $\delta^{13}C$ ($F_{1,23}$=22.91, p=<0.001). Maize and cattle feed both had very high $\delta^{13}C$ values and were combined into one prey source 'C4 farm feed' (Figure 3.1).

Temporal patterns in badger isotope values

The isotopic values of individual badgers varied temporally, both between seasons and within seasons along the length of individual vibrissae (Figure 3.2). Isotopic changes in both $\delta^{15}N$ and $\delta^{13}C$ along vibrissae and between seasons also varied spatially across the study site (Figure 3.2, significant three way interaction between vibrissa section, season and location, $\delta^{15}N$- $X^2_{3,18}$=23.3, p=<0.001, $\delta^{13}C$ - $X^2_{3,18}$=48.9, p=<0.001 , Tables 3.3 & 3.4). Individual badgers captured in the spring, had higher $\delta^{13}C$ values than in the autumn, with individuals in the western area of Woodchester Park captured in spring also exhibiting an increase in $\delta^{13}C$ in older vibrissae tissue closer to the vibrissa tip (Figure 3.2), suggesting the consumption of C4 resources several months into the past.

There was no trend in $\delta^{15}N$ values along vibrissae collected in spring, but $\delta^{15}N$ values in vibrissae collected in autumn increased from the tip to the base, particularly in the core of the study area, indicating the consumption of higher $\delta^{15}N$ values, prior to capture (Figure 3.2). Vibrissa $\delta^{15}N$ values were similar in both seasons, with the exception of the west of the study area where vibrissae in the autumn had lower values than those

in spring (Figure 3.2). Seasonal changes in $\delta^{13}C$ also varied across the two years of the study (year by season two-way interaction, $X^2_{1,18}$=31.0, p=<0.001), with a larger shift in $\delta^{13}C$ between spring and autumn in 2011 than 2010.

Isotope mixing models

The diets of badgers sampled in spring were estimated using either five or six prey sources depending on location within the study site. Beetles, woodland OI (other invertebrates, worms, slugs and snails), farmland OI, and insect larvae were used in models in all locations, with maize worms also included in models for social groups in the west of the study site due to the presence of maize fields. In addition, although there was no evidence of maize or farm feed in the spring faecal samples (Table 3.1), the prey source C4 farm feed was included in all spring diet models due to the isotopic evidence of a C4 influence in the badger isotope values (Figures 3.1 & 3.2). Peanuts were excluded from diet models, as although they are consumed by badgers at this location, they are available for only a short window of time, and therefore unlikely to significantly contribute to diet. Isotopic differences between prey sources across the study site meant that diets of badgers in the east west and core of the study were estimated using prey in their respective locations (Figure 3.1). Autumn diet models included all of the same prey sources, with the addition of the two sources; wheat and fruit/nuts (Figure 3.1).

Mixing model results indicate that badgers in the spring consumed roughly even proportions of beetles, insect larvae and OI (other invertebrates) from farmland and woodland habitats, with values of these prey slightly lower in the west of the study area due to the inclusion of maize worms (Table 3.4). C4 farm feed (maize and animal feed) was estimated at 12% of overall spring diet (mean modal value across all animals, Table 3.4), but consumption of these resources varied across the study area, with higher consumption in the west ($F_{2,132}$=4.43, p=0.01, Table 3.4). Total consumption of earthworms and OI was high, the two groups comprised 45% of total diet (Table 3.4).

Mixing model results from badger vibrissae sampled in the autumn suggested a seasonal shift in diet with reduced consumption of invertebrate prey sources and consumption of wheat and fruit (Table 3.4), which is a similar pattern observed in the

faecal analysis. The estimated consumption of beetles, insect larvae, woodland OI, farmland OI and maize worms were all lower in autumn than in the spring (Table 3.4). Although faecal analysis indicated maize was consumed in autumn in the west of the study area (Faecal analysis, Table 3.4), the estimated consumption of C4 farm feed was also lower in autumn than spring and did not vary across locations (ANOVA, $F_{2,58}$=0.66, p=0.52, Table 3.4). Estimated consumption of fruit was low, constituting on average 11% of individual diets, with significantly lower consumption in the west of the study area (ANOVA,$F_{2,58}$=18.56, p=<0.001, Table 3.4)

Faecal analysis indicated a higher consumption of wheat in summer 2011 than summer and autumn 2010 (Table 3.1), although the difference between years in estimated wheat consumption from SIAR models was almost significant ($F_{1,59}$=3.9, p=0.052), the pattern was the opposite, with slightly higher consumption in 2010 (mean modal estimate=0.18, 95%CI=0.01) than 2011(mean modal estimate=0.17, 95%CI=0.01). Consumption of C4 farm resources in the spring also varied across years ($F_{1,133}$=30.5, p=<0.001), with higher consumption in 2011 (mean modal estimate=0.14, 95%CI=0.01) than 2010 (mean modal estimate=0.09, 95%CI=0.01). Despite apparent isotopic similarity among some prey sources (figure 3.1) correlations between SIAR posterior estimates for different prey sources were low (correlation <0.5) suggesting models did not struggle to separate prey.

Individual dietary variation

Within the population, there was a large amount of variation in the modal (most likely) contribution of different prey sources to individual diets, particularly in spring. For example woodland OI contributed all little as 2%, or as much as 50% to individual diets (Table 1). This variation could partially be explained by differences between social groups, with significant differences between social groups in modal estimates for the consumption of beetles ($F_{19,115}$=4.92, p=<0.001), woodland OI ($F_{19,115}$=4.73, p=<0.001), farmland OI ($F_{19,115}$=10.9, p=<0.001), insect larvae ($F_{19,115}$=6.1, p=<0.001) and C4 farm feed ($F_{19,115}$=6.1, p=<0.001) in spring. However, within some social group there was also individual variation

in the estimated consumption of some prey sources, particularly woodland OI and C4 farm resources (Figure 3).

3.5 Discussion

Comparisons between techniques

The vast majority of studies of badger foraging ecology and diet have been carried out via the analysis of faeces (Roper 2010), which is the case in most terrestrial carnivorous mammals. Using faecal analysis we confirmed that that badgers at Woodchester Park consume a diversity of prey sources and undergo a pronounced seasonal dietary shift. Faecal analysis not only reveals broad seasonal patterns but also provides detailed dietary information, as prey can be identified to species level via the inspection of indigestible components. However, faecal analysis suffers from several limitations. For example, the contents of faeces reflect diet over a narrow temporal window, are potentially biased towards indigestible prey and do not provide information on the energetic importance of differing prey sources which may vary in energy content and digestibility (Putman 1984; Reynolds & Aebischer 1991).

Stable isotope analysis of badger vibrissae also demonstrated a seasonal change in diet, with changes in $\delta^{15}N$ and $\delta^{13}C$ both among seasons and along the length of individual vibrissae. As badger vibrissae take several months to grow (Robertson *et al.* 2012) the isotopic information within vibrissae collected over several days of sampling reflects diet over a much longer temporal scale than faecal samples collected over a period of a week. For example, despite no evidence of maize or cattle feed consumption in spring faecal samples, vibrissae collected within a similar period contained evidence of the utilisation of these resources in the recent past. However, raw $\delta^{13}C$ or $\delta^{15}N$ values, although providing continuous quantitative measures of diet can be difficult to interpret ecologically, particularly where there is a large number of potential prey sources. Interpretation of isotopic patterns requires data on isotopic variation within the prey base, which in this study was found to be substantial, with large and localised variation in both $\delta^{13}C$ and $\delta^{15}N$ between potential prey sources (Table 3.2). Analysis of prey indicated that several prey sources that are difficult if not impossible to distinguish using faecal analyses were highly

distinct isotopically. For example, invertebrates in woodland habitats had lower $\delta^{15}N$ than those in farmland, and C4 farm resources (maize and cattle feed) had much higher $\delta^{13}C$ than other prey types. However, in several cases prey sources which are easy to differentiate using faecal analysis were isotopically similar, such as woodland OI and Fruit, or snails and earthworms. As a consequence, significant changes in diet which are detectable using faecal analysis could potentially result in little if any change in isotopic values.

Some of these issues can be addressed by combining the two sets of information in isotopic mixing models, as the probability of inclusion or exclusion of prey sources can be informed by faecal analysis. We found that the results from dietary mixing models were qualitatively similar to those from faecal analyses, although it is not possible to directly compare these two sets of data, as FA was quantified as frequency of occurrence, while mixing models estimated the proportion diet contribution of differing prey sources. However, despite this limitation we did see a similar general pattern in the data obtained by the two techniques. For example, both techniques estimated a high importance of beetles, insect larvae and other invertebrates (slugs, snails and earthworms) relative to other prey sources in spring, and reduced consumption of these prey in autumn corresponding with increased consumption of fruit and wheat. However, mixing models often struggle to provide accurate representations of diets when there are large numbers of prey sources, and/or where prey sources are isotopically similar (Phillips 2012). For example, this may explain why despite higher wheat consumption in 2011 (as indicated by faecal analysis) the mixing model results indicated no difference between years, as wheat is in the centre of the mixing space and therefore difficult to differentiate (Figure 3.1).

The results in this study confirm that different approaches to investigating diet (faecal analysis or SIA) both have limitations. Stable isotope analysis has the potential to elucidate patterns which are not possible via the analysis of faeces such as variation in the utilisation of farmland or woodland habitats (figure 3.3). Several other studies have also demonstrated the ability of SIA as a tool to investigate the utilisation of prey or habitats which are difficult to measure using traditional methods (Stewart *et al.* 2003; Ogden & Hobson 2006; York & Billings 2009). We also found substantial individual dietary variation

within the population, both within and between social groups, which has not previously been demonstrated in badgers. Although some studies have used faecal genotyping to investigate individual patterns in other species, the sample sizes required to characterise an individual's diet breadth are substantial, with up to 35 samples required (Prugh *et al.* 2008). This would require substantial effort to measure individual diet over several months on the scale of the present study. However, our results also highlight the strength of combining faecal analysis and SIA, as that the two methods can potentially determine different aspect of an animal's foraging ecology, while also informing the interpretation of results obtained.

Seasonal changes in badger diet at Woodchester Park

Faecal analysis indicates that the badgers in this location in the spring consume a diet of predominantly invertebrates, with a particularly high occurrence of earthworms. This changed significantly in the summer and autumn, with a reduction in the consumption of invertebrate prey sources and an increase in the consumption of fruit, wheat and maize. These results are similar to those of previous studies of badger diet in the UK and other parts of Europe which demonstrate a seasonal dietary shift from animal prey in spring to plant resources in autumn (Fischer, Ferrari & Weber 2005; Palphramand, Newton-cross & White 2007).

Dietary changes observed via faecal analysis also coincided with isotopic changes in the $\delta^{15}N$ and $\delta^{13}C$ of badger vibrissa. Badgers in spring had higher $\delta^{13}C$ than those in autumn and social groups in the western area of the study site had a significant change in $\delta^{13}C$ values along their vibrissae, with higher values close to the vibrissa tip. Seasonal changes in $\delta^{13}C$ corresponded with a higher estimated consumption of C4 farm resources, which mixing models estimated were higher in spring than summer, and also varied across years with greater consumption in 2010 than 2011. Although 'C4 farm' resources were an amalgamation of both maize and cattle feed it seems most likely that these higher $\delta^{13}C$ values in the west of the study area are primarily driven by the consumption of maize or of worms from within maize fields, as maize is cultivated within and adjacent to several badger group territories in this location. This result is surprising given that maize is grown

in the winter and is harvested in November-December. However, although an average length vibrissa used in this study (45mm) reflects on average around 3 months worth of growth, longer vibrissa (>60mm) may potentially reflect 5 months of growth (Robertson *et al.* 2012). Values near the tip of longer vibrissae collected in spring (May –June) may therefore, potentially reflect diet in January or February, not long after the harvest of maize. Although the faecal samples collected in the current study do not span this period, previous studies have also shown that badgers may consume large quantities of maize late into the spring, as maize cobs are available buried in ploughed fields for several months after harvest (Fischer, Ferrari & Weber 2005). However, given that high $\delta^{13}C$ values outside of the range of natural prey were also found in social groups far from an obvious source of maize, it is also possible in some instances that badgers are also consuming other C4 source such as farm animal feed which they can potentially obtain from farm buildings (Garnett, Delahay & Roper 2002).

Although maize was detected in faecal samples collected in summer (August) prior to the sampling of autumn vibrissa samples (September –October), $\delta^{13}C$ values of badgers were well within the range of invertebrate prey sources and estimated consumption of maize was low. We suggest that this is due to fact that the majority of maize was still unripe at this time and that consumption of maize was still at too low a level as to significantly affect individual isotope values. Also the sample size of individuals in autumn in the west of the study area was quite low (n=8) such that individuals consuming maize may not have been sampled. Worms are potentially available from maize fields prior to harvest, but the lower $\delta^{13}C$ in badgers in autumn, and the low estimated consumption of these resources (mean modal estimate 2%), we believe further suggest that high $\delta^{13}C$ in spring is primarily due to the consumption of C4 plant resources and not worms from maize fields.

Mixing models carried out using isotopic data from vibrissa sampled in spring also indicate that in addition to C4 farm resources, badgers on average consume a diet of 45% OI (worms, slugs and snails), 20% insect larvae and 20% beetles. Previous studies have shown that in the UK in the winter to spring period earthworms comprise on average 61% (Kruuk & Parish 1981), 70% (Palphramand *et al.* 2007) and 58% (Hofer 1988) of resources

consumed by badgers (by either mass or volume), which is slightly higher than that of this study. Estimates of beetles consumed in the current study is also considerably higher than that of beetles in other studies carried out in the UK (<2%, Palphramand *et al.* 2007), but similar to some studies from other parts of Europe, such as Ireland (17%, Cleary *et al.* 2009). It is possible that given the isotopic similarity between farmland OI and beetles (Figure 1), SIAR models may have slightly overestimated beetle consumption and correspondingly underestimated the consumption of farmland OI. However, although we did not quantify the relative volume or mass of prey in faecal samples, SIAR estimates in spring are qualitatively similar to that of %FO values obtained, with high importance of OI (snails/worms), beetles and insect larvae relative to other prey sources.

In autumn estimated consumption of all invertebrate prey sources were significantly lower than those in spring, and in addition to these resources, badgers also consume moderate amounts of fruits and wheat. The estimated dietary contribution of fruit from mixing models is low (average of 11%) relative to other studies of badger diet in autumn the UK (20% mass, Palphramand *et al.* 2007). However, as previously mentioned the isotopic similarity between prey sources means that mixing model results from the autumn should be cautiously interpreted, as although inspection of SIAR diagnostic plots (matrix correlations) indicated sources were not highly correlated, fruit was similar isotopically to woodland OI. However, although fruit was isotopically similar to woodland OI we would predict that if the majority of badgers changed from consuming higher $\delta^{15}N$ invertebrate prey to increasingly large amounts of fruit (as is suggested by faecal analysis) we would observe a corresponding isotopic shift in badger raw $\delta^{15}N$ values.

We found that badgers in the western part of the study area had lower $\delta^{15}N$ values in autumn than those in spring, but in the core and eastern areas $\delta^{15}N$ values were similar in both seasons. In addition vibrissae sampled in the autumn in all locations exhibited an increase in $\delta^{15}N$ from tip to base, which is contrary to the pattern expected. One possible explanation for this is that fruit and other plant resources contain little protein and N, relative to invertebrate prey. For example, in the current study, fruit and wheat were approximately 1% N, compared with around 10-12% N for invertebrate prey. A given mass of a plant prey source will therefore contribute significantly less N to the $\delta^{15}N$

composition of the badgers tissues than an equivalent mass of animal prey, due to differences in elemental composition, or 'concentration dependence' (Phillips & Koch 2002). Also, as badgers are still consuming large quantities of high protein invertebrate prey in the autumn, a greater proportion of N in vibrissa tissue may come from these resources due to isotopic routing (Kelly & Del Rio 2010). For example the $\delta^{15}N$ of tissues of birds fed high protein diet closely matches that of dietary protein, while non-dietary protein is only significantly incorporated into tissues if the protein content of the diet is low (Podlesak & McWilliams 2006). Badgers could therefore potentially consume high volumes of fruit without significantly altering the isotopic composition of their proteinaceous tissues, such as vibrissae.

3.6 Conclusions

This study highlights the advantages to using SIA and that this technique may facilitate the investigation of previously unexplored behaviour even within a species such as badgers which have been the subject of intense research for several decades. In particular the presence of high degrees of individual variation had not previously been demonstrated in this species. Niche variation may occur for a variety of reasons, either between age, classes, sexes or individuals, with important ecological implications (Bolnick *et al.* 2003; Dall *et al.* 2012). Further studies using SIA may allow for further investigation of this behaviour. However, we also suggest several potential limitations of SIA and highlight the importance of combining a stable isotope approach with traditional methods such as faecal analysis in order to avoid coming to inaccurate conclusions.

Table 3.1 Percentage frequency of occurrence of prey items in faecal samples analysed at Woodchester park (April 2010-August 2011) separated into three sub locations within the study site.

	Spring 2010				Summer 2010				Autumn 2010				Spring 2011				Summer 2011			
	West	East	Core	**Total**	West	East	Core	**Total**	West	East	Core	**Total**	West	East	Core	**Total**	West	East	Core	**Total**
Prey type																				
Earthworms	81	93	100	**90**	93	86	80	**87**	70	46	42	**51**	92	73	100	**87**	77	64	70	**70**
Snails	75	67	80	**73**	20	43	90	**46**	0	8	0	**3**	38	40	70	**47**	8	7	10	**8**
Insect larvae	75	73	80	**76**	7	21	0	**16**	0	15	0	**6**	62	80	90	**76**	23	0	30	**16**
Beetles	63	87	90	**78**	67	86	90	**79**	10	23	8	**14**	85	67	90	**79**	46	43	50	**46**
Mammals	0	13	10	**2**	13	0	10	**8**	0	8	0	**3**	8	0	10	**5**	0	0	0	**0**
Birds	0	0	0	**0**	0	7	0	**3**	0	0	0	**0**	8	0	10	**5**	0	0	20	**5**
Fruit	0	0	0	**0**	60	64	40	**56**	50	69	42	**54**	0	0	0	**0**	62	36	50	**49**
Maize	0	0	0	**0**	13	0	0	**5**	8	0	0	**9**	0	0	0	**0**	31	0	0	**11**
Wheat	0	0	0	**0**	27	21	10	**21**	14	0	8	**3**	0	0	0	**0**	0	64	60	**48**
n	16	15	10	**41**	15	14	10	**39**	10	13	12	**35**	13	15	10	**38**	13	14	10	**37**

Table 3.2 Table 1 – Mean $\delta^{13}C$ and $\delta^{15}N$, standard error of the mean, and minimum and maximum values of badger prey sources collected in 2010 and 2011 at Woodchester Park, Gloucester UK. Sample sizes analysed from each year and C:N mass ratios are also displayed.

		$\delta^{13}C$				$\delta^{15}N$				n			
Prey type	Species	mean	SE	Min	Max	Mean	SE	Min	Max	2010	2011	total	C/N (SD)
Worms	*Lumbricus terrestris, L.rubellus*	-26	0.2	-27.5	-21	1.9	0.5	-4.9	10.9	33	50	83	3.5 (0.2)
Beetles	*Abax parallelipipidus, Pterostichus madus*	-25.7	0.1	-28.5	-22.7	4.8	0.3	0.4	12.2	39	28	67	3.5 (0.3)
Snails	*Arianta arbustorum, Capaea nemoralis, C.nemoralis*	-27.1	0.2	-29.2	-25.4	-0.1	0.6	-6.5	7.9	21	18	39	4.0 (0.4)
Slugs	*Arianta ater, Deroceras sp.*	-26.8	0.2	-28.6	-23.2	-0.1	0.6	-4.5	6.5	14	14	28	4.4 (0.6)
Insect larvae	*Noctuid sp., Tipulid sp.*	-28.5	0.3	-30.2	-23.7	2.5	0.5	-1.4	9.3	20	14	34	4.6 (1.2)
Peanuts		-24.9	0.3	-26.6	-22.5	0.1	0.2	-0.7	1.7	7	10	17	5.4 (0.9)
Cattle feed		-17.7	0.2	-17.2	-18.3	3.1	0.1	3	3.2	5	5	10	17.8 (0.7)
Fruit	*Rubus fruticosus, Punus spinosa,Taxus baccata, Rosa canina, Malus domestica, Pyrus sp.,*	-25.8	0.3	-31.3	-22.3	-2.9	1.3	-8.4	3.5	41	45	86	72.4 (40.2)
Nuts	*Quercus sp., Coryllus avellana, Fagus sylvatica*	-26.3	0.5	-29.1	-20.8	-3.0	0.5	-6	-0.2	12	7	19	14.0(9.0)

Table 3.3 Fixed effects and model coefficients included in final minimum adequate models explaining variation in $\delta^{15}N$ and $\delta^{13}C$ in badgers at Woodchester Park. Section refers to vibrissa section numbered 7(base) to tip (5-1)

Isotope	Fixed effects	Estimate	SE	t value
$\delta^{15}N$	(Intercept)	2.92	0.46	6.39
	Section	0.36	0.07	5.39
	Season(spring)	1.82	0.46	3.93
	Location(east)	0.43	0.66	0.66
	Location(west)	-0.34	0.88	-0.39
	Season(spring) X Location(east)	-0.55	0.67	-0.82
	Season(spring) X Location(west)	-0.03	0.9	-0.03
	Section X Location(east)	0.01	0.1	0.06
	Section X Location(west)	-0.12	0.14	-0.87
	Section X Season(spring) X Location(core)	-0.35	0.08	-4.28
	Section X Season(spring) X Location(east)	-0.23	0.08	-2.84
	Section X Season(spring) X Location(west)	-0.16	0.13	-1.19
$\delta^{13}C$†	(Intercept)	74.4	6.2	12.015
	Section	-2.42	1	-2.426
	Season(spring)	-36.41	6.92	-5.26
	Year(2011)	6.82	2.59	2.659
	Location(east)	-12.23	8.82	-1.397
	Location(west)	-35.55	12.54	-2.838
	Season(spring) X Year(2011)	-18.51	2.76	-6.715
	Season(spring) X Location(east)	11.1	9.83	1.117
	Season(spring) X Location(west)	-17.3	13.42	-1.292
	Section X Location(east)	2.19	1.42	1.549
	Section X Location(west)	4.29	2.05	2.089
	Section X Season(spring) X Location(core)	6.23	1.2	5.19
	Section X Season(spring) X Location(east)	3.3	1.22	2.716
	Section X Season(spring) X Location(west)	8.11	1.96	4.143

†$\delta^{13}C$ values Box-Cox transformed – estimates and SE values for $\delta^{13}C$ are all $X10^{e17}$

Table 3.4 SIAR mixing model estimates of prey source diet proportions for badgers at Woodchester Park in spring and autumn in 2010 and 2011 . Displayed is the mean (averaged across all individuals) of the modal (most likely) estimate for each prey source, with the standard deviation of modal values (across all badgers) in brackets. Also displayed is the mean of the 95% credibility interval range for each prey source.

	Spring 2010					Autumn 2010				
	West	Core	East	**Total**		West	Core	East	**Total**	
Prey type				**mode**	**95% CI**				**mode**	**95% CI**
Beetles	0.20	0.22	0.25	**0.22 (0.07)**	**0.38 (0.05)**	0.08	0.15	0.18	**0.17 (0.06)**	**0.29 (0.05)**
Insect larvae	0.21	0.25	0.24	**0.23 (0.03)**	**0.39 (0.03)**	0.09	0.17	0.16	**0.16 (0.03)**	**0.29 (0.01)**
Farmland OI	0.17	0.26	0.24	**0.22 (0.06)**	**0.39(0.06)**	0.07	0.18	0.17	**0.17 (0.03)**	**0.31 (0.03)**
Woodland OI	0.23	0.2	0.17	**0.20 (0.08)**	**0.34 (0.05)**	0.22	0.12	0.09	**0.11 (0.07)**	**0.26 (0.05)**
Worms(maize fields)	0.10	-	-	**0.11 (0.05)**	**0.23 (0.03)**	0.02	-	-	**0.02(0.02)**	**0.14 (0.01)**
C4 farm feed	0.10	0.09	0.09	**0.10 (0.05)**	**0.18 (0.06)**	0.09	0.08	0.08	**0.08 (0.02)**	**0.12 (0.03)**
Fruit/nuts	-	-	-	-	-	0.02	0.18	0.12	**0.12 (0.05)**	**0.26 (0.04)**
Wheat	-	-	-	-	-	0.13	0.12	0.18	**0.18 (0.02)**	**0.31 (0.2)**
Total Worm/OI	0.50	0.46	0.41	**0.45 (0.07)**	-	0.3	0.30	0.27	**0.28 (0.06)**	-
n	21	21	26	**68**		2	17	19	**38**	

	Spring 2011					Autumn 2011				
	West	Core	East	**Total**		West	Core	East	**Total**	
Prey type				**mode**	**95% CI**				**mode**	**95% CI**
Beetles	0.19	0.2	0.23	**0.21 (0.07)**	**0.37 (0.06)**	0.12	0.17	0.18	**0.16 (0.06)**	**0.29 (0.05)**
Insect larvae	0.17	0.24	0.23	**0.22 (0.05)**	**0.38 (0.04)**	0.14	0.18	0.16	**0.16 (0.03)**	**0.28 (0.02)**
Farmland OI	0.15	0.25	0.21	**0.21 (0.06)**	**0.38 (0.06)**	0.11	0.18	0.17	**0.16 (0.05)**	**0.30 (0.03)**
Woodland OI	0.21	0.2	0.2	**0.21 (0.08)**	**0.33 (0.05)**	0.20	0.11	0.09	**0.13 (0.08)**	**0.26 (0.05)**
Worms(maize fields)	0.12	-	-	**0.12 (0.06)**	**0.23 (0.03)**	0.03	-	-	**0.03(0.03)**	**0.15 (0.02)**
C4 farm feed	0.19	0.13	0.13	**0.15 (0.06)**	**0.19 (0.06)**	0.06	0.08	0.07	**0.07 (0.02)**	**0.14 (0.03)**
Fruit/nuts	-	-	-	-	-	0.03	0.13	0.12	**0.09 (0.05)**	**0.23 (0.05)**
Wheat	-	-	-	-	-	0.15	0.18	0.18	**0.17 (0.01)**	**0.30 (0.2)**
Total Worm/OI	0.48	0.45	0.41	**0.45 (0.08)**	-	0.34	0.29	0.27	**0.29 (0.06)**	-
n	19	27	21	**67**		6	11	6	23	

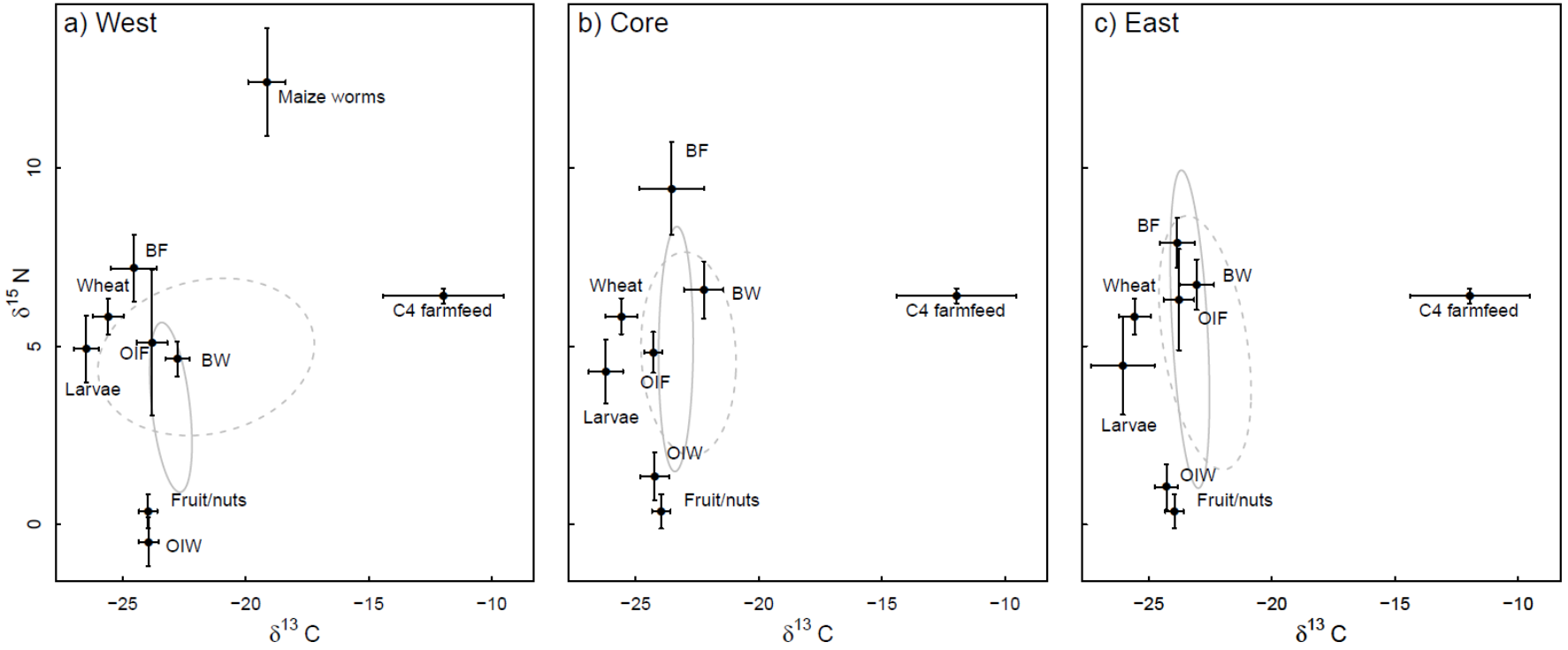


Figure 3.1 Isotope values of badgers from vibrissae sampled in spring and autumn along with potential prey in the west, east and core of Woodchester Park, UK. Ellipses are 95% probability ellipses encompassing badger isotope values from vibrissae sampled in the spring (dashed line) and autumn (solid line) drawn using the standard ellipse function in SIAR. Ellipses are shown for clarity of presentation. Prey values are means with 95% confidence intervals. Prey are OIF (other invertebrates, slugs, snails earthworms in farmland habitats) and BF (beetles in farmland habitats) and OIW (other invertebrates, slugs, snails earthworms in woodland habitats) and BW (beetles in woodland habitats).

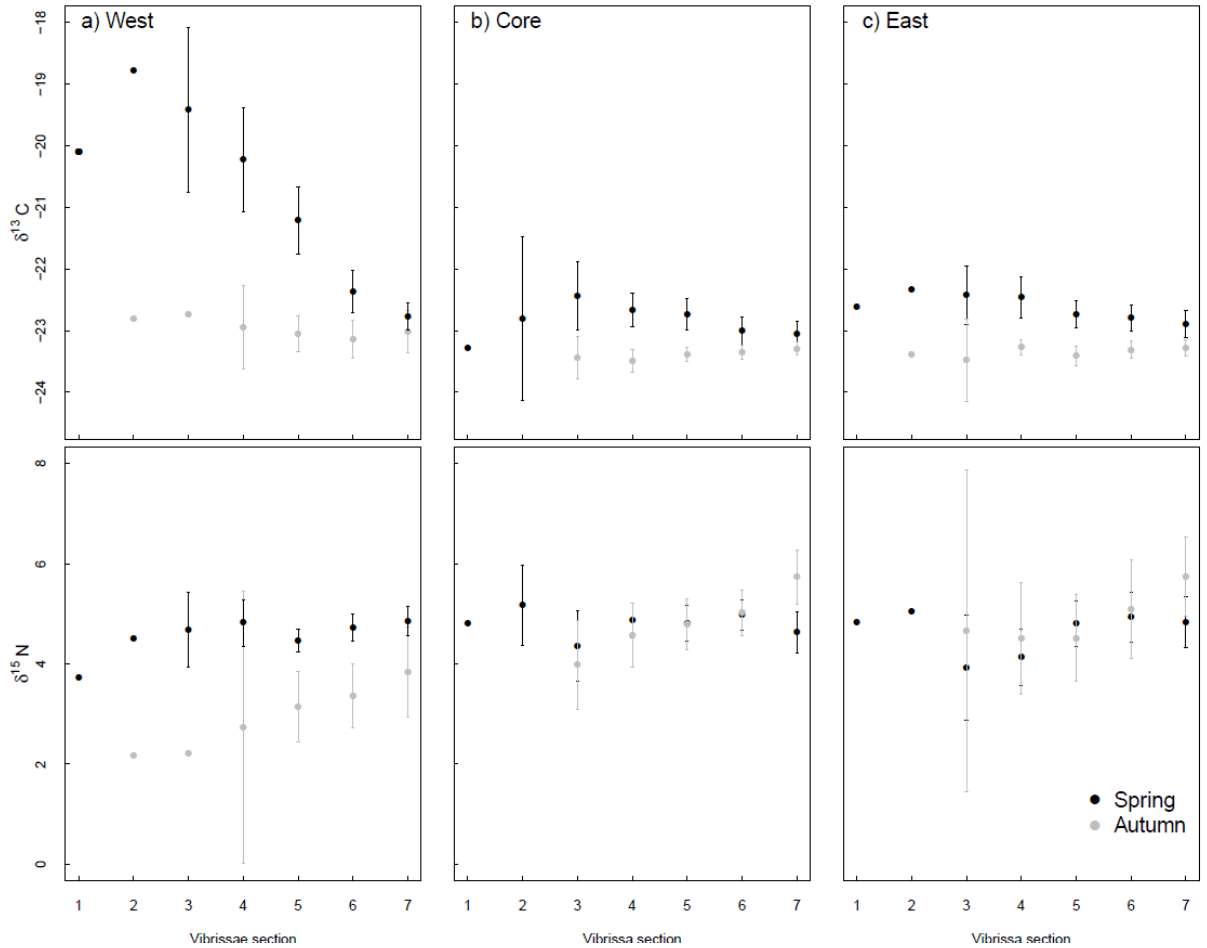


Figure 3.2 Mean $\delta^{13}C$ and $\delta^{15}N$ of serially sampled badger vibrissae in Spring and Autumn within the west, core and east of Woodchester park. Vibrissae (whiskers) were serially sampled into ~0.4mg sections from base (section 7) to tip (section 5 to 1 depending on length). Error bars are 95% confidence intervals. Points without error bars had only one or a small number of values.

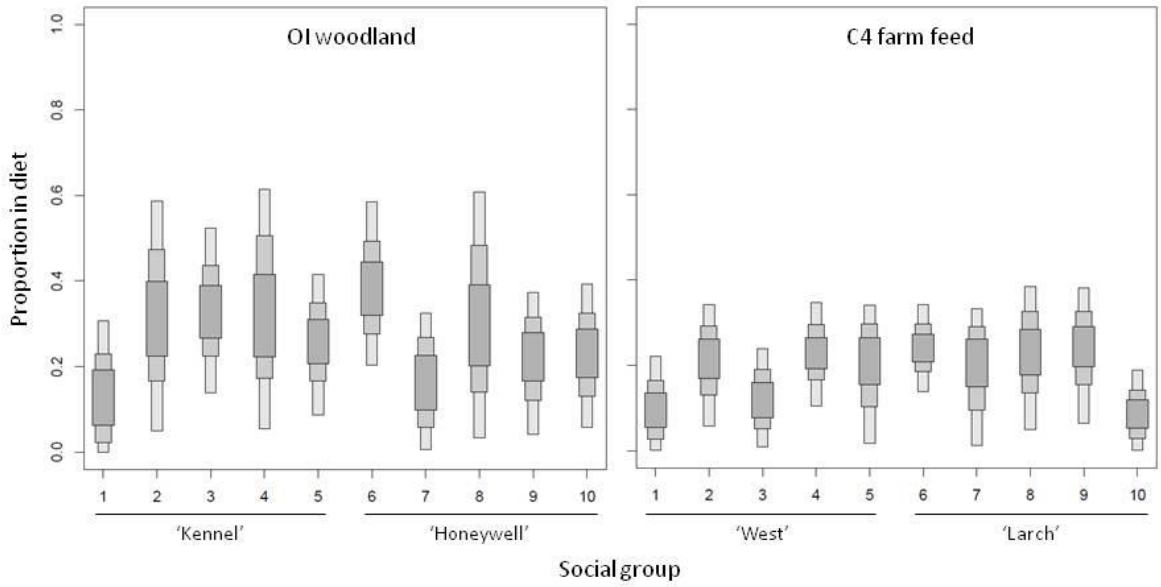


Figure 3.3 Example of individual dietary variation within four badger social groups ('Kennel', 'Honeywell', 'West' and 'Larch') in spring. Bars are the Range of possible contributions of OI woodland (other invertebrates – worms, snails and slugs, in woodland habitats) and C4 farm feed (maize and cattle feed) to the diets of individual badgers, within the four different social groups. Decreasing bar widths represent 50%, 75% and 95% credibility intervals estimated from using the Bayesian mixing model SIAR. In each case all individuals within a group are sampled in the same year. Prey consumption for each individual (grey bar) is estimated using multiple isotope samples obtained for each badger via the sub sampling of an individual vibrissae collected in spring.

CHAPTER 4: Individual foraging specialisation in a social mammal: the European badger *Meles meles*

4.1 Abstract

1. Individual foraging specialisation has been identified in an increasing number of animal species and populations. However, in some groups, such as terrestrial mammals, it is difficult to disentangle individual niche variation from spatial variation in resource availability. Social and territorial species present an opportunity to discern individual specialisation, since individuals within groups share the same foraging environment and similar constraints on resource availability.
2. In the present study we investigate individual foraging niche variation in the European badger (*Meles meles*), a social carnivore that lives in a shared group territory, but predominantly forages alone. Using stable isotope analysis, we distinguish the extent to which foraging variation in badgers is determined by social and spatial constraints and by individual differences within groups.
3. We found that individual badgers within social groups differed markedly and consistently in their isotope values, indicating that individuals living with the same patterns of resource availability consistently occupied distinctive foraging niches. Although sex had a significant effect on isotope values, substantial variation within groups occurred independently of age and sex.
4. Individual differences were consistent over a period of several months and in some instances were highly consistent across the two years of the study, suggesting individual foraging specialisations are sustained in the longer-term.
5. Individual specialisation in foraging niche may therefore persist in populations of territorial species not simply as a result of spatial resource variation, but may also arise from individuals selecting differently from the same pool of available resources.
6. Although the exact cause of this behaviour is unknown, we suggest specialisation may occur due to learning tradeoffs which may limit individual

niche widths. However, variation in the degree of specialisation between groups also suggests that ecological factors at the group level may influence the degree of specialisation.

4.2 Introduction

Many populations of animals that might ordinarily be described as generalist foragers, are in fact composed of aggregations of ecologically heterogeneous individuals that specialise on a sub-component of the population's overall niche (Bolnick *et al.* 2003; Araújo, Bolnick & Layman 2011). This within-population niche variation can arise from differences between sexes (e.g. Bearhop *et al.* 2006), age classes (e.g. Newland *et al.* 2009) or morphological groups (e.g. Smith & Skulason 1996). Alternatively, this variation may occur due to 'individual specialisation', such that individuals differ significantly in their niche, independently of age, sex or morphological group (Bolnick *et al.* 2003). Individual specialisation has important evolutionary implications, as long term ecological divergence of individuals represents a potential mechanism for adaptive radiation, and may lead to the formation of resource polymorphisms and ultimately speciation (Bolnick *et al.* 2003; Ackermann & Doebeli 2004; Rueffler, Egas & Metz 2006; Knudsen *et al.* 2010). Individual differences in niche utilisation also have important ecological and management implications, as interactions between predator, prey and environment are not manifested homogeneously across the population. For example, individuals which specialise on certain resources may be more susceptible to environmental or anthropogenic changes which reduce the availability of these resources (Thiemann *et al.* 2011). Some specialists may also be more likely to encounter pathogens (Johnson *et al.* 2009) or come into conflict with humans (Cerling *et al.* 2006). As a consequence, assessments made at the level of the individual, rather than at the population level, can play an important role in developing an understanding of ecosystem processes and function (Hawes *et al.* 2005; Post *et al.* 2008; Cianciaruso *et al.* 2009), species interactions (Duffy 2009) and land management (Searle, Hunt & Gordon 2009).It may therefore be important for researchers to take an individual approach to investigating niche use in animal populations, particularly where there is a conservation or management interest.

Many animals are territorial or occupy a home range which is only a proportion of the population's range. In these species, foraging niche may be strongly influenced by spatial and temporal variations in resource availability, which may constrain individual niches relative to the population. Investigating individual specialisation in such species is therefore challenging, as it is difficult to determine if niche variation is the product of variation in individual resource preferences, or due to variation in resource availability. This is crucial, as the potential causes and consequences of 'individual specialisation' differ between these two scenarios (Bolnick *et al.* 2003).

Territorial behaviour is particularly common in terrestrial mammal species. Individual specialisation in this group may be of particular interest, given the importance of terrestrial mammals as food sources, pest species, disease vectors and foci for human-wildlife conflict. Many terrestrial mammals also play pivotal roles in many ecosystems as top predators or keystone species, and are currently threatened, or are declining rapidly (Ceballos and Ehrlich 2002). However, despite the potential importance of individual specialisation within terrestrial mammals fewer than 10% of documented cases of individual specialisation occur within this group (Araújo et al. 2011). In addition, in the majority of cases where both individual foraging variation and habitat variation have been measured in terrestrial mammals, individual resource use correlates with local resource availability (Angerbjörn et al. 1994; Mattson and Reinhart 1995; Ben-David et al. 1997; Sidorovich et al. 2001; McEachern et al. 2006; Prugh et al. 2008), suggesting that individuals are not specialising on a subset of available resources, but are instead simply utilising what is available within their range. One exception is the grey wolf *Canis lupus*, where several studies have shown that within packs, individual wolves may vary in their resource use, suggesting individuals may sample differently from the available prey base (Urton and Hobson 2005; Darimont et al. 2009). However, in these studies, age and sex effects on individual niches were not considered, so it is therefore difficult to determine if this individual variation is due to individual specialisation or a combination of other class effects.

In this study we use analysis of the stable isotopes of carbon and nitrogen to investigate individual niche variation in a population of Eurasian badgers (*Meles meles*)

that has been the subject of a long term study. In recent years stable isotope analysis (SIA) has emerged as a useful tool for investigating individual niche variation (Bearhop et al. 2004; Newsome et al. 2009). SIA works on the premise that the isotopic composition of a consumer's proteinaceous tissues reflects those of their diet (Deniro and Epstein 1978, 1981). The most common isotopes measured in foraging studies are ^{13}C to ^{12}C (expressed as $\delta^{13}C$) and ^{15}N to ^{14}N (expressed as $\delta^{15}N$), which vary predominantly with habitat and trophic level respectively (Crawford et al. 2008). By repeatedly measuring the isotopic signatures of individuals over time it is therefore possible to quantify along these isotopic axes a representation of the range of habitats and food resources utilised, effectively describing the 'isotopic niche' of that individual (Bearhop et al. 2004; Newsome et al. 2007). Variance between and within individuals can then be compared to that represented across the population in order to quantify individual foraging specialisation (Newsome et al. 2009).

Badgers are a particularly good candidate to investigate individual niche variation. In Britain and Ireland, badgers live in territorial social groups containing on average approximately 6 individuals (range 2 to >20) sharing a centrally located burrow system or 'sett' (Roper 2010). Foraging studies at the population and social group level have shown that large variations in foraging niche may occur between and within populations, and this has been ascribed to variation in resource availability (Hofer 1988; Roper 1994). Despite their social nature, badgers forage predominantly alone within the group territory (Kruuk 1978), although individuals have largely overlapping foraging ranges such that the group territory is a resource shared by all individuals (Kruuk 1978; Shepherdson et al. 1990, Roper 2010). Individual foraging specialisation has not been explored in this species, although radio tracking and gut content studies suggest some degree of individual-level variation in habitat and resource use (Garnett et al. 2005; Cleary et al. 2009). However, it has also been suggested that badgers are purely opportunists and that they utilise whatever resources are available and are therefore unlikely to specialise (Roper 1994, Roper 2010).

We quantify individual niche variation in badgers in order to determine if individual niches are determined primarily by resource availability or if badgers are

individual specialists and consistently utilise only a subset of the group's shared resources. We hypothesise that if habitat, and thereby resource availability, is the key driver of resource use then the majority of niche variation within the population will be among social groups, due to differences in territory composition. Alternatively if individual specialisation occurs in this species we predict that individuals within groups will differ consistently in their foraging niches, despite having a shared territory, and that this variation is independent of age or sex effects.

4.3 Methods

Study area and badger sample collection

This study was carried out at Woodchester Park (WP), Gloucestershire, UK (51°43' N, 2°16' E) where the resident badger population has been the subject of a long-term study. The study area is approximately 7km^2 supporting a resident badger population of approximately 22 social groups. The majority of these groups (~19) occupy setts within a central wooded valley, with territories extending out onto a surrounding landscape of grassland (pasture, hayfields and amenity land) and arable land. The three remaining social groups occupy territories south-west, but adjacent to the main study area, with the habitat consisting of patches of woodland surrounded by highly improved pasture and some arable. Social group territory boundaries are determined annually in spring by bait marking (Delahay *et al.* 2000). Badgers were live captured in cage traps placed at active setts within territories in spring 2010 (11th May – 16th June) and 2011 (3rd-25th May) as part of a long-term disease study and following standard trapping protocols (see Delahay *et al.* 2006 for detailed description of study site and trapping protocol). Vibrissae were collected for SIA from anaesthetised badgers, by cutting as close to the skin as possible using scissors. Badger vibrissae grow at a rate such that an average length vibrissa used in this study reflects on average 104 days (range 72-160) days of growth (Robertson *et al*. 2012). All work involving the capture and sampling of live badgers was carried under English Nature and UK Home Office licences, in accordance with the Animals (Scientific Procedures) Act 1986 and was subject to an internal ethical review process.

Faecal sampling and prey source identification

For the purposes of informing stable isotope analyses, prey sources were identified by consulting the literature on the foraging ecology and diet of badgers in Britain, and through collecting fresh faeces from badger latrines in the period prior to trapping. A total of 100 faecal samples were collected from latrines in 10 badger social groups (five per social group per year) spread evenly across the site over the month prior to trapping. Following collection, faecal samples were first soaked in >90% ethanol for 24 hours as a precaution against infection and then analysed following Kruuk (1987). Samples were broken up and washed through a 1.3mm sieve, with small particles and liquid from the first wash being collected in a 500ml beaker. This was allowed to settle for 10 minutes and a 1.5ml subsample was taken from the bottom of the beaker using a pipette. This was placed on glass slide and viewed at X40 using a binocular microscope to detect the presence of earthworm chaetae. The remaining solid fraction was then washed thoroughly and examined under water in a large white dish. Undigested prey remains were identified using a reference collection and a variety of commercially available identification guides. Percentage frequency of occurrence (%FO) was recorded for each prey type identified.

Prey collection

Badger prey samples were collected from April to June in both 2010 and 2011. We collected samples from prey categories with >5% frequency of occurrence from faecal analysis which included; earthworms, snails, carabid beetles and insect larvae. Vertebrate prey (small mammals, rabbits and birds) may be consumed by badgers, but these were not collected as faecal analysis indicated they did not feature regularly in the diet at this study site (FO <5%) which is also similar to other foraging studies of badgers in the UK (Roper 2010). Large lumbricid worms (*Lumbricus terrestris*, and *L. rubellus*) were collected at night during wet weather and by digging and sifting soil. Only these species were sampled as they have been shown to be those primarily consumed by badgers (Kruuk & Parish 1981). Snails (primarily *Capaea nemoralis, C. Hortensis* and *Arianta arbustorum*)

and slugs (primarily *Arionater sp.*) were collected opportunistically during damp weather. Insect larvae (*Tipulidae* and *Noctuidae*) were collected by digging in grassland habitats. Beetles (predominantly *Pterostichus madus* and *Abax parallelepipedus*) were collected using pitfall traps. All prey types were collected in both years, with sampling spread across the study site and encompassing the variety of habitats where prey exist and where badgers may be foraging. In addition to these natural prey sources, we also collected samples of peanuts (used to bait traps), however, it should be noted that peanuts were available for a relatively short period relative to that reflected by a vibrissa. Cattle feed was also collected as this may be consumed by badgers (Garnett, Delahay & Roper 2002). As part of ongoing dietary monitoring and prey sample collection we also collected maize cobs and worms from maize fields within the study area in winter 2010, as maize is potentially consumed by badgers into the spring (Fischer, Ferrari & Weber 2005). All prey were frozen on the day of collection and stored at -20°C until analysis.

Sample preparation and stable isotope analysis

Prey samples were defrosted and rinsed in distilled water to remove soil or detritus. Prey were then prepared with reference to faecal analysis, and where necessary indigestible material was removed. This included snail shells, beetle exoskeletons and the thick skins of insect larvae. Earthworms were defrosted and gut contents were removed via dissection and flushing with distilled water, as we assumed that earthworm gut contents (i.e. soil and detritus) were likely to be largely indigestible to badgers. Prey were then dried for 72 hours at 50°C and free lipids were extracted using a Soxhlet apparatus and 2:1 chloroform:methanol solvent. Prey were then homogenised using a pestle and mortar and ~0.8mg of material was sealed in a tin capsule for analysis.

Badger vibrissae (one from each individual capture) were thoroughly rinsed in distilled water, scraped with fine forceps to remove surface contaminants and then dried. Vibrissae were then sub-sampled into ~0.4mg sections using a scalpel and sealed in tin capsules for analysis. Each vibrissa was cut into an average of 4 sub-sections (sd=1, range = 3-7). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios were determined using an

elemental analyzer EA 1108 (Carlo Erba Instruments, Milan, Italy) coupled to an Isoprime IRMS (GVI, Manchester, UK) configured for simultaneous carbon and nitrogen stable isotope analysis. Isotope ratios are expressed as δ values, which is reported in parts per mil/thousand (‰) with reference to international standards following the equation $\delta X = 1000 [(R_{sample} / R_{standard}) - 1]$. Where R_{sample} is the ratio of heavy to light isotopes ($^{13}C:^{12}C$ or $^{15}N:^{14}N$) and $R_{standard}$ is that of the standard for that element (C = Vienna-Pee Dee Belemnite, N = atmospheric nitrogen). Within-run mean accuracy of a collagen standard was 0.05‰ for $\delta^{13}C$ and 0.11‰ for $\delta^{15}N$.

Statistical analysis

In order to compare $\delta^{13}C$ and $\delta^{15}N$ values of invertebrate prey types, in different habitats and years we carried out a MANOVA analysis followed by univariate ANOVA tests. To investigate the causes of individual variation in badger vibrissa isotope values we fitted a series of general linear mixed models and evaluated their relative performance using Akaike's information criterion (AICc). The response variables used were isotope values of individual vibrissae sections with separate models created for $\delta^{13}C$ and $\delta^{15}N$. Fixed effects included in models were age (yearling=1 year old, adult= 2-3 years, or old = ≥4 years) and sex. A further fixed effect was included, which divided the study area into four broad 'locations' to control for isotopic variation in prey sources (WP-east, WP-west, WP-core and WP-south). Potential two way interactions among fixed factors included were, location X age, location X sex and age X sex. In order to investigate the importance of individual and social group variation, individual badger nested within social group was included as a hierarchical random effect. Year (2010 or 2011) was also included as a random effect.

AIC model selection was then carried out using the MuMIn package in R 2.14.1 (cran.r-project.org). Input variables were standardised with mean=0 and sd=0.5, following Gelman (2008) prior to analysis. This is carried out so that all input variables used in the analysis have comparable scales. Potential models were restricted to a top model set with ΔAIC<3 and average model coefficients were than calculated using this refined model set.

To provide an assessment of model fit Nagelkerke R^2 (Nagelkerke 1991) was also calculated for each model. To test the importance of individual and social group random effects, top models were compared with and without these effects and tested for significant changes in variance explained using maximum likelihood ratio tests. In addition, variance components were calculated from top models included in model averaging analyses to estimate % variance not explained by fixed effects. As an additional measure of among individual variation within groups we also calculated an index of individual specialisation; WIC/TNW (Bolnick *et al.* 2002), for $\delta^{13}C$ and $\delta^{15}N$ where WIC is the within individual component and TNW is total niche width. This is calculated for each social group using analysis of variance with individual as a factor, where WIC is the within group sum of squares and TNW the total sum of squares (Newsome *et al.* 2009). This index varies from 0 (high among individual variation) to 1 (no among individual variation).

Long term consistency in foraging niche

As a number of individual badgers were captured in both 2010 and 2011 it is possible to investigate longer term between year consistencies in isotopic niche to see if individual patterns are stable over time. In order to do this we fitted general linear mixed models to test if $\delta^{13}C$ and $\delta^{15}N$ values in 2010 were significantly related to those in 2011. Response variables were isotopic values for individual vibrissae segments in 2011 with a single fixed effect of isotopic values at the corresponding vibrissae segment in 2010. Random variables included were individual nested within social group, nested within location. In addition, we also repeated this analysis with $\delta^{13}C$ and $\delta^{15}N$ standard deviation in 2011 as the response variable and $\delta^{13}C$ and $\delta^{15}N$ standard deviation in 2011 as a single fixed effect, to test whether individual niche widths were consistent across years. Only the three basal vibrissa segments were included in the analysis as the length of vibrissa obtained may differ amongst years.

4.4 Results

In total 155 vibrissae were analysed from 115 individual badgers; 78 individuals from 18 social groups sampled in spring 2010 and 77 individuals from 16 social groups sampled in

spring 2011. The number of individuals sampled per group varied from 2-9 with a mean of 5. A total of 40 individual badgers were caught in both years.

Prey source isotope values

Analysis of badger prey items indicated that the potential isotopic niche available to the badger population was broad (Table 4.1). The stable isotope ratios of some prey types were highly variable, with ranges of >10‰ in some invertebrates (Table 4.1). MANOVA analysis indicated that this variation could be explained by significant isotopic differences between habitat types ($Pilai_{1,176}$=99.99, p=<0.001) and locations ($Pilai_{3,345}$ = 34.94, p=<0.001), but not years ($Pillai_{1,176}$=0.002, p=0.80). All invertebrate prey types in the south west of the study area had higher $\delta^{15}N$ values than in the other three locations, while beetles slugs, snails and worms in the west of the study area had slightly lower $\delta^{15}N$ values than other areas (Figure 4.1).Within locations, invertebrate prey collected in woodland habitats had lower $\delta^{15}N$ values than those in farmland habitats (Figure 4.1). Worms collected from maize fields also had higher $\delta^{13}C$ values than other habitats.

Individual and group niche variation

Badgers varied widely in their $\delta^{15}N$ and $\delta^{13}C$ values suggesting a variety of foraging strategies within the population (Figure 4.1). Model selection indicated that variation in isotope values could be explained by a 95% top model set containing 2 and 4 top models for $\delta^{15}N$ and $\delta^{13}C$ respectively ($\delta^{13}C$ values were Box-Cox power transformed to conform with normality assumptions), with models containing combinations of age, sex and location as fixed effects (Table 4.2). Badgers in the south of the study area had significantly higher $\delta^{15}N$ values than those in other locations, while those in the western area had higher $\delta^{13}C$ values (Table 4.3, Figure 4.1). Age was included in top models for both isotopes (Table 4.2), however, inspection of the averaged model coefficients indicated that differences between age classes were small and variable, suggesting that age did not have a marked effect on foraging niche (Table 4.3). Similarly, although sex was included in several top models explaining variation in $\delta^{13}C$, differences between sexes in $\delta^{13}C$ were also small and variable. All top models explaining variation in $\delta^{15}N$ contained

sex and an interaction between sex and location (Table 4.2) with male badgers in the west and east of the study area having consistently lower $\delta^{15}N$ values than females (Table 4.3), suggesting sex related differences in foraging niche. However, the total variance explained by fixed effects in top models was small for both $\delta^{15}N$ (mean Nagelkerke R^2=0.07, Table 4.2) and $\delta^{13}C$ (mean Nagelkerke R^2=0.03, Table 4.2), indicating a significant amount of isotopic variation that was not attributable to fixed effects included in models.

Within locations, isotope values varied significantly among social groups (Table 4.3, Figure 4.2) with the removal of the social group random effect significantly reducing variance explained by all top models for both isotopes ($\delta^{15}N$, X^2= 15.5-17.1, p=<0.001 for all models, $\delta^{13}C$, X^2=33.4-38.6, p=<0.001 for all models). Within groups, large isotopic variation existed among individuals with consistent non overlapping differences of up to 4‰ in some groups (Figure 4.3). This is supported by results of the variance components analysis which found that individual nested within social group explained on average 30% (sd=0.7) and 44% (sd= 0.9) of the variance not explained by fixed effects averaged across top models for $\delta^{15}N$ and $\delta^{13}C$ models respectively (Table 4.3). This compares to a social group effect of 30% (sd=0.7) for $\delta^{15}N$ and much lower effect of 2% (sd=1) for $\delta^{13}C$. In addition, the removal of the individual random effect significantly reduced the variance explained by all top models for $\delta^{15}N$ (X^2= 135.7-136.2,p=<0.001 for all models, Table 4.2) and $\delta^{13}C$ (X^2=156.7-157.3, p=<0.001 for all models, Table 4.3) indicating a significant amount of variation in isotope values among individuals within social groups. The degree of individual specialisation measured using WIC/TNW was high for $\delta^{13}C$ (mean =0.47, sd=0.27, Figure 4.4) and $\delta^{15}N$ (mean =0.55, sd=0.22, Figure 4.4), but varied markedly across social groups (Figure 4.4).

Consistency of individual variation

Individual foraging patterns were consistent across the two years of the study (Figure 4.5). Within locations and social groups, there was a significant positive correlation between individual vibrissa section isotope values in 2010 with those of the corresponding vibrissa sections in 2011 for both $\delta^{13}C$ (likelihood ratio test using maximum-likelihood simplification of minimal adequate REML model, X_1 =107.73, p= <0.001, Nagelkerke R^2= 0.61, Figure 4.6) and

$\delta^{15}N$ (X^2_1 =41.6, p= <0.001, Nagelkerke R^2= 0.31, Figure 4.6). Individual isotopic niche widths were also consistent across years, with individual standard deviations in $\delta^{13}C$ in 2010 significantly related to those in 2011 (X^2_1=12.7, p= <0.001, Nagelkerke R^2= 0.64, $\delta^{13}C$ σ^2 log10 transformed), particularly in the western part of the study area (Figure 4.7). However, there was no significant relationship between $\delta^{15}N$ standard deviation in 2010 and 2011 (X^2_1=0.24, p= 0.62, $\delta^{15}N$sd log10 transformed).

4.5 Discussion

We found that the badger population living in the comparatively small area of Woodchester Park occupied a broad isotopic niche with large carbon and nitrogen ranges (Figure 4.1). Although significant isotopic differences occurred among social groups (Figure 4.2, Table 4.2), individual differences within groups explained a significant amount of isotopic variation, with up to ~4‰ differences between individuals in some cases (Figure 4.3). This is equivalent to ~1 trophic level (Deniro & Epstein 1981) or the difference between woodland and farmland habitat types (Figure 4.1). Age and sex did explain some of this within group variation, with isotopic differences between males and females in some parts of the study area (Table 4.2). However, the variation explained by age and sex effects was generally small, such that individual foraging variation within groups primarily occurred independently of these factors due to 'individual specialisation' (Bolnick *et al.* 2003).

The degree of individual specialisation measured as WIC/TNW (within individual component / total niche width, varied markedly across social groups (Figure 4.3) but on average was significantly higher (mean $\delta^{13}C$= 0.47, mean $\delta^{15}N$ = 0.55) than recent examples of individual specialisation in other species (mean=0.66, n=78, Araújo et al. 2011), with values as low as <0.2 for some social groups in the current study. Individual diet variation may potentially result from individuals randomly sampling from among shared resources over short temporal scales (Bolnick *et al.* 2002, 2003), which may contribute to the high levels of specialisation observed in some species where resource use is observed for several days or weeks (Araújo, Bolnick & Layman 2011). This is unlikely to be the case in the current study, as a vibrissa of the average length used in this study

(45mm) requires on average 104 days (range 72-160 days) to grow (Robertson *et al.* 2012) and therefore contains isotopic dietary information over a long timescale. In addition, we also found evidence of consistent year to year individual differences in foraging niche, with correlations between $\delta^{13}C$ and $\delta^{15}N$ values in 2010 with those in 2011 (Figures 4.5 and 4.6). This suggests long-term individual differences in foraging niche, similar to those recorded in several other mammal and bird species (Estes *et al.* 2003; Woo *et al.* 2008; van de Pol *et al.* 2009). For example, individual foraging preferences in populations of sea otters and Brünnich's guillemot *Uria lomvia* have been shown to persist for multiple years in some cases (Estes *et al.* 2003; Woo *et al.* 2008).

Within the population, social group explained a significant amount of isotopic variation, with substantially greater variation in $\delta^{15}N$ than $\delta^{13}C$ between social groups (Figure 4.2). Anthropogenic nitrogen input to pasture may increase the $\delta^{15}N$ values of primary consumers (Vander Zanden *et al.* 2005) and application of fertiliser such as cattle slurry has been shown to result in elevated earthworm $\delta^{15}N$ values (Schmidt 1999). Location and group level differences in $\delta^{15}N$ may therefore occur due to variations in farmland management. In addition, we also observed a large $\delta^{15}N$ difference between invertebrate prey in woodland and farmland habitats (Figure 4.1). Group level differences in $\delta^{15}N$ may therefore be due to variations in the contribution of woodland and farmland to group territories. In contrast $\delta^{13}C$ variation between natural prey sources and social groups were small in the majority of cases (Figures 4.1 and 4.2), with the exception of some social groups within the west of the study area where there seems to be consumption of C4 resources (maize or cattle feed, Figure 4.1).

Group level differences in isotope values are not surprising, as social groups at Woodchester Park differ in their territory habitat composition (Delahay *et al.* 2006a), which has been shown to result in corresponding group level dietary variation in other badger populations (Hofer 1988). Variation in territory composition has also been found to correlate with within-population foraging niche variation in American mink *Neovison vison* (Sidorovich *et al.* 2001), European mink *Mustela lutreola* (Sidorovich *et al.* 2001), grizzly bears *Ursus arctos* (Mattson & Reinhart 1995), American martens *Martes americana* (Ben-David, Flynn & Schell 1997) and coyotes *Canis latrans* (Prugh *et al.* 2008),

suggesting that resource use in territorial species is primarily determined by resource availability. However, if individual foraging niches are primarily determined by resource availability we would expect that variation between individuals within social groups would be relatively small, as all individuals in the social group occupy the same territory. In the present study, individual badgers within social groups differed markedly in their isotope values, with greater variation between individuals within groups, than between groups with different territories. This clearly suggests that individual foraging niches are not purely the product of resource availability and that individuals within the same environment may occupy distinct foraging niches.

Although the majority of the isotopic variation observed within groups is associated with individual differences, we did find that sex and an interaction between sex and location explained variation in $\delta^{15}N$ values. European badgers exhibit sexual dimorphism (Johnson & Macdonald 2001), which is common in mustelids and may result in differences in foraging niche in some species(McDonald *et al.* 2002). Our results suggest that male and female badgers may differ in their foraging niches, with males utilising lower $\delta^{15}N$ prey sources than females. However, the magnitude of this difference appears small and varied with location in the study area. Sex related differences in foraging niche in badgers may therefore be dependent on environmental conditions. Within groups, age may also potentially determine foraging niche either through the effects of ontogenic niche shifts (Newland *et al.* 2009), or as a result of dominance hierarchies (Murray 2006). Previous studies have shown contrasting effects of age and dominance on foraging behaviour in badgers. For example, Revilla (2001) showed that age and dominance affected the utilisation of key habitat in a population of badgers in Spain, however, Macdonald et al. (2002) found no evidence of a social feeding hierarchy in artificially provisioned badgers in the UK. Within the age classes that we sampled in our study (i.e. those over 1 year of age), we found no evidence of an effect of age on isotope values. This suggests that there is no noticeable niche shift with age, and also that differences in social dominance are unlikely to contribute to the patterns of individual foraging variation observed within social groups. It is also worth noting that isotopic variation among individuals may occur due to physiological differences independent of diet. For

example,δ^{15}N values may fluctuate as a product of nutritional stress (Hobson & Alisauskas 1993). However, the magnitude of isotopic variation observed between individuals in the present study (up to 4‰) is much larger than that expected due to stress alone(~0.5–2.0‰ , Hobson & Alisauskas 1993; Cherel, Hobson, & Bailleul 2005). We also observed large variations in δ^{13}C which are unlikely to be affected by these factors.

Our results therefore provide strong evidence that badgers exhibit marked individual specialisation, with consistent foraging preferences expressed by individuals over significant temporal scales. Given that within social groups all individuals have the same local resource availability, why would individuals consistently differ in their foraging niches and utilise only a portion of the available resources? Badgers live in a diverse environment and can potentially utilise a variety of prey sources and habitat types. Although badgers are described primarily as 'gleaners' of relatively immobile prey (Roper 2010), different food may require differing foraging behaviours. For example, behavioural observations of badgers foraging for worms describe specialised foraging behaviour (Kruuk 1978b) which differs from that used when foraging for insect larvae (Pigozzi 1989).Within badger territories prey sources such as worms or insect larvae may also be patchily distributed and vary in their accessibility due to weather conditions (Kruuk 1978). Efficient foraging may therefore require knowledge of prey locations and foraging techniques which may be learned by interacting with the foraging environment, or with other individual badgers. Foraging experiments have shown that badgers are able to remember foraging patch locations and sizes (Mellgren & Roper 1986). Studies of sea otters and dolphins also indicate that foraging specialisations may be learned from conspecifics (Tinker *et al.* 2007; Sargeant & Mann 2009) and several other studies suggest that learned behaviours may lead to individual specialisation (Araujo & Gonzaga 2007; Woo *et al.* 2008), especially if tradeoffs limit the range of behaviours an individual can effectively exhibit (Tinker, Mangel & Estes 2009).

Although behavioural differences may potentially cause individual foraging specialisation in badgers, it is clear from the current study that the degree of specialisation varies between different social groups (Figure 4.3), suggesting that social and environmental factors at the group level may also be important. Intraspecific

competition and resource availability are two factors which are predicted to influence individual foraging variation within populations (Araújo, Bolnick & Layman 2011). For example, individual specialisation is positively correlated with intraspecific competition in sticklebacks (Svanbäck & Bolnick 2007) and with resource diversity in wolves (Darimont, Paquet & Reimchen 2009). Variations in social group size and territory composition may therefore result in varying degrees of specialisation within badger groups, by determining the diversity, abundance and level of competition for available resources.

In summary, this study suggests that individual specialisation may occur within territorial species not only due to variations in territory composition, but also due to individual phenotypic differences independent of resource availability. Intra-specific variation in such traits may have important ecological implications (Bolnick *et al.* 2011; Violle *et al.* 2012). Future research should therefore aim to further investigate the causes and consequences of this behaviour (Bolnick *et al.* 2003; Araújo, Bolnick & Layman 2011)

Table 4.1 Mean $\delta^{13}C$ and $\delta^{15}N$, standard error of the mean, 95% confidence intervals, and minimum and maximum values of badger prey sources collected in 2010 and 2011 at Woodchester Park, Gloucester UK. Sample sizes analysed from each year, C:N mass ratios and dietary (%FO) are also displayed. Dietary (%FO) is the percentage frequency of occurrence of prey types from 100 faecal samples collected across both years.

	$\delta^{13}C$				$\delta^{15}N$				n				
Prey type	Mean	SE	Min	Max	Mean	SE	Min	Max	2010	2011	total	C/N (SD)	(% FO)
Worms	-26	0.2	-27.5	-21	1.9	0.5	-4.9	10.9	33	50	83	3.5 (0.2)	86.3
Beetles	-25.7	0.1	-28.5	-22.7	4.8	0.3	0.4	12.2	39	28	67	3.5 (0.3)	71.6
Snails	-27.1	0.2	-29.2	-25.4	-0.1	0.6	-6.5	7.9	21	18	39	4.0 (0.4)	52.6
Slugs	-26.8	0.2	-28.6	-23.2	-0.1	0.6	-4.5	6.5	14	14	28	4.4 (0.6)	
Insect larvae	-28.5	0.3	-30.2	-23.7	2.5	0.5	-1.4	9.3	20	14	34	4.6 (1.2)	75.8
Peanuts	-24.9	0.3	-26.6	-22.5	0.1	0.2	-0.7	1.7	7	10	17	5.4 (0.9)	
Cattle feed	-17.7	0.2	-18.3	-17.2	3.1	0.1	3	3.2	5	5	10	17.8 (0.7)	0
Maize	-11.3	0.2	-11.3	-11.7	3.7	0.1	3.5	3.7	5		5	12.5 (0.7)	0

Table 4.2 Top models with a ΔAICc values of ≤3 explaining variation in individual badger $\delta^{15}N$ and $\delta^{13}C$ values. Degrees of freedom, ΔAICc, weight and Nagelkerke $R^2$for each model are displayed. The chi squared and p values were calculated to measure the significance of removing individual and social group random effects. The % variance components are also displayed for random effects for each model.

						random effects					
Isotope	Model fixed effects	*df*	ΔAICc	weight	Nagelkerke R^2	Social group (location)			Individual (Social group)		
						% σ2	x^2	P	% σ2	x^2	P
$\delta^{15}N$	Location + Sex + Location X Sex	12	0	0.8	0.07	30.4	17.13	<0.001	29.9	135.7	<0.001
	Location + Sex + Age + Location X Sex	14	2.83	0.2	0.07	29.4	15.51	<0.001	30.4	136.2	<0.001
$\delta^{13}C$	Location	8	0	0.32	0.02	2.8	38.2	<0.001	42.8	157.3	<0.001
	Location + Age	10	0.07	0.31	0.03	1.1	33.4	<0.001	44.4	157.2	<0.001
	Location + Sex	9	0.82	0.21	0.02	2.0	38.3	<0.001	43.2	156.7	<0.001
	Location + Sex + Age	11	1.40	0.16	0.03	0.6	33.6	<0.001	44.6	156.9	<0.001

Table 4.3 Average model coefficients and relative importance of variables included in top model set (AICc of ≤3) explaining individual badger $\delta^{15}N$ and $\delta^{13}C$ variation. Parameter names with brackets show the effect of that parameter category with reference to the reference category (location = wp-core, sex = female, age= adult). Parameters highlighted in bold are those with confidence intervals not spanning zero indicating a consistent directional effect on isotope values.

Isotope	Parameter	Coefficient Estimate	SE	5% CI	95% CI	Relative importance
$\delta^{15}N$	(Intercept)	5.00	0.25	4.59	5.41	~
	Sex(male)	**0.41**	**0.24**	**0.01**	**0.81**	**1**
	Location(wp-east)	0.33	0.36	-0.26	0.93	1
	Location(wp-south)	**3.68**	**0.54**	**2.78**	**4.57**	**1**
	Location(wp-west)	-0.30	0.39	-0.94	0.34	1
	Sex(male) X Location(wpeast)	**-1.08**	**0.34**	**-1.64**	**-0.52**	**1**
	Sex(male) X Location(wp-south)	-0.31	0.51	-1.14	0.52	1
	Sex(male) X Location(wp-west)	**-0.69**	**0.34**	**-1.25**	**-0.12**	**1**
	Age(old)	-0.17	0.15	-0.42	0.07	0.2
	Age (yearling)	-0.05	0.13	-0.27	0.16	0.2
$\delta^{13}C$ †	(Intercept)	35.50	3.20	30.23	40.77	~
	Location(wp-east)	-3.04	2.34	-6.88	0.80	1
	Location(wp-south)	1.58	3.04	-3.43	6.58	1
	Location(wp-west)	**-1.09**	**2.40**	**-14.87**	**-6.98**	**1**
	Age(old)	-2.73	1.80	-5.69	0.23	0.47
	Age (yearling)	1.66	1.70	-1.14	4.46	0.47
	Sex(male)	1.75	1.73	-1.10	4.59	0.37

†$\delta^{13}C$ values Box-Cox transformed – coefficient, SE and CI values are all $X10^{e-14}$

Figure 4.1 Mean vibrissae $\delta^{15}N$ and $\delta^{13}C$ values of individual badgers (Woodchester Park, Gloucestershire, UK) in 2010 (n=77, grey points) and 2011(n=78, black crosses) and their potential prey sources. Individual badgers and prey sources values are displayed in four sub locations within study area, with isotopically and ecologically similar prey sources merged together for clarity. Prey sources are; carabid beetles collected in woodland (BW) or farmland (BF), 'other invertebrates (worms, slugs and snails) in woodland (OIW) and farmland(OIF), worms in maize fields (WM), insect larvae (IL) , peanuts (P) and 'C4 farm resources' (CF) which includes maize and cattle feed.

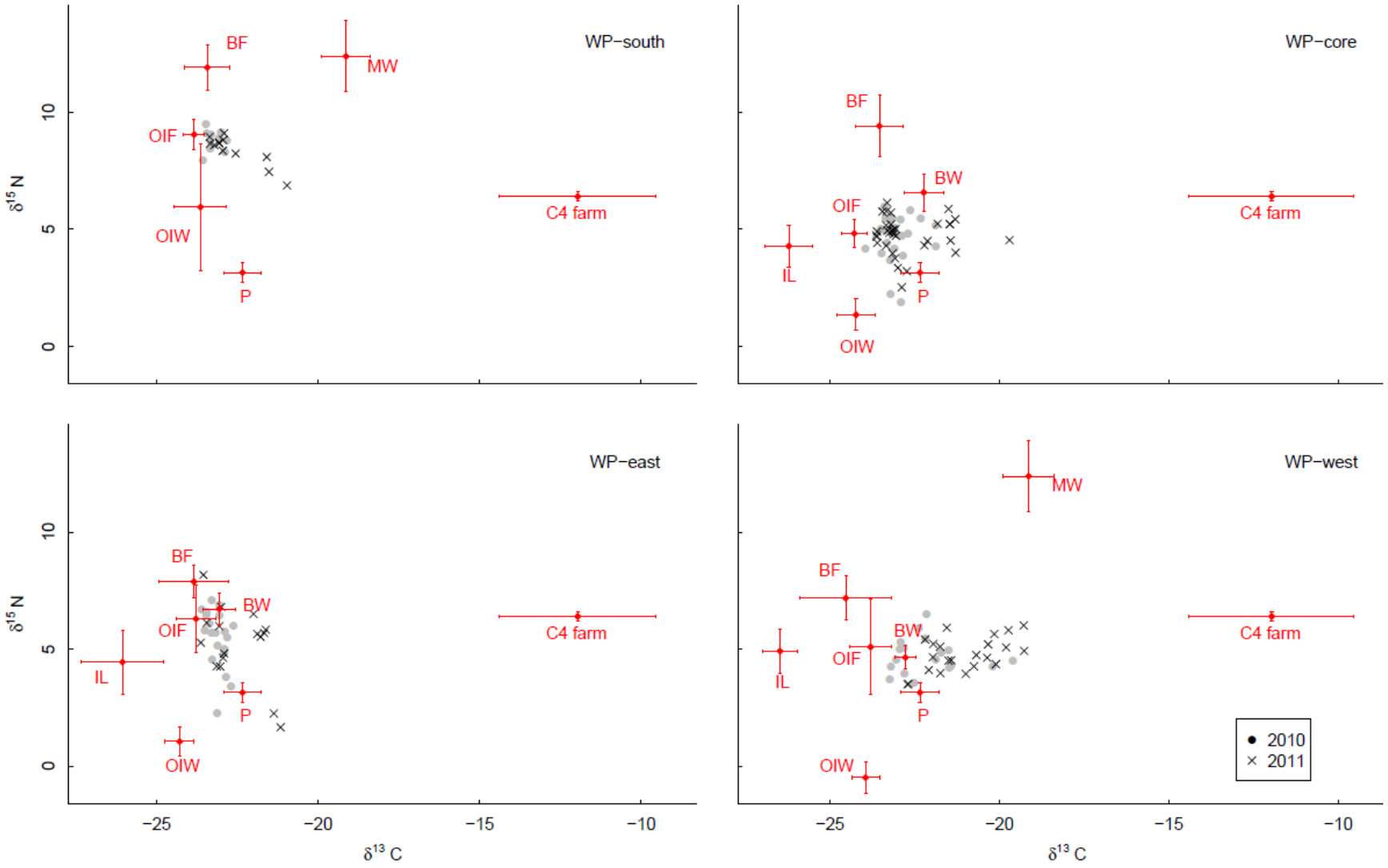

WP−south
WP−core
WP−east
WP−west
BF
MW
OIF
OIW
P
C4 farm
BW
IL
δ15 N
δ13 C
2010
2011

Figure 4.2 Mean vibrissae $\delta^{15}N$ and $\delta^{13}C$ values of badger social groups in four sub-locations within the Woodchester park study site in spring 2010 and 2011.Error bars are standard error of the mean for clarity of presentation

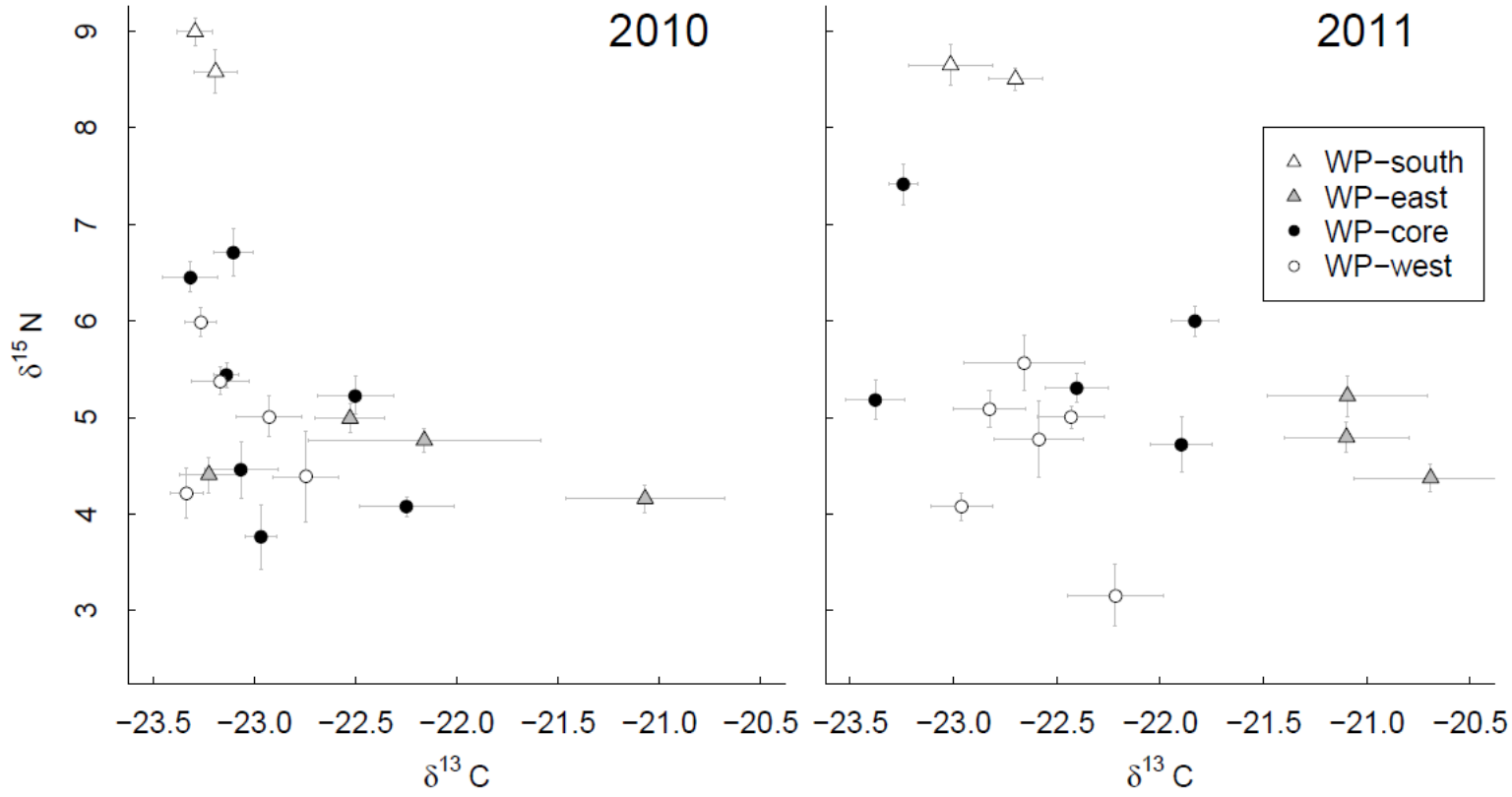


Figure 4.3 Examples of individual isotopic niche variation within four badger social groups ('Beech', 'Breakheart', 'Kennel' and 'Honeywell') in spring 2010. Points are mean $\delta^{15}N$ and $\delta^{13}C$ values for individual badgers in 2010. Error bars are 95% confidence intervals.

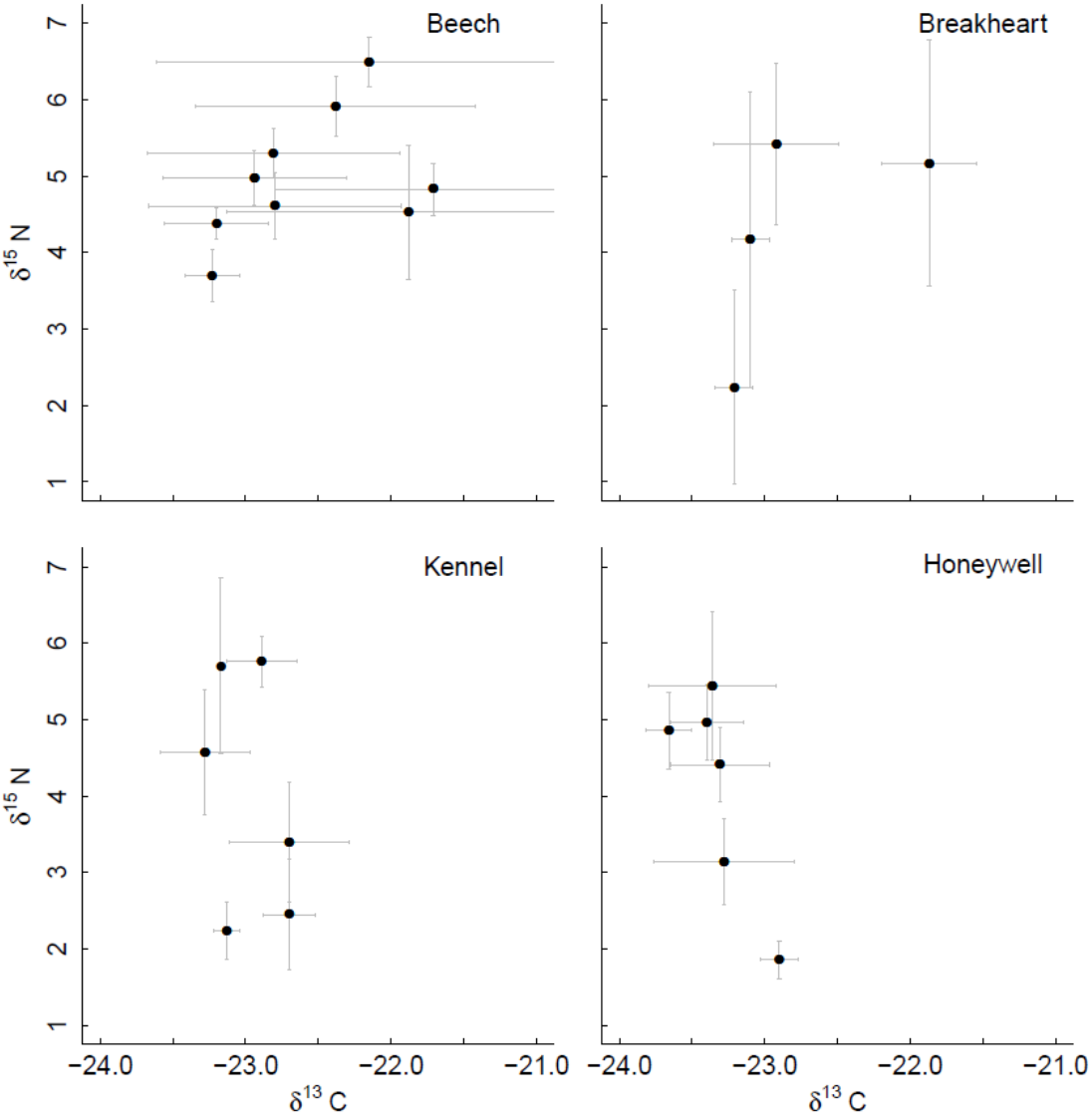


Figure 4.4 Frequency histograms of the degree of individual specialisation (measured as the within individual component of niche width WIC/total niche width TNW) in $\delta^{15}N$ and $\delta^{13}C$ values within badger social groups. The WIC/TNW metric for each social group is calculated using sum of squares values obtained from analysis of variance with individual as a fixed effect.

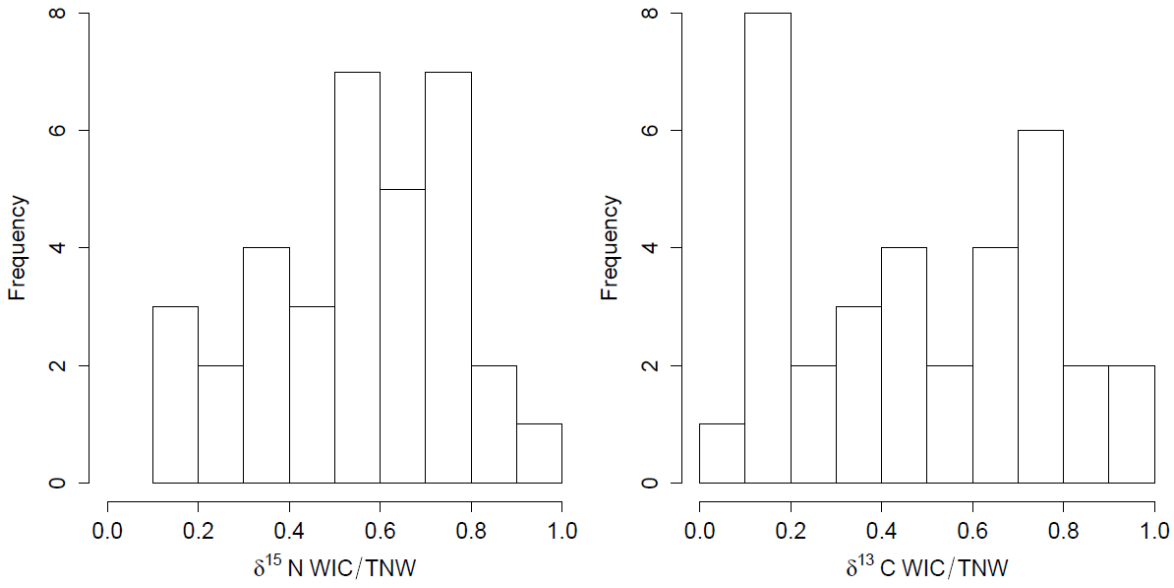


Figure 4.5 Examples of consistent individual isotopic variation within badger social groups across years. Values are mean vibrissae $\delta^{15}N$ and $\delta^{13}C$ values of individual badgers (identifiable by four digit i.d codes) in two social groups, error bars are standard error of the mean for clarity of presentation.

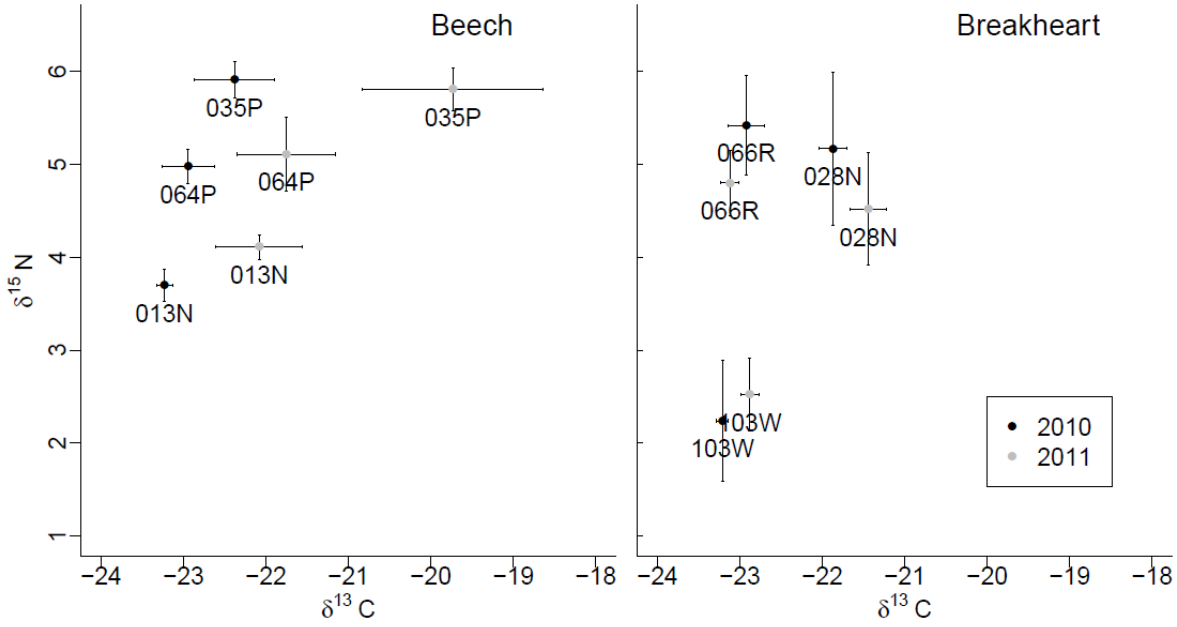


Figure 4.6 Correlation between individual badger vibrissa segment $\delta^{13}C$ and $\delta^{15}N$ values in 2010 with those in the same vibrissa segment on the same individual in 2011. Values are labelled by location (Figure 1).

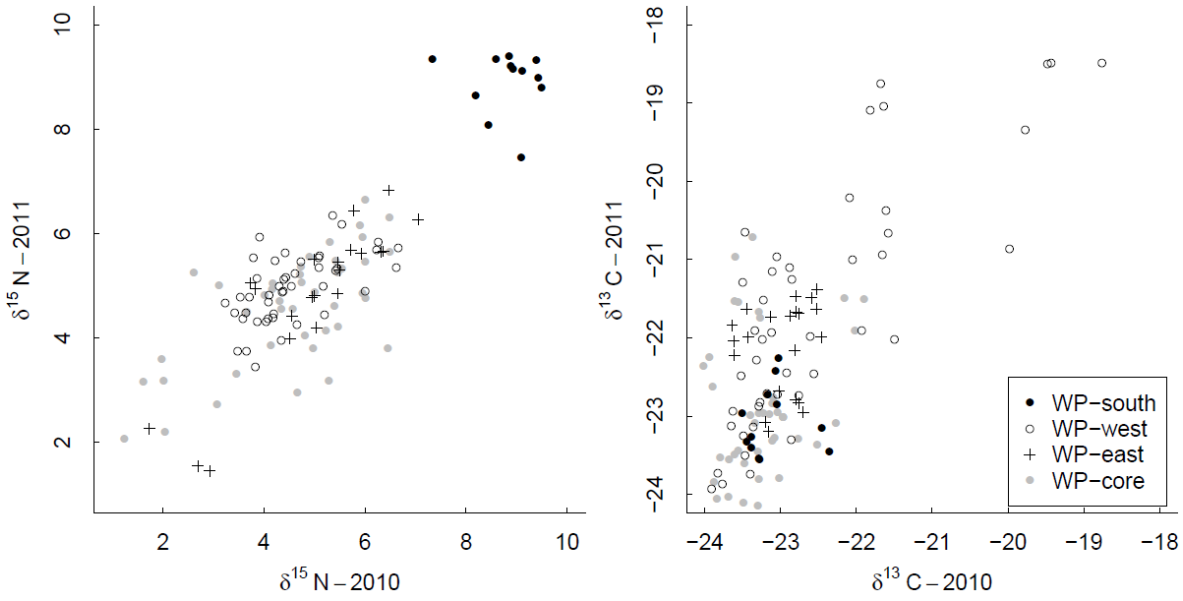
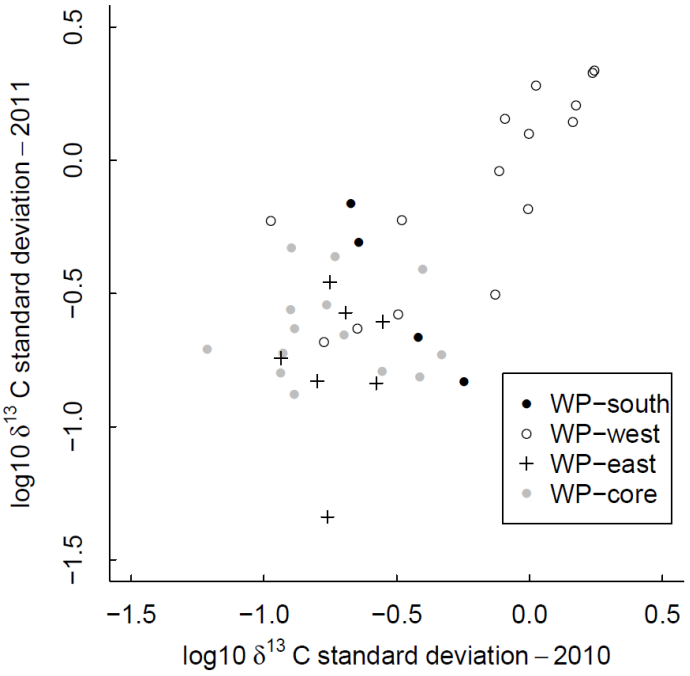


Figure 4.7 Correlation between the standard deviation in individual badger $\delta^{13}C$ values (log 10 transformed) calculated from serial sampling of individual vibrissa in 2010, with that of the same individual in sampled in 2011. Individual badgers are labelled by location.



CHAPTER 5: Intraspecific competition and resource availability affect the incidence and consequence of niche specialisation in a social carnivore

5.1 Abstract

1. Individual niche variation is widespread in animal populations with important implications for their, ecology, evolution and management. However, to date the causes and consequences of this behaviour remains poorly understood.
2. In the current study we use stable isotope analysis to infer diets and thereby investigate the causes and consequences of individual niche variation in the European badger; a mustelid that lives in territorial social groups, but forages alone. We aim to determine how ecological factors at the group level influence individual niche variation within groups, as well as the fitness effects of specialised foraging strategies.
3. We find that the degree of individual niche variation within groups is positively related to the group size. Individual niche variation was also negatively related to the availability of farmland habitats (an important foraging habitat for badgers) and the consumption of anthropogenic foods, supporting the idea that resource competition results in increased individual specialisation.
4. We also find that the degree of individual specialisation is related to an individual's body condition, but that this effect varies with the ecological context. Specialisation has a stronger positive relationship with body condition in larger social groups and those with reduced availability of key farmland habitats.
5. This study demonstrates that competition plays a crucial role in determining patterns of individual niche variation and may also influence the fitness consequences associated with divergent foraging strategies. Intraspecific competition may therefore act to both cause and maintain individual niche specialisation within populations.

5.2 Introduction

Niche variation within biological systems is ubiquitous, occurring at a variety of levels from communities to individuals (Devictor *et al.* 2010) and plays an important role in stabilising species interactions and maintaining biodiversity (Levine & HilleRisLambers 2009). Individual niche variation, although previously believed to be unimportant, is increasingly being recognised as key component of a population's niche width (Bolnick *et al.* 2003). In addition, there is growing recognition that individual niche variation has important implications for ecological and evolutionary processes, both at the population and community level (Bolnick *et al.* 2011; Violle *et al.* 2012; Sih *et al.* 2012; Dall *et al.* 2012). In some instances individual variation is the product of class differences, with differing ages or sexes occupying distinct niches (Bolnick *et al.* 2003). However, in large number of cases this variation occurs independent of such factors and is termed 'individual specialisation' (Bolnick *et al.* 2003).The widespread occurrence of individual niche variation may seem surprising, as foraging theory suggests that individuals should focus on those resources which are most valuable, and where fitness is maximised, effectively reducing variation (Pyke, Pulliam & Charnov 1977; Bolnick *et al.* 2003). Despite this, the number of species where individual specialisation has been documented continues to grow, with this behaviour recorded in over 180 species to date (Araújo, Bolnick & Layman 2011). Due to the widespread occurrence of individual specialisation and its important implications, evolutionary biologists and ecologists are becoming increasingly interested in investigating the forces which cause and maintain niche variation within wild populations.

According to optimal foraging theory (OFT), and individual's resource use is determined by an interaction between an individual's phenotype and its foraging environment, with the ultimate aim of maximising energy intake while minimising energy expended (Pyke, Pulliam & Charnov 1977; Persson 1985). An individual's phenotype influences its resource use via its effects on an individual's abilities to identify, handle and consume resources, which determines its resource preferences or 'fundamental niche'(Bolnick *et al.* 2003, Hutchinson 1957). Morphological, behavioural or physiological phenotypic variation may therefore result in individual niche variation via its effects on an individual's preferences or efficiency (Svanback & Eklov 2003, 2004). However, an

individual's actual resource use or 'realised niche' is not only determined by its phenotype, but also by its environment (Hutchinson 1957). The environment determines resource availability, and also the abundance, degree of competition and predation risk associated with those resources present. Individual specialisation may therefore occur if individuals within a population experience different environmental conditions, for example due to territoriality (Prugh *et al.* 2008). Individuals may also vary in their patterns of resource use if phenotypic differences mean that they vary in their competitive ability, or in their response to changes in the foraging environment (Svanbäck & Bolnick 2005, 2007). As a consequence, ecological factors which affect the foraging environment at the individual and population level also play an important role in driving patterns of niche variation (Araújo, Bolnick & Layman 2011).

Two key ecological factors, which are not necessarily mutually exclusive, that are predicted to increase individual niche variation within populations are intraspecific competition and resource limitation (Araújo, Bolnick & Layman 2011). Competition and resource limitation are predicted to cause the population niche to expand as individuals are forced to utilise less profitable resources (Schoener 1971; Roughgarden 1972). If individuals differ in their ranked prey preferences, or their competitive ability, individual niche variation will therefore increase as a result, as individuals diversify in their foraging niche when the availability of the most prized resources is limited (Svanbäck & Bolnick 2005; Tinker, Mangel & Estes 2009). These predictions have been confirmed by several recent empirical studies which demonstrate that population density (a common proxy for competition) and resource limitation correlate with increased individual niche variation within populations (Svanbäck & Bolnick 2007; Tinker, Bentall & Estes 2008; Svanbäck & Persson 2009). Populations occupying broader niches, either due to increased competition or due to ecological opportunity, may also be composed of specialised individuals if constraints limit individual niche widths, such that individuals occupy a smaller proportion of the population's niche as it expands (Bolnick *et al.* 2007; Darimont, Paquet & Reimchen 2009). This is the basis of Van Valen's (1965) niche variation hypotheses (NVH) which predicts that populations with broader niches are also more heterogeneous. Although this hypothesis received limited support in the decades after its conception (Bolnick *et al.*

2003, 2007), several recent studies have found a positive correlation between population niche width and individual specialisation in a variety of taxa, confirming the predictions of the NVH (Bolnick *et al.* 2007; Tinker, Bentall & Estes 2008; Costa *et al.* 2008; Darimont, Paquet & Reimchen 2009). However, Agashe & Bolnick (2010) recently found no support for the NVH in lab populations of flour beetles, indicating that this relationship may not be as widespread as previously thought (Bolnick *et al.* 2007).

Although an increasing number of studies are moving from simply documenting individual niche variation to investigating its causes (Araújo, Bolnick & Layman 2011), the drivers of this behaviour still remain unknown in the majority of cases where it occurs. Moreover, in many populations it is also unclear whether specialised individuals achieve higher fitness than generalists, or whether differing specialist strategies are equivalent (Bolnick *et al.* 2003; Woo *et al.* 2008). This is important, as although ecological factors such as competition may initially lead individuals to specialise, fitness tradeoffs associated with differing strategies may act to maintain them in the long term (Bolnick *et al.* 2003; Bolnick 2004; Martin & Pfennig 2009; Cucherousset *et al.* 2011)

It is generally assumed that specialists are more efficient foragers than generalists (Bolnick *et al.* 2003) and as a consequence specialisation will be maintained within populations via disruptive selection away from generalist phenotypes (Bolnick *et al.* 2003; Bolnick 2004; Martin & Pfennig 2009; Cucherousset *et al.* 2011). To date, studies have found mixed effects of niche variation on fitness, with niche variation resulting in fitness consequences in some cases (Golet *et al.* 2000; Votier *et al.* 2004; Darimont, Paquet & Reimchen 2007; Johnson *et al.* 2009; Cucherousset *et al.* 2011; Authier *et al.* 2012), but with no effect in others (Katzner *et al.* 2005; Woo *et al.* 2008; Chilvers & Wilkinson 2009; Kobler *et al.* 2009; Whitfield *et al.* 2009; van de Pol *et al.* 2009). In some studies researchers quantify specialisation along a continuous axis (e.g. Woo et al. 2008), however, some populations contain differing types of specialist such that fitness varies not only between generalists and specialists but between differing specialist types. For example, egg size and volume are greater in Great Skuas *Stercorarius skua* specialising on sea birds than those predominantly eating fish (Votier *et al.* 2004). Some authors have also suggested that the fitness benefits and costs associated with differing foraging

strategies may be context dependent (Woo *et al.* 2008; Matich, Heithaus & Layman 2011), and although this has been demonstrated in some cases (Van de Pol *et al.* 2009; Svanbäck & Persson 2009), few studies have investigated how the ecological context may influence the consequences of individual niche variation for fitness.

In order to investigate individual niche specialisation within populations, researchers ideally require repeat measures of resource use which are obtained simultaneously and from a large number of individuals (Araújo, Bolnick & Layman 2011). This is difficult, or often impossible to obtain for the majority of species, particularly using traditional method such as gut and stomach content analysis (Bolnick *et al.* 2002). In recent years stable isotope analysis has emerged as a widespread tool in the field of foraging ecology (Crawford, McDonald & Bearhop 2008), and is increasingly being applied to studies of individual specialisation (Bearhop *et al.* 2000; Newsome *et al.* 2009). Stable isotope analysis works on the premise that the stable isotope ratios of a consumer's proteinaceous tissues reflect that of their diet, over the period of tissue synthesis (Deniro & Epstein 1978, 1981; Hobson & Clark 1992). Dietary studies typically measure ratios of C^{13}:C^{12} and N^{15}:N^{14} which vary predominantly with habitat and trophic level respectively, and depending on the tissue analysed, can yield months to years worth of foraging information (Crawford, McDonald & Bearhop 2008). Variation in isotopic values within and between individuals, can therefore provide information on the degree of foraging niche, or 'isotopic niche' variation at the individual and population level (Bearhop *et al.* 2004; Layman *et al.* 2007, 2011). Isotopic values of individuals and their prey sources can also be transformed by the use of stable isotope mixing models in order to estimate the contributions of dietary sources to an individual's diet (Phillips 2012). Individual isotopic data in the form of 'δ-space' (bivariate C and N data) or 'p-space' (isotope derived dietary proportions) can therefore provide several meaningful quantitative measures of individual specialisation (Newsome *et al.* 2007, 2012).

In this study we use stable isotope-derived metrics in order to investigate individual foraging specialisation in a population of European badgers (*Meles meles*) in the UK. Badgers are large omnivorous mustelids which in the UK live in territorial social groups with a shared burrow system, known as a 'sett'. Although badgers are social animals,

individuals within social groups forage predominantly alone and previous studies using stable isotope analysis have found that significant long-term isotopic differences occur between individual badgers within social groups, suggesting individual foraging specialisation (Chapter 4). The degree of individual niche variation varies at the group level, hinting that ecological effects may influence patterns of individual resource use within the population (Chapter 4). Badger social groups vary in their size and in the composition of their territories; as a consequence, individuals within different groups may experience varying levels of competition and resource availability. Badgers therefore make an ideal model species to study the ecological causes and consequences of individual niche variation.

We aim to quantify the degree of individual specialisation within badger social groups and then use ecological information about individuals and groups in order to answer several questions relating to the causes and consequences of this behaviour. Specifically we aim to test whether individual niche variation is related to levels of competition (using group size as a proxy), resource availability (using habitat composition), or group niche width (in accordance with the niche variation hypothesis). Second, we aim to investigate whether individual specialisation is related to individual body condition (a common proxy for fitness, Kobler *et al.* 2009), and whether the relationship between body condition and specialisation varies with the ecological context.

5.3 Methods

Study area and sample collection

This study was carried out at Woodchester Park, Gloucestershire, UK where the resident badger population has been the subject of a long-term capture-mark-recapture study for over thirty years. The study site is approximately 7km^2 and consists of a mosaic of mixed woodland and farmland habitats, supporting a badger population of approximately 22 social groups, whose territory boundaries are determined annually (Delahay *et al.* 2000, 2006a). Detailed habitat maps are available for the study area (Delahay *et al.* 2006a) and major changes to habitat were updated for the current study. Badgers were live captured in spring 2010 (11th May – 16th June) and 2011 (3rd-25th May) following standard trapping protocols. Once captured, vibrissae (one per individual) were sampled from anesthetised badgers using steel scissors by cutting as close to the base as possible. Vibrissae sampled from badgers measured on average 45mm in length, which reflects 3-5 months of growth, therefore providing long-term isotopic dietary information (Robertson *et al.* 2012). Badger prey types were identified from faecal analysis and a large number of samples were collected for SIA from different habitats across the study site as described in (Chapters 3 and 4). All work involving the capture and sampling of live badgers was carried under English Nature and UK Home Office licences, in accordance with the Animals (Scientific Procedures) Act 1986 and was subject to an internal ethical review process.

Sample preparation and stable isotope analysis

Badger vibrissae were rinsed thoroughly in distilled water and scraped with fine forceps to remove dirt and potential surface contaminants and then dried. Once prepared each vibrissa was sub-sampled into ~0.4mg sections using a scalpel (average of 4 sections, sd=1, range = 3-7), and each section was sealed in tin capsules for analysis. Prey samples were defrosted and cleaned in distilled water to remove soil or detritus. Preparation of prey samples was carried out with reference to faecal samples. Only digestible components were used and indigestible components such as invertebrate exoskeletons or snail shells were removed. Prey were then dried for 72 hours at 50°C and free lipids were extracted using a Soxhlet apparatus and 2:1 chloroform:methanol solvent. Each sample was then

homogenised and ~0.8mg of powdered material was sealed in a tin cup for SIA. All stable isotope analysis was carried out at the Food and Environment Research Agency mass spectrometry facility in York, UK using a Fisons 1108 elemental analyser (EA) linked to a continuous-flow isotope-ratio mass spectrometer (Isoprime – GV instruments). Isotope ratios are expressed as δ values, which is reported in parts per thousand (‰) with reference to international standards. Within-run mean accuracy of a collagen standard was 0.05‰ for $\delta^{13}C$ and 0.11‰ for $\delta^{15}N$.

Quantifying individual specialisation

We used two approaches in order to quantify the degree of individual specialisation exhibited by individual badgers within social groups in relation to the breadth of their isotopic niche and in relation to their diet or niche position.

Niche width

In order to quantify individual specialisation in niche width we used Roughgarden's WIC/TNW metric (Roughgarden 1974), where WIC corresponds to the within-individual-component of the population's niche width and TNW is the total niche width of the population. The degree of specialisation ranges from 0-1 with high values indicating low specialisation (as individual niche widths are comparable to that of the population) and low values indicating high degrees of individual specialisation (as individuals utilise a narrow range of the population's niche). In order to make this more intuitive we converted this to the index $IS_{W/T}$ = 1-WIC/TNW so that higher values equal higher levels of specialisation.

In order to calculate niche widths for individual badgers and their social groups we build on theoretical work by Layman *et al.* (2007) and Bearhop *et al.* (2004) which suggests that a population's or individual's isotopic variation or 'isotopic niche' reflects the range of resources utilised and is therefore analogous to its ecological niche (Bearhop *et al.* 2004; Newsome *et al.* 2007; Layman *et al.* 2011). We calculated the isotopic niche width of an individual badger (δWIC) as the total area (TA) convex hull (Layman *et al.* 2007) in $\delta^{13}C$ - $\delta^{15}N$ bi-plot space encompassed by isotopic values from serial samples

along that individual's vibrissa (Figure 5.1). The total isotopic niche width of a social group (δTNW) is the total area convex hull in $\delta^{13}C$- $\delta^{15}N$ bi-plot space encompassed by all vibrissa section isotopic values from all individuals within that badger social group (Figure 5.1). The degree of isotopic niche specialisation for individual *i* in social group *j* is then calculated as $IS_{W/T}$= 1- δWIC*i*/ δTNW*j*. As individuals varied in the length of the sampled vibrissa and therefore the number of vibrissa sections analysed, we carried out all δWIC/δTNW calculations using only the three most basal vibrissa sections from each vibrissa in order to keep the temporal scale approximately equal for all individuals, and so that social group hull areas were not biased towards individuals with a greater number of measurements (longer vibrissae). This method provides a relative measure of how isotopically variable individual badgers are in relation to their social group. In addition, although it is also possible to calculate isotopic niche width in bivariate space using ellipses, this is not accurate with only three isotope measurements (Jackson *et al.* 2011)

Niche position

The metric WIC/TNW classifies a specialist as an individual whose niche is smaller than that of its group or population, which fits with conventional definitions of individual specialisation (Bolnick *et al.* 2003). However, by using this metric, two individuals with equal niche widths (range of resources consumed) are classed as equally specialised, even if the individuals in question differ substantially in their actual resource use or niche position (Figure 5.1). In order to address this, we also calculated individual specialisation in niche position or diet, using the proportional similarity (PS) index (Bolnick *et al.* 2002). This quantifies the degree of dietary overlap, or similarity between an individual and the population according to the equation:

$$PS_i = 1 - 0.5 \sum_j | p_{ij} - q_j | = \sum_j \min(p_{ij}, q_j)$$

where p_{ij} is the proportion of prey source j in individual i's diet, and q_j is the proportion of prey source j in the population's diet. Values of PS vary from 0-1, with low values indicating high degrees of specialisation (as individual diet is different to the population) and high values close to 1 indicating low degrees of specialisation (as individual and

populations diet becomes more similar). As with WIC/TNW, in order to make this more intuitive we used the index IS_{PS} = 1-PS so that high values equal high levels of specialisation (Figure 5.1).

Diets of individual badgers were estimated using the Bayesian mixing model SIAR (Parnell *et al.* 2010), which uses isotopic variation in individual consumers and their prey sources in order to estimate the likely contribution of those prey sources to the consumer's diet. Trophic discrimination factors of 2.55‰ and 3.05‰ were used for $\delta^{13}C$ and $\delta^{15}N$ respectively and were calculated by averaging across published values for mammalian carnivore hair (Caut, Angulo & Courchamp 2009; Newsome *et al.* 2010a; Lecomte *et al.* 2011). Standard deviations of 0.7‰ and 0.6‰ were used for $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors respectively, based on published values for population variation in another mustelid; the sea otter (Newsome *et al.* 2010a). All SIAR models included C:N ratios of potential prey sources to control for concentration dependence (Phillips *et al.* 2012). Due to spatial variation in prey sources isotope values the study location was divided into four areas (west, core, east and south, Chapters 3 and 4) and SIAR calculations carried out using badgers and prey matched within locations. Some prey types which were ecologically similar and isotopically indistinct were merged for use in mixing models. Prey sources used were; 1) beetles, 2) insect larvae (Tipulidae and Noctuidae), 3) C4 farm resources (cattle feed and maize), 4) OIF (other invertebrates - worms/snails/slugs in farmland habitats), 5) OIW (other invertebrates in woodland habitats), and in the west of the study area 6) worms collected from maize fields. Proportional Similarity values were calculated using the modal (most likely) estimate of prey source contributions to individual diets (p_{ij}) compared to that of their social groups diet (q_j), with social group diets calculated using isotopic values from all individuals, except individual *i,* within that group. We excluded individuals from their group diet calculations, as the number of individuals sampled per group varied from 2-9, resulting in a corresponding variation in the contribution of an individual's data to the group's diet calculations. This would potentially underestimate PS in small groups and inflate values in large groups. As with measures of $IS_{W/T}$ only the three basal vibrissa sections were used in

IS_{PS} calculations, so that the timescale of foraging information was equal for all individuals and groups.

Body condition

In order to calculate the body condition of individual badgers we used the scaled mass index, which standardizes body mass at a fixed value of a linear body measurement based on the scaling relationship between mass and length (Peig & Green 2009). This has been demonstrated as performing better than other conventionally used measures of body condition estimated from similar mass length relationships (Peig & Green 2009, 2010). We used mass in kilograms and body length in cm as our variables and calculated scaling coefficients for males and females separately using data from >2000 individuals (all over 1 year old) in each case obtained from the Woodchester Park data base.

Statistical analysis

To investigate which factors influenced the degree of individual specialisation within badger social groups we fitted a series of generalised linear mixed models and evaluated these using Akaike's information criterion (AICc). Two separate analyses were carried out with either IS_{PS} or $IS_{W/T}$ as the response variable. Both IS_{PS} and $IS_{W/T}$ were logit transformed order to meet normality assumptions (Warton & Hui 2011). We excluded social groups from the analysis where <40% of the estimated total group size had been analysed. We did this in order to remove large social groups where only a small proportion of the group had been sampled which will potentially result in a misrepresentation of the group's total isotopic niche/diet and therefore bias assessments of the degree of individual specialisation. Fixed effects in both models included individual age (three categories: yearling=1 year old, adult= 2-4 years, or old = >4 years) and sex. Group size (total number of adult individuals captured in a given group in the past 12 month period) was also included in models as a measure of within group competition. 'Prop farmland', the proportion of the territory which was farmland (all arable and grassland categories) was included in models as a measure of resource availability. Arable fields and grassland are key foraging habitats for badgers, as they contain a much higher biomass of earthworms

(which in the UK is their primary prey source, Kruuk 1978) compared to woodland habitats (Hofer 1988). Although mature deciduous woodland (excluding Beech dominated stands) also has a high abundance of earthworms (Hofer 1988), this habitat is rare or absent from the vast majority of the study site, so for simplicity badger territories were divided into woodland and farmland habitats based on habitat data. In order to test the NVH and whether individual specialisation correlates with niche width (TNW), we included social group δTNW in models explaining variation in $IS_{W/T}$. We also included group mean $\delta^{13}C$ as an additional variable to account for variation in the availability of C4 farm resources in group diets, which could potentially influence the effects of competition and natural prey availability on the degree of specialisation. Potential two-way interactions included in models were; group size X prop farmland, group mean $\delta^{13}C$ X group size, group mean $\delta^{13}C$ X prop farmland, age X prop farmland, age X group size, sex X group size, sex X prop farmland and age X sex. Random effects in models were social group and year.

In order to investigate the relationship between the degree of individual specialisation and individual body condition we used a similar approach, fitting general linear mixed models to assess variation in the scaled mass index of individual badgers. Fixed effects included in models were, $IS_{W/T}$, $IS_{PS,}$ age, sex, group size, proportion of farmland and group mean $\delta^{13}C$. To test whether the effect of individual specialisation on body condition varied with competition and resource availability we included potential two way interactions between $IS_{W/T}$/IS_{PS} and group size, prop farmland and group mean $\delta^{13}C$. We also included two dietary variables to test whether individuals specialising on specific resources were in better condition. Variables were; 'diff OIW' (the difference between the estimated contribution of OIW; other invertebrates in woodland habitats, in the individual diet to that of its group) and 'diff C4' (the difference between the estimated contribution of C4 farm resources; maize and farm feed, in the individual diet to that of its group).

For all three analyses, models containing different combinations of fixed effects were compared using the package 'MuMIn' using R 2.15.0 (cran.r-project.org). Prior to analysis input variables were standardised to a mean of zero and a standard deviation of two (Gelman 2008; Grueber *et al.* 2011). Potential models were restricted to top model

set with <2 delta AIC (Burnham and Anderson 2002). Average model coefficients were then calculated using this refined model set. Variables were deemed to have an effect on the degree of individual specialisation if the coefficient confidence intervals did not span zero (Grueber *et al.* 2011). To provide an additional method of model fit, Nagelkerke R^2 (Nagelkerke 1991) were calculated and displayed for each top model.

5.4 Results

Individual specialisation indices were calculated for a total of 144 badgers from 19 social groups across the two year study period. Of these, nine badgers from four social groups were removed from the following analyses as <40% of the group was sampled. This left a final data set of 135 samples from 106 different individuals, with 63 individuals from 12 social groups caught in 2010 and 72 individuals from 14 social groups caught in 2011. The degree of individual specialisation varied between individuals and the two metrics used, with a mean (sd) of 0.15 (0.08) and 0.94 (0.08) for $IS_{W/T}$ and IS_{PS} respectively. Individual IS_{PS} and $IS_{W/T}$ values were not significantly correlated (r=0.13, t_{133} = 1.561, p=0.12).

Variation in the degree of individual specialisation was best explained by a top model set of three and ten models for IS_{PS} and $IS_{W/T}$ respectively, containing a total of six single variables and five two way interactions (Table 5.1). Fixed effects in top models explained on average 16% (sd=2) of the variation in $IS_{W/T}$ and 19% (sd=2) variation in IS_{PS}. The degree of individual specialisation measured both as IS_{PS} and $IS_{W/T}$ was related to availability of farmland in the social group's territory (IS_{PS}, estimate=-0.73, 95%CI = -1.04 to -0.44, relative importance=1; $IS_{W/T}$, estimate=-0.60, 95%CI = -1.00 to -0.20, relative importance=1). Both metrics indicated greater specialisation in territories with a lower proportion of farmland. The relationship between the proportion of farmland in a group's territory and IS_{PS} varied with age, with a negative relationship for adult badgers (2-4 years old), but a slight, positive relationship in yearlings and older (>4years old) badgers (variable relative importance=1, Tables 5.1 and 5.2). The degree of specialisation measured using both indices was also related to the size of the social group, with higher levels of specialisation in larger groups (IS_{PS}, estimate= 0.32, 95%CI = 0.10-0.55, relative importance=1, $IS_{W/T}$, estimate= 0.45, 95%CI = 0.07-0.83, relative importance=1, Tables 5.1

and 5.2). Group size was included as a measure of competition, however the size of the social group was also significantly correlated with social group territory size (t_{28}=3.45, p=0.001, Pearsons r=0.56), indicating it is both a measure of number of competitors, as well as the spatial extent of the territory.

For both indices of specialisation the relationship with group size varied according to the group's mean $\delta^{13}C$, with a reduced effect of group size in social groups with higher $\delta^{13}C$ values suggesting consumption of anthropogenic C4 resources. This was confirmed by a positive correlation between the proportion of C4 resources in individual diets and the mean $\delta^{13}C$ of the group (r=0.71, t_{133}=11.5, p=<0.001, Figure 5.3). There was also an interaction between sex and group size, with a greater effect of group size on $IS_{W/T}$ in male than female badgers (Tables 5.1 and 5.2).

All three top models explaining variation in $IS_{T/W}$ also included group δTNW (variable relative importance =1, Table 5.1) with a strong positive relationship between $IS_{T/W}$ and δTNW indicating that groups with broader isotopic niches had higher levels of individual specialisation (Figure 5.3). This occurred despite a positive relationship between δWIC and δTNW (likelihood ratio test, $X^2_{1,133}$=6.18, p=0.01), indicating individual isotopic niche widths were larger in social groups with broader isotopic niches (Figure 5.4).

Effects of specialisation on body condition

Variation in the scaled mass index of badgers was potentially explained by a top model set (<2 ΔAICc) of 16 candidate models, 12 of which contained either $IS_{W/T}$ or IS_{PS}. The degree of individual specialisation exhibited by badgers was related to their body condition, and the effect of specialisation was dependent on both the proportion of farmland within the group's territory for both specialisation indices (IS_{PS}, estimate=-0.77, 95%CI = -1.31 to -0.22, relative importance=0.55; $IS_{W/T}$, estimate=-0.60, 95%CI = -1.16 to -0.05, relative importance=0.48) and the size of the group for IS_{PS} (estimate=0.88, 95%CI = 0.14 to 1.62, relative importance=0.55,Tables 5.3 and 5.4). More specialised individuals (for both indices) in social groups with low availability of farmland habitats were in relatively better body condition than those which were less specialised (Figure 5.5). Similarly, individuals with more specialised diets (higher IS_{PS}) were also in better condition in larger social

groups (Figure 5.5). Although the dietary variables diff OIW (the difference between the estimated contribution of OIW; other invertebrates in woodland habitats, in the individual diet to that of its group) and diff C4 (the difference between the estimated contribution of C4 farm resources; maize and farm feed, in the individual diet to that of its group) were included in top models the average model coefficients spanned zero, suggesting a small or inconsistent effect of these variables.

5.5 Discussion

Individual specialisation was higher in social groups with low availability of key farmland resources and in social groups of larger size, indicating that competition and resource limitation both act to drive individual niche variation. We also found that social groups occupying broader isotopic niches were made up of more individuals specialising on a subset of resources, confirming the predictions of the NVH and suggesting that generalisation at the group level is achieved via increased individual specialisation. Finally, our results demonstrate that the degree of individual specialisation is related to body condition, a potential proxy for fitness. However, the effect of individual specialisation is context dependent, varying with group level measures of resource availability. We discuss the details and implications of our findings below.

Causes of individual niche variation

Individual niche variation is predicted to correlate with resource competition by traditional optimal foraging theory models and by more recent models investigating the causes of individual specialisation (Svanbäck & Bolnick 2005; Tinker, Mangel & Estes 2009). Under conditions of high competition, access to the most valuable resources will decrease, forcing individuals to utilise secondary or tertiary resources. This will result in increased individual variation if individuals differ in their ranked preferences, or the rate at which they accept lower ranked resources (Svanbäck & Bolnick 2005).

We found that niche specialisation measured both as IS_{PS} and $IS_{W/T}$ was negatively related to the proportional availability of farmland habitats within the group's territory,

such that individual specialisation increased where farmland resources were relatively limited. Individual niche variation was also positively related to the size of the group, with higher levels of niche specialisation in larger groups. Within groups, individual badgers may fight over food resources (Macdonald *et al.* 2002) and individuals may monopolise profitable foraging habitats in cases where resources are limited (Revilla 2001). Individual badgers in territories with a low availability of farmland habitats and with a larger numbers of conspecifics are therefore likely to experience increased competition for key resources as a result. However, group size was also correlated with territory size. This may indicate that there may be a spatial effect on the degree of specialisation. In larger territories individual may be able to separate spatially from one another to a greater extent. However to further confirm this we would require data on individual movement patterns which are lacking for this population.

We also found that individuals in social groups with higher mean $\delta^{13}C$ values (indicating a higher consumption of anthropogenic C4 resources) had lower degrees of specialisation and utilised a larger proportion of the group niche (Tables 5.1 and 5.2). In addition, the effect of group size on both measures of specialisation varied with the mean $\delta^{13}C$ of the group, with a reduced effect of group size in groups with isotopic values indicating a greater reliance on C4 resources. This is also likely linked to levels of resource competition as anthropogenic resources such as maize or animal feed may occur in high concentrations and require little energy to obtain.

Our results therefore strongly suggest that individual niche specialisation within badger social groups is influenced by levels of competition, as both measures of niche specialisation were positively related to group size and negatively related to the availability of farmland foraging habitats and the consumption of anthropogenic C4 resources. These results agree with other recent studies which demonstrate that individual niche variation is correlated with the density of conspecifics (Svanbäck & Persson 2004, 2009; Svanbäck & Bolnick 2007; Fontaine, Collin & Dajoz 2008) and increases when resource availability is low (Tinker, Bentall & Estes 2008; Kobler *et al.* 2009; Tinker *et al.* 2012).

Although important variables in models explaining variation in individual specialisation were primarily related to ecological factors, age and sex were also included in several top models (Tables 5.1 and 5.2). We also found that the effect of group size on individual niche specialisation differed between sexes, with stronger relationship with $IS_{W/T}$ in males than females (Table 5.1) and the effect of the proportion farmland varied with age, with a stronger effect on adult badgers (2-4 years old) than yearlings or old individuals (>4 years old). The extent that an individual specialises relative to its group is therefore not only determined by group level ecological factors, but also by differences at the class level. These sex and age effects may be due to differences in the competitive pressure experienced by individuals in these different groups. Badgers may engage in fights over reproductive rights, territoriality or access to food (Macdonald *et al.* 2002, Roper 2010). Previous studies have shown that bite wounding from is more common in males and adults than females or cubs (Delahey *et al.* 2006). Greater levels of aggression and competition between males and adults may mean an increased degree of competition, particularly in larger groups, resulting in increased niche specialisation to avoid conflict.

Finally, one of the most important variables explaining values of $IS_{W/T}$ within the badger groups was the group's δTNW, or total isotopic niche width. At the population level, niche expansion is predicted to occur in one of two ways. Either individual niche widths expand with that of the population, such that individuals utilise the full range of the populations niche ('parallel release', Bolnick *et al.* 2010). Alternatively, niche expansion occurs primarily due to differences between individuals, resulting in increased individual niche variation (NVH, Van Valen 1965). We found that at the group level, individual isotopic niche widths were correlated with that of their social group, suggesting some degree of parallel release. However, the strength and slope of this relationship was low, such that groups with broader niches still exhibited higher levels of individual specialisation in agreement with the NVH (Figure 5.2). This study therefore adds to a growing list of species which indicate that more generalised groups of animals are also more ecologically heterogeneous, confirming the once discredited NVH (Bolnick *et al.* 2007, 2010; Tinker, Bentall & Estes 2008; Costa *et al.* 2008; Araújo *et al.* 2009; Darimont,

Paquet & Reimchen 2009). The implications of this relationship are that individual niche widths do not expand to match that of the population (or in this instance social group) due to trade-off induced constraints on individual niche widths (Bolnick *et al.* 2003, 2007). In badgers, these constraints could be cognitive, due to an inability to learn the locations or foraging behaviours associated with a potential diverse prey base, or due to other phenotypic differences which determine an individual's fundamental niche. Alternatively, other individuals may monopolise resources such that individuals disperse themselves in an ideal free manner (Haugen 2006). The effects of such fundamental differences seems likely, as although we did find significant effects of the ecological factors on individual niches in this study, a significant proportion of the variance in this behaviour remains unexplained (~80%, Table 5.1).

Consequences of individual niche variation

We found that within social groups, individual body condition was related to niche specialisation exhibited by individual badgers. However, the effect of niche specialisation on body condition was context dependent. There was no significant effect of dietary variables on the relationship between specialisation and body condition, indicating that it was specialisation per se which was important, rather than specialisation on a particular resource type, as has been found in other studies (e.g Votier et al. 2004). The effect of IS_{PS} and $IS_{W/T}$ on body condition varied with the availability of farmland foraging habitats in the group's territory, with a more positive relationship between specialisation and body condition in social groups where farmland resources were limited (Figure 5.4). Similarly the relationship between IS_{PS} and body condition varied with group size, with a stronger relationship in larger social groups. As previously mentioned, a large group size and a low availability of farmland resources likely indicate higher competition for resources within groups. This result therefore suggests that individual specialisation may result in higher fitness, but only in situations where competition for resources is intense. Individuals which are more specialised may have a high foraging efficiency, for example, specialist sea otters have markedly reduced handling times and increased efficiency relative to generalists (Tinker, Bentall & Estes 2008; Tinker *et al.* 2012). This may therefore result in higher body

condition, but only when resources are limiting. Alternatively, in the current study specialisation may correlate with body condition in social groups with high resource competition as individuals in poor body condition are consistently restricted to lower quality resources due to reduced competitive ability.

Although it is difficult to determine if body condition is a cause or consequence of individual specialisation, the results of this study demonstrate that the relationship between body condition (a common measure of individual fitness, Kobler *et al.* 2009) and the extent that an individual specialises in its foraging niche is not homogeneous across the population. Variation in ecological factors at small spatial scales within the population results in positive and negative effects of specialisation depending on the ecological context. Such ecological effects may therefore explain why the relationship between individual specialisation and fitness is not detected in some studies (Woo *et al.* 2008). Previous studies have also shown that selection towards different foraging phenotypes is context dependent, with stronger selection when competition for resources increases (Bolnick 2004; Svanbäck & Persson 2009). In Oystercatchers, selection towards specialised foraging behaviours varies temporally due to changing environmental conditions, with specialists performing better than generalists in cold harsh winters (Van de Pol *et al.* 2009). Our results therefore further support the findings that the costs and benefits associated with specialisation may vary depending on the environmental conditions, with an increased benefit of specialisation when key foraging resources are limiting.

In conclusion, this study demonstrates that resource competition results in increased individual niche variation and also potentially changes the consequences for individuals that specialise. Intraspecific competition for resources may therefore be a key factor in both causing and maintaining individual niche variation with wild populations (Svanbäck & Bolnick 2007). This study also demonstrates that the incidence and consequence of foraging specialisation may vary over small spatial scales, as the current study population occupies an area of only $7km^2$. This differs markedly from previous studies which investigate the effects of ecological factors on niche variation at primarily the population level (e.g. Tinker *et al.* 2008; Newsome *et al.* 2012). Future research aimed at further understanding individual specialisation should therefore not only attempt to

quantify intra-population variation in foraging niche, but also intra-population variation in ecological factors that may determine resource use and its consequences.

Table 5.1 Details of top models with a ΔAICc<2 explaining variation the degree of individual specialisation measured as $IS_{W/T}$ (an individual's isotopic niche width divided by that of its group) and IS_{PS} (dietary similarity between an individual and its group). Each row in the table indicates a model, with a + indicating the inclusion of a given variable within each model. Degrees of freedom, ΔAICc, model weight and Nagelkerke R^2 values are also included for each model.

	Variables included in top models														
	Age	Sex	GroupδTNW	Group mean δ13C	Prop farmland	Group size	Age X Propfarmland	Sex X Groupsize	Group mean δ13C X Groupsize	Group mean δ13C X Propfarmland	Prop farmland X Groupsize	*df*	ΔAICc	weight	Nagelkerke R^2
$IS_{W/T}$		+	+	+	+	+		+		+		11	0	0.16	0.16
		+	+	+	+	+		+	+	+	+	12	0.32	0.14	0.19
		+	+	+	+	+				+		10	0.35	0.14	0.16
		+	+	+	+	+		+	+	+	+	13	0.95	0.10	0.20
		+	+	+	+	+			+	+		11	1.01	0.10	0.17
			+	+	+	+				+		9	1.06	0.09	0.14
		+	+	+	+	+		+		+	+	12	1.3	0.08	0.16
			+	+	+	+						8	1.68	0.07	0.12
		+	+	+	+	+		+				10	1.96	0.06	0.15
		+	+	+	+	+				+	+	11	1.99	0.06	0.14
IS_{PS}	+				+	+	+					10	0	0.14	0.18
	+			+	+	+	+		+			12	0.09	0.13	0.22
	+				+	+	+				+	11	1.95	0.05	0.18

Table 5.2 Average model coefficients calculated for variables included in top models (table 5.1) explaining variation in the degree of individual specialisation measured as $IS_{W/T}$ (1-an individual's isotopic niche width divided by that of its group) and IS_{PS} (1-dietary similarity between an individual and its group). Average coefficient estimates, 95% confidence intervals and relative importance is displayed for each variable. Variables in bold are those with 95% confidence intervals which do not span zero, indicating a consistent directional effect on the degree of specialisation. $IS_{W/T}$ and IS_{PS} were both logit transformed and predictor variables were standardised to mean zero and standard deviation of 2 prior to analysis. In both cases higher values = higher degrees of individual specialisation.

Response	Fixed effects	Estimate	5% CI	95%CI	Relative importance
$IS_{W/T}$	(Intercept)	3.4	3.21	3.6	-
	GroupδTNW	**1.32**	**0.85**	**1.79**	**1**
	Group mean δ13C	**-0.87**	**-1.36**	**-0.37**	**1**
	Prop farmland	**-0.60**	**-1.00**	**-0.20**	**1**
	Groupsize	**0.45**	**0.07**	**0.83**	**1**
	Group mean δ13C X Groupsize	**-1.09**	**-1.96**	**-0.23**	**0.87**
	Sex	**-0.39**	**-0.75**	**-0.03**	**0.84**
	Sex X Groupsize	**0.75**	**0.05**	**1.46**	**0.54**
	Group mean δ13C X Prop farmland	0.64	-0.06	1.33	0.34
	Prop farmland X Groupsize	0.73	-0.3	1.75	0.24
IS_{PS}	(Intercept)	-2.08	-2.23	-1.94	-
	Prop farmland	**-0.73**	**-1.04**	**-0.44**	**1**
	Age(old) X Prop farmland	**0.88**	**0.52**	**1.25**	**1**
	Age(yearling) X Prop farmland	0.22	-0.22	0.68	1
	Age(old)	-0.05	-0.24	0.15	1
	Age(yearling)	-0.03	-0.24	0.17	1
	Groupsize	**0.32**	**0.10**	**0.55**	**1**
	Group mean δ13C	-0.02	-0.27	0.22	0.41
	Group mean δ13C X Groupsize	**-0.57**	**-1.02**	**-0.14**	**0.41**
	Propfarm X Groupsize	-0.21	-0.74	0.32	0.16

Table 5.3 Details of top models with a ΔAICc<2 explaining variation in body condition (scaled mass index). Each row in the table indicates a model, with a + indicating the inclusion of a given variable within each model. Degrees of freedom, ΔAICc, model weight and Nagelkerke R^2 values are also included for each model. Abbreviated variable are

Variables included in top models													
Sex	diff OIW	diff C4farm	$IS_{W/T}$	IS_{PS}	Prop farmland	Group size	$IS_{W/T}$ X Propfarmland	IS_{PS} X Propfarmland	IS_{PS} X Groupsize	*df*	ΔAICc	weight	Nagelkerke R^2
+				+	+	+		+	+	10	333.93	0.1	0.4
+			+	+	+	+	+	+	+	12	334.02	0.1	0.43
+		+		+	+	+		+	+	11	334.05	0.1	0.41
+		+	+	+	+	+	+	+	+	13	334.12	0.09	0.44
+			+		+		+			9	334.67	0.07	0.39
+						+				6	334.79	0.07	0.35
+			+		+		+			8	334.9	0.06	0.37
+		+	+		+	+	+			10	335.07	0.06	0.4
+		+	+		+		+			9	335.13	0.06	0.39
+		+				+				7	335.19	0.05	0.36
+	+		+	+	+	+	+	+	+	13	335.57	0.04	0.43
+			+	+	+	+		+	+	11	335.62	0.04	0.42
+										5	335.62	0.04	0.33
+		+								6	335.68	0.04	0.34
+		+	+	+	+	+		+	+	12	335.82	0.04	0.42
+	+			+	+	+		+	+	11	335.89	0.04	0.4

Table 5.4 Average model coefficients calculated for variables included in top models explaining variation in body condition (scaled mass index). Average coefficient estimates, 95% confidence intervals and relative importance is displayed for each variable. Variables in bold are those with 95% confidence intervals which do not span zero. $IS_{W/T}$ (1-an individual's isotopic niche width divided by that of its group) and IS_{PS} (1-dietary similarity between an individual and its group) were both logit transformed and all predictor variables were standardised to mean zero and standard deviation of 2 prior to analysis. In both cases higher values = higher degrees of individual specialisation. Variable diff OIW is the difference between the estimated contributions of OIW (other invertebrates in woodland habitats) in an individual's diet to that of its group, while diff C4 is the difference between the estimated contributions of C4 farm resources(maize and cattle feed) in an individual's diet to that of its group.

Fixed effects	Estimate	5% CI	95%CI	Relative importance
(Intercept)	7.17	6.96	7.38	-
Sex	**1.12**	**0.84**	**1.39**	**1**
Prop farm	0.12	-0.24	0.48	0.8
Group size	0.29	-0.03	0.62	0.8
IS_{PS}	0.03	-0.26	0.33	0.55
IS_{PS} X Propfarm	**-0.77**	**-1.31**	**-0.22**	**0.55**
IS_{PS} X Groupsize	**0.88**	**0.14**	**1.62**	**0.55**
$IS_{W/T}$	0.15	-0.13	0.44	0.56
$IS_{W/T}$ X Propfarm	**-0.60**	**-1.16**	**-0.05**	**0.48**
diff OIW	-0.20	-0.46	0.07	0.43
diff C4farm	-0.11	-0.37	0.16	0.08

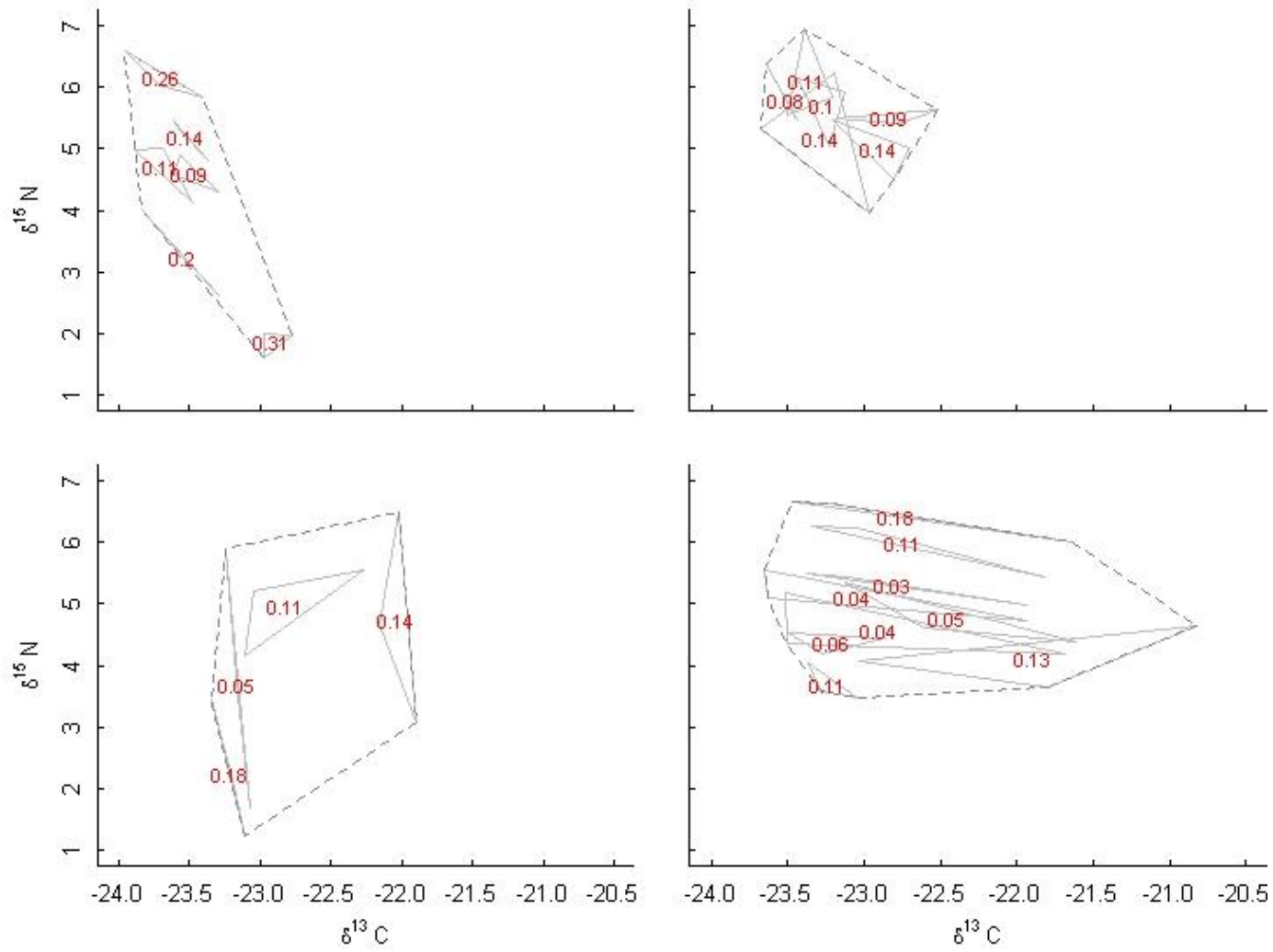


Figure 5.1 Examples of Isotopic metrics used to quantify individual specialisation in four badger social groups at Woodchester Park in 2010. Dashed line is total convex hull area encompassing all isotopic data from the group, describing the group's total isotopic niche width (δTNW). Grey triangles represent individual badgers, with the hull areas encompassing the three basal vibrissa section isotopic values for that badger (individual isotopic niche width or δWIC). Values in red represent the IS_{PS} values (1- the Proportional similarity index) of individual badgers calculated by comparing individual diets (calculated using the mixing model SIAR) to those of the rest of the social group, higher values denote higher levels of individual specialisation. IS_{PS} values are located at the mean $\delta^{15}N$ and $\delta^{13}C$ for each individual.

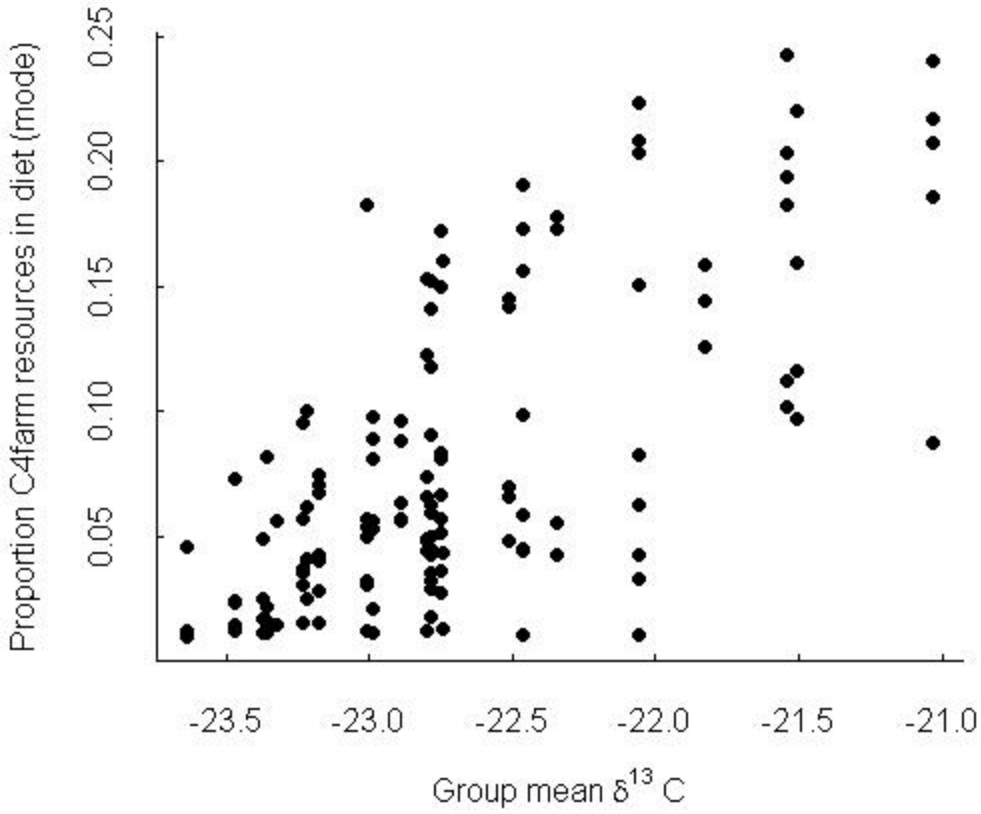


Figure 5.2 Correlation between the $\delta^{13}C$ mean of a badger social group with the estimate modal (most likely) proportion of 'C4 farm resources' (an amalgamation of maize and cattle feed) in individual badgers diets estimated using the Bayesian mixing model SIAR.

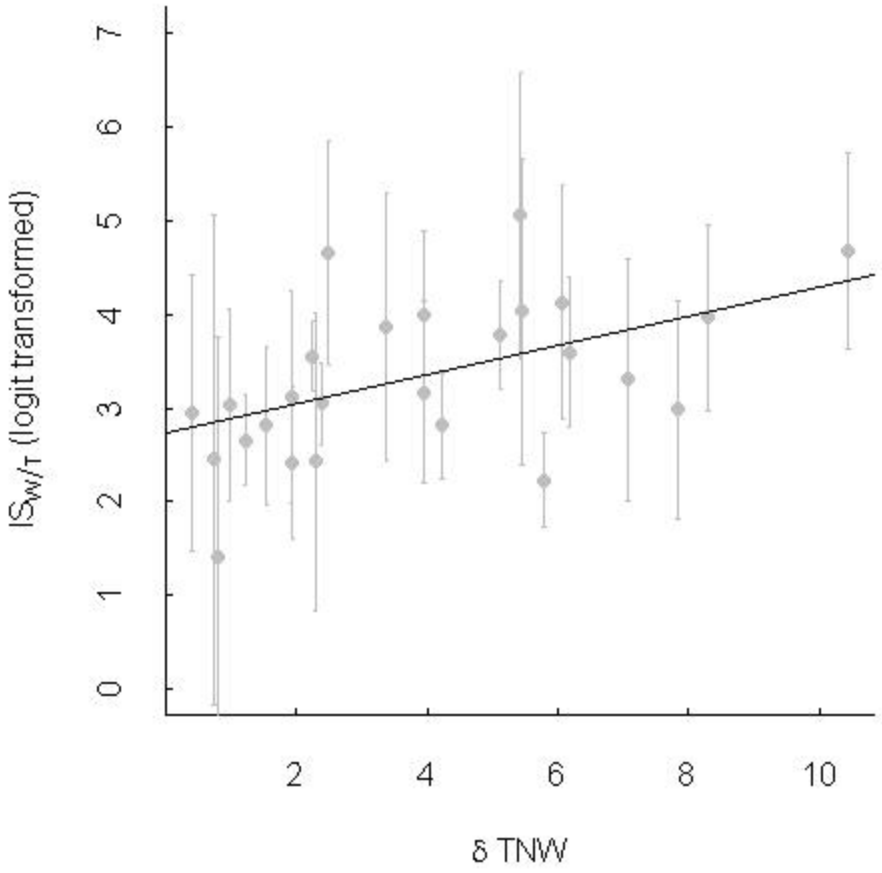


Figure 5.3 The relationship between the degree of Individual niche specialisation ($IS_{W/T}$) within badger social groups and the group's total isotopic niche width (δTNW). Values are the mean $IS_{W/T}$ (logit transformed)values for social groups, with the error bars illustrating the 95%CI. The line is the fitted relationship between the two variables from the top model explaining variation in $IS_{W/T}$ (Table 1), demonstrating that social groups with broader isotopic niche widths (δTNW), have higher degrees of specialisation ($IS_{W/T}$) .

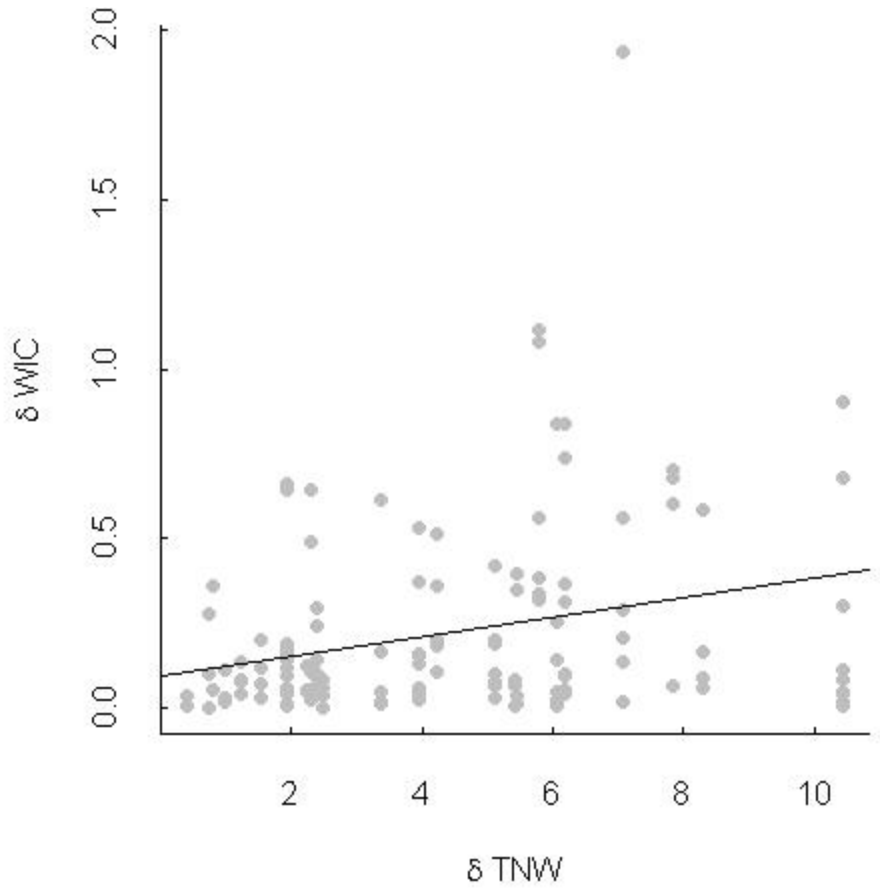


Figure 5.4 Correlation between the isotopic niche widths of individual badgers(δWIC) with that of their social group (δTNW). The metrics δWIC and δTNW are calculated from the TA convex hulls encompassing isotopic values of individual badgers and their social groups respectively (Figure1). Individual specialisation is therefore higher in social groups with broad niches (Figure 5.3), despite an increase in individual niche widths (δWIC) with δTNW.

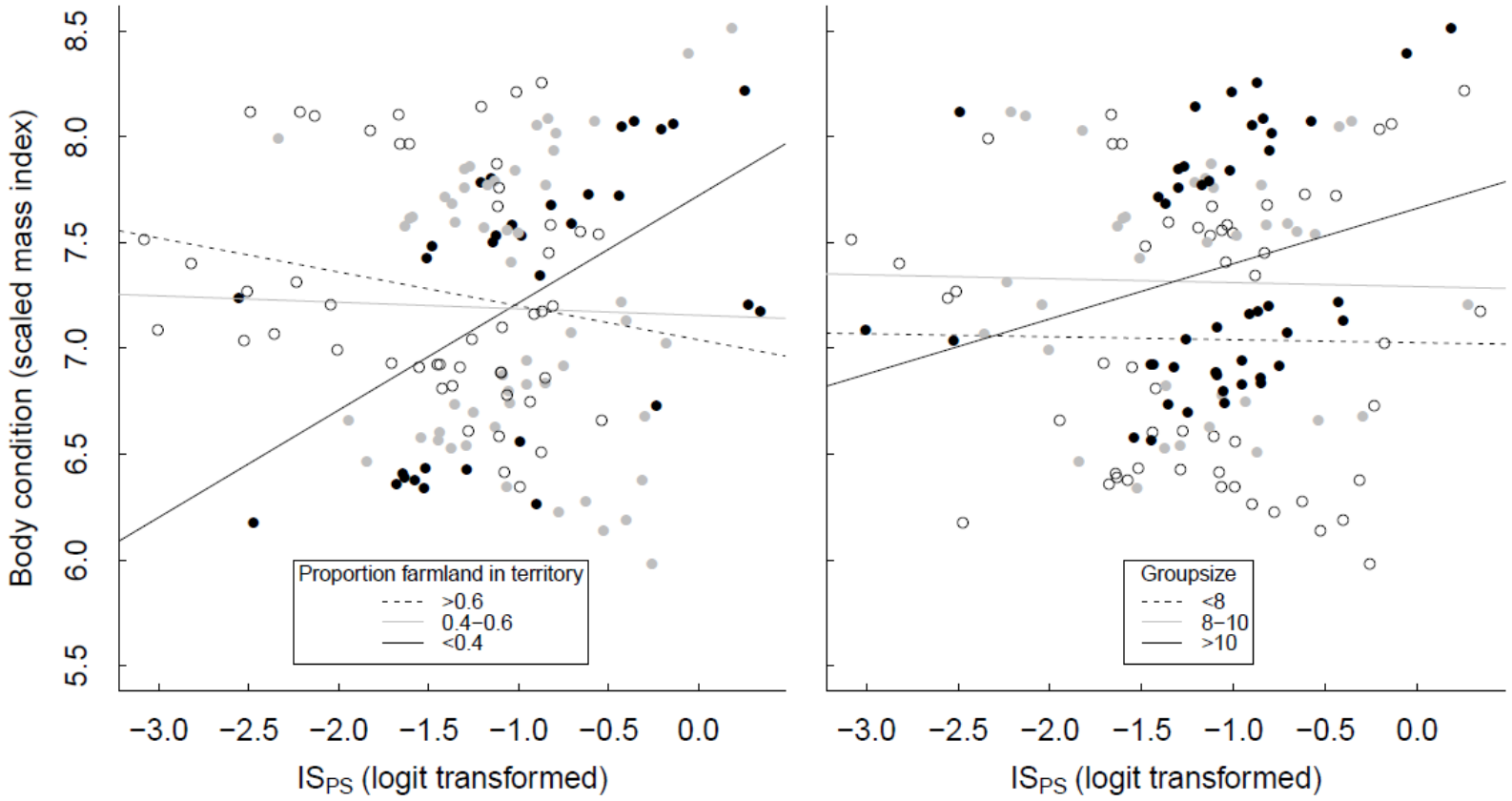


Figure 5.5. Relationship between body condition and the degree of individual specialisation measured as IS_{PS} (values are logit transformed, higher values equal higher levels of specialisation) in relation to the proportion of the groups territory which is farmland and in relation to the size of the group. Both group size and proportion farmland are continuous variables and have been split into categories of roughly equal sample sizes for illustrative purposes. Points are predicted values from the top model explaining variation in body condition (Table 3).

CHAPTER 6: General discussion

The primary aim of this thesis was to use badgers as a model species to investigate individual niche variation, via the application of stable isotope analysis. In this final chapter I will discuss how my research has expanded knowledge in three areas; Individual specialisation, stable isotope analysis and badger ecology and suggest future directions for research.

6.1 Individual specialisation

Stable isotope analysis of badger vibrissae in this thesis indicated that individual badgers within the population have highly variable foraging niches (Chapter 4). Individual differences in foraging niche or 'individual specialisation' is a widespread phenomenon and has been recorded in a large number of species. However, in many cases the long-term consistency of this behaviour is unknown, as foraging behaviour or diet is only measured over weeks or days (e.g Cook, Cherel, & Tremblay 2006; Costa *et al.* 2008; Kotzerka, Hatch, & Garthe 2011). In addition, in many cases of reported 'individual specialisation' the effects of age or sex on foraging niche are not investigated (e.g. Cherel *et al.* 2006; Prugh *et al.* 2008; Darimont *et al.* 2009), despite the fact individual specialisation by definition is only niche variation independent of these class effects (Bolnick *et al.* 2003). I found individual variation in badgers independent of age or sex which is consistent over several months (length of a single vibrissa) and across years, strongly suggesting long-term individual specialisation in this species. Long-term individual specialisation has been demonstrated previously in seabirds (Woo *et al.* 2008), marine mammals (Estes *et al.* 2003; Chilvers 2008) and also sea turtles (Vander Zanden *et al.* 2010). However, few previous studies of individual specialisation have investigated this behaviour in terrestrial mammals and in those that have found that niche variation was primarily the product of localised habitat variation (Angerbjörn *et al.* 1994; Ben-David *et al.* 1997; Sidorovich *et al.* 2001), suggesting individuals merely utilised the prey available to them.

We demonstrated that individual specialisation may occur in terrestrial mammals independent of resource availability and is therefore the product of behavioural differences among individuals (Chapter 4). Individual specialisation in this group is potentially important, as terrestrial mammals are often of high management, conservation and research interest. Individual difference in foraging niche may result in some individuals or subsets of the population being more or less susceptible to changes in resource availability (Chilvers & Wilkinson 2009; van de Pol *et al.* 2009). Individual niche variation also has potential ecological implications by changing the number and strength of ecological interactions which effects density dependence, interspecific competition and food web structure (Bolnick *et al.* 2011). Variation in the strength of ecological interactions may also be of particular importance in species such as badgers which occupy the upper trophic levels within food webs (Woo *et al.* 2008; Matich, Heithaus & Layman 2011). Individual specialisation within these species may potentially change how they interact with lower trophic levels and influence community structure (Rooney *et al.* 2006; Araújo, Bolnick & Layman 2011). For example, mobile generalist predators may have stabilising effect on community dynamics, by linking separate food chains and controlling a diverse range of prey (McCann & Hastings 1997; Rooney *et al.* 2006; Matich, Heithaus & Layman 2011). However, if certain individuals or subsets of the population continually specialise on certain prey types or environments independent of availability this may potentially have a destabilising effects. The importance of individual specialisation in feedback loops with community structure remains unstudied, and has been suggested as a promising area for future research (Araújo, Bolnick & Layman 2011).

The growing interest in individual niche variation has lead to a recent rise in studies attempting to investigate the ecological factors which determine the degree of specialisation within populations (Tinker *et al.* 2012). We found that the degree of individual specialisation within badger social groups was positively related to group size and negatively related to the availability of key resources (Chapter 5). This result is consistent with several theoretical models investigating the causes of niche variation that predict competition for resources will result in increased levels of individual specialisation (Svanbäck & Bolnick 2005; Tinker, Mangel & Estes 2009). This is also consistent with

recent empirical studies which have found a correlation between individual niche variation with population density (Svanbäck & Bolnick 2007) and resource limitation (Tinker, Bentall & Estes 2008).

Although specialisation occurs in a large number of species the fitness implications of this niche variation remains poorly understood. In some cases individual differences in foraging niche correlate with measure of individual fitness (Golet *et al.* 2000; Votier *et al.* 2004; Darimont, Paquet & Reimchen 2007; Johnson *et al.* 2009; Cucherousset *et al.* 2011; Authier *et al.* 2012). Fitness benefits associated with occupying a more specialised niche may therefore maintain individual specialisation within populations via disruptive selection away from less profitable generalist phenotypes (Bolnick *et al.* 2003; Bolnick 2004; Martin & Pfennig 2009; Cucherousset *et al.* 2011). However, despite a strong link between niche variation and fitness in some cases, several studies have also found no relationship between these factors (Katzner *et al.* 2005; Woo *et al.* 2008; Chilvers & Wilkinson 2009; Kobler *et al.* 2009; Whitfield *et al.* 2009; van de Pol *et al.* 2009). In some cases the opposite relationship is also observed, such that more generalised individuals achieve higher fitness (Iguchi *et al.* 2004; Whitfield *et al.* 2009).

I found that there was a relationship between a potential measure of fitness (body condition) and the degree an individual specialises in its foraging niche, but this relationship varied with the level of resource competition (Chapter 5). Previous studies have shown that the fitness consequences of differing foraging strategies may be context dependent such that specialisation results in higher fitness when resource competition is most intense (Bolnick 2004; van de Pol *et al.* 2009; Svanbäck & Persson 2009). Fluctuating environmental conditions may therefore result in a fluctuating fitness landscape such that divergent foraging strategies may be maintained within the same population over time (Van de Pol *et al.* 2009). Although in the case of badgers it is difficult to disentangle whether body condition is a direct consequence or cause of differences in foraging niche (Chapter 5), future research focused on determining the fitness implications of individual niche variation should consider the strength of competition and how this could influence such relationships. Further investigations of the consequences of individual specialisation should also focus on identifying differing types of specialist, as current metrics quantify

specialisation along a continuous axis from generalised to specialised (Bolnick *et al.* 2002), where in reality individual may specialise on different resources which vary in their fitness consequences (Votier *et al.* 2004). I attempted to address this by including dietary variables in models explaining the relationship between specialisation and body condition to test for interactions between diet and specialisation (Chapter 5). The results suggested that diet, or which resource you specialise upon, was not an important factor. However, the statistical approach used is not ideal, as this increases the number of variables in the analyses and potentially complicates the interpretation of results. Further advances in indices used to quantify specialisation may help address such problems.

Although we found a relationship between individual specialisation and resource competition, a large proportion of variation in the extent that individuals specialise was not explained by models in our analyses (Chapter 5). This may be due to inherent inaccuracies in our specialisation indices or in our measures of competition. Alternatively this may suggest that individual specialisation is also determined by intrinsic factors which influence an individual's fundamental niche and are independent of ecological factors considered. How intrinsic factors determine an individual's fundamental niche and how this may lead to individual specialisation has been investigated in relatively few studies and is a promising and important area for future research (Bolnick *et al.* 2003).

One potential cause is that individual foraging behaviours are learned either from conspecifics or via interaction with the foraging environment, which has been shown to be cause of foraging specialisation in both in sea otters and dolphins (Estes *et al.* 2003; Tinker *et al.* 2007; Sargeant & Mann 2009). Alternatively, foraging differences may be the product of inherent behavioural differences between individuals. Consistent individual differences in behaviour in the form of 'behavioural syndromes', 'coping styles' and 'personality' are also widely documented within animal populations (Sih, Bell & Johnson 2004; Réale *et al.* 2007), and may correlate with differences in foraging behaviour in some cases (e.g Farwell & McLaughlin 2009). To date individual specialisation and personality have been viewed as distinct separate fields although, it has been recently been suggested that they should be more closely integrated (Dall *et al.* 2012). Differences in personality have also been shown to be heritable suggesting an genetic component to individual

differences (Dingemanse *et al.* 2010). To date the genetic basis of individual specialisation has not been investigated, although previous studies have shown a link between elements of foraging behaviour and morphology which may be linked to resource use (Bolnick *et al.* 2003). This is an area which urgently requires further research, if the fundamental causes of individual specialisation are to be understood. Combined studies of personality and niche specialisation may therefore help to shed light on the behavioural and genetic factors which shape an individual's fundamental niche and may result in individual specialisation.

6.2 Stable isotope analysis

Stable isotope analysis is a potentially powerful tool for investigating individual niche variation within animal populations and is increasingly being applied to this subject (Hückstädt *et al.* 2012). One of the primary advantages of this technique is that a single sampling event can potentially provide long term foraging information (Crawford, McDonald & Bearhop 2008). However, in order for this information to be correctly interpreted, researchers require knowledge of the rates of growth or turnover in the tissues sampled (Newsome, Clementz & Koch 2010). In chapter 2 I quantified the growth rate of badger vibrissae, which was crucially important for the interpretation of future results in the following research (Chapters 4, 5 & 6). Although previous studies have measured vibrissae growth, the list of species where this has been investigated remains very small. As a consequence researchers often have to rely on measures of vibrissae growth from different often unrelated species (e.g Newland et al. 2011; Newsome et al. 2009), which may be problematic as growth patterns may differ, even between closely related species (Hirons, Schell & St. Aubin 2001). In addition, although earlier studies have suggested that individual characteristics such as age or body condition may influence vibrissae growth (Wright 1965; Ibrahim and Wright 1975), no studies had investigated how vibrissae growth varied within large wild populations. I quantified vibrissae growth in badgers in a large number of wild individuals and found that age, sex and body condition had small insignificant effects on vibrissae growth rates (Chapter 3). These results are

potentially useful for any future research using vibrissae in combination with stable isotope analysis or other biomarkers.

In addition to information on growth rate or tissue turnover it is important that researchers have accurate trophic enrichment factors (TEFs) that quantify the isotopic fractionation between the consumer's diet and tissues. Published TEF values for badger vibrissae are unavailable, instead I had to use values for hair from other mammalian carnivores (Foxes, seals etc). As a consequence, the results of dietary mixing models may contain additional sources of variation (Chapters 3 and 5). Future lab based feeding studies are required to provide further accurate TEF values particularly for vibrissae, as these have only been determined in a handful of studies (e.g Newsome, Bentall, et al. 2010).

Stable isotope analysis was the primary method used in the research within this thesis and was fundamentally important in quantifying and investigating individual level niche variation. Crucially SIA facilitated the measurement of long term individual resource use simultaneously in a large numbers of badgers, which made it possible to test various hypotheses (Chapters 3, 4 & 5). However, the first step in this process is to use using $\delta^{13}C$ and $\delta^{15}N$ data to generate quantitative measures of individual niche variation.

Several analytic methods are potentially available for measuring consistent differences in behaviour. A widespread approach applied to animal behaviour studies is the use of measures of repeatability (Bell *et al.* 2009). Repeatability (r) is generally measured as $r=s^2_A / s^2_A + s^2$ where s^2_A is the behavioural variation among individual and s^2 is the variation within individuals. The measures s^2_A and s^2 are essentially identical to 'between individual component' (BIC) and 'within individual component' (WIC) of a populations niche as described by Bolnick *et al.* (2003). Variance among and between individuals can be estimated using a mixed model or analysis of variance (Anova) approach, whether quantifying behavioural repeatability (Nakagawa and Shielzeth 2010) or 'individual specialisation' (Bolnick *et al.* 2003, Bolnick 2002).In chapter 4 I investigated individual within group variation in foraging niche by analysing variance in $\delta^{13}C$ and $\delta^{15}N$ using a mixed model and variance components approach, which has been used in several other studies (Newsome *et al.* 2009; Hückstädt *et al.* 2012). Although informative, this

statistical approach is not ideal as it requires separate analyses for each isotope and it also ignores potential individual variation in trophic enrichment factors (TEFs) between diet and consumer (Matthews & Mazumder 2004). In addition, statistical methods such as these which partition variance to quantify repeatability or niche specialisation only provide population or group level estimates and do not provide individual measures.Recently, Bayesian hierarchical mixing models have been developed as a tool to measure individual diet variation using isotopic data (Semmens *et al.* 2009; Derbridge, Krausman & Darimont 2012). These models estimate individual variation in diet by the use of a dietary mixing model approach with a hierarchical variance structure which aligns with ecological grouping variables such as geographic location or social unit (Semmens *et al.* 2009). This is advantageous as it produces an estimate of dietary variation along a single continuous axis, while also permitting the inclusion of variance in TEFs and other values (Semmens *et al.* 2009). However, these models are highly complex and currently do not account for other potential sources of variance such as age, or sex which are important in separating individual specialisation from class differences in niche. Furthermore, as with other indices these the models as of yet do not provide quantified measures of the degree of specialisation for each individual in the analysis, but give a single estimate of variation within the population. This is problematic, as individual measures of the degree of specialisation are required to test hypothesis related to the causes or consequences of this behaviour.

Quantifying niche specialisation at the individual level is not a straight forward process, as the diversity of potential metrics can make it confusing for researchers to identify the best method to use. Bolnick (2002) outlined several different indices which can be used to quantify the level of individual niche variation within populations depending on type data available. Sargeant (2007) further highlighted four differing measures of niche specialisation with reference to an individual's niche width, richness, evenness or overlap. Several recent studies have also proposed further metrics or indices, such as the use of network analysis to make comparisons between individual niches (Araújo *et al.* 2008) or to look at the connectivity between an individual and the prey sources available to them (Tinker *et al.* 2012).

Using isotopic data Indices of individual specialisation can be calculated either using raw δ values, estimated dietary proportions from dietary mixing models, or transformed indices using either of these data types (Newsome *et al.* 2012).Although, not aimed at use with stable isotope data I adapted two of the metrics as proposed by Bolnick (2002) to quantify the degree of specialisation for individual badgers within social groups ($IS_{W/T}$ and $IS_{PS,}$ chapter 5). One metric quantified isotopic niche variation of an individual relative to the group using $\delta^{13}C$ and $\delta^{15}N$ data ($IS_{W/T}$, Chapter 5), while the other measured the diet similarity using mixing model output (IS_{PS}, Chapter 5). However, it is possible that individuals may potentially be specialised with regard to one metric but generalised with regard to another. For example, two individuals may have equally specialised diets that differ from their group (high IS_{PS}) but vary in their consistency or niche breadth ($IS_{W/T}$). By analysing these two characteristics of an individual's niche in separate analyses it is therefore not possible to detect such differences. Some authors have suggested grouping individuals into categories based on their values along two differing specialisation axes (e.g. narrow niche/specialist diet, narrow niche/generalist diet, broad niche/specialist diet or broad niche/ generalist diet, Newsome *et al.* 2012). However, this approach relies on the use of arbitrary cut off values to determine which category an individual belongs to such that individuals which have very similar values may be classified as differing types. This also produces multilevel response variable which may be difficult to analyse.

As previously mentioned, the majority of current metrics used to measure individual specialisation also quantify specialisation along a continuous axis from specialist to generalist, treating all specialists as equal, even if they differ in their actual resource use (e.g. worm specialists or beetle specialists).Future research into individual specialisation ideally requires composite metrics which make it possible to simultaneously combine separate niche characteristics (e.g. niche breadth, diet similarity, most commonly consumed prey item) into a single measure of specialisation. Future developments of Bayesian analyses, particularly Bayesian hierarchical mixing models (Semmens *et al.* 2009) may make it possible to achieve this. For example, changes to current mixing models may make it possible to add individual variables (e.g. age, sex or season) to analyses, such that

these sources of variance are incorporated into dietary estimates and used to quantify specialisation (Semmens pers. coms).

However, one current limitation with using data from Bayesian mixing models is they produce a very large amount of information which is difficult to work with. For example, In Chapter 4 I calculated measures of individual specialisation using the modal values for prey consumption as estimated by SIAR. In reality SIAR and other mixing models generate a whole distribution of values, with many thousands of estimates. It may therefore be possible to take upper and lower quartiles from this distribution which would produce in turn several different measures of specialisation. Although potentially informative, this may however be difficult work with statistically as this would produce many differing response variables, especially if models involve a large number of potential prey sources. However, choosing a small range of values may help demonstrate that observed relationships with specialisation or diet metrics are not too heavily influenced by the uncertainty within the mixing models used to generate these values. Future advancements in mixing model calculations and software may make it possible to estimate diet in a mixing model framework while also using the whole posterior distribution of diet estimates to calculate the degree of specialisation, and how this varies relative to other individuals to produce combined diet/specialisation metrics.

6.3 Badger Ecology

Badger foraging ecology has been the subject of a substantial body of scientific research and it continues to be an active area of scientific interest (Roper 2010). Badgers have been previously described as generalist omnivores, with their resource use primarily determined by local resource availability (Roper 1994). I found that individual badgers in the same social group can differ significantly in their foraging niche indicating that badgers do not utilise the whole range of resources, but instead they may specialise on a narrow range of those available (Chapter 4). Despite a substantial body of work on badger foraging ecology long-term individual diet variation has not previously been demonstrated. This is not only applicable to studies of individual specialisation, but may be applied to future research on badger foraging ecology. In particular, using stable

isotope analysis it may be possible to determine the extent that individuals utilise isotopically distinct C4 based farm resources as well as the extent that individuals forage on high nitrogen farm habitats (Chapter 3). The consumption of these resources may potentially bring badgers into close contact with cattle which has been suggested as an important potential route for disease transmission (Garnett, Delahay & Roper 2002; Tolhurst *et al.* 2009). Previous studies investigating the consumption of farm resources by badgers have been carried out on a relatively small scale (Garnett et al. 2002, n=2 social groups, Tolhurst et al. 2009, n=6 farms) due to the expense and effort required for the methods used (faecal analysis and video surveillance). Limitations on sample size make it difficult to investigate a link with disease transmission as hypotheses cannot be statistically validated. Due to its relatively low cost stable isotope analysis may therefore be a potential method to investigate the interaction between badgers and farms over large spatial scales and with a large enough sample size to statistically investigate a link between foraging behaviour and disease transmission.

I also observed significant isotopic variation between social groups and sub-locations within our study area (Chapter 4). Theoretically it may therefore be possible to use stable isotope analysis as a technique to quantify movement within badger populations, as resident individuals may have distinct isotopic signatures from those from other areas or social groups. Stable isotope analysis has previously been used to infer movement in mammals, however primarily over a large spatial scale (Hénaux *et al.* 2011; Pauli, Smith & Ben-David 2012). Stable isotope analysis may therefore also be a potential future method to investigate movement and dispersal in badgers which is also of interest for studies of disease dynamics, particularly in combination with management activities such as culling (Donnelly *et al.* 2006; Woodroffe *et al.* 2006; Woodroffe & Donnelly 2009). In order to make this possible future work would be needed to validate the relationships between isotopic variation and badger movement.

6.4 Conclusions

Individual niche variation is widespread in animal populations and given the potential importance of this behaviour it is necessary that the incidence, cause and consequence of

this behaviour is further understood. My research demonstrates that this behaviour is present in badgers, a well studied and ecologically important species. Using this species as a model I have shown that the causes and consequences of niche variation may vary at a fine scale within populations due to ecological factors, particularly competition for resources. In addition I have shown that by applying stable isotope analysis it is possible to provide new insights into the complex behaviours of species which has been the subject of intense research effort for several decades.

However, there are many unanswered questions and several potential avenues for future research. For example, the underlying genetic or behavioural causes of an individual's resource preference or fundamental niche remain poorly understood, and likely play a large role in driving individual differences in foraging niche. In addition, although individual specialisation has been recorded in close to 200 species the causes and consequences of this behaviour still remain unknown in a majority of cases. The research within this thesis adds to a growing list of studies investigating this behaviour, however, it is still unknown how general these patterns are, and whether they are consistent across differing species and contexts.

Investigating individual niche variation within wild populations is clearly a challenge, as it requires information not only on niche variation itself, but also detailed individual and population level ecological information. However, the research in this thesis demonstrates that may be achievable by combining novel methodological approaches with long-term study populations of well studied species.

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