Intraspecific variation in the foraging ecology and morphology of kea

Nestor notabilis

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Abstract

Intraspecific variation can have knock-on ecological consequences on resource use, morphology and population dynamics. Kea parrots, *Nestor notabilis*, have a number of attributes that suggest that intraspecific variation in their foraging ecology may exist: their bill is sexually dimorphic, they inhabit two very different environments (montane and temperate rainforest), and they have a protracted juvenile period during which time they may learn to exploit their environment more effectively, suggesting foraging differences among age classes.

In this thesis, I investigated intraspecific variation in the foraging ecology of kea, and its link with variation in morphological traits. Firstly, I conducted field observations and faecal sample analyses and found that kea in the rainforest habitat ate invertebrates three times as frequently as those in the high-altitude habitat and that adult males ate more roots and invertebrates than immature kea. I then established kea-specific diet-tissue discrimination factors for carbon and nitrogen stable isotope ratios and regression equations to convert between the stable carbon and nitrogen isotope ratios of kea blood and feather samples. I subsequently used stable isotope mixing models, based on these kea-specific values, to establish the contribution of plant and animal matter to the kea's diet. I confirmed that the diet of kea in the rainforest habitat is mainly animal-based, whereas the diet of kea in high-altitude habitat is mainly plant-based, and also found that, in the rainforest, males ate more animal matter than females. Additionally, I found that birds sampled in the rainforest had longer bills and heads than those in high-altitude regions, which suggests a link between kea bill and head length and foraging ecology. I then measured the strength of the relationship between bill/head length and the consumption of animal matter. I found a larger effect size than has been recorded between diet and morphology in other species, which demonstrated that this relationship is strong enough that changes in the degree to which kea rely on invertebrate foraging could result in changes in their morphology. Finally, I examined differences in the kea's isotopic niche and found the first evidence for niche partitioning among male and female kea in high-altitude habitat.

This work has demonstrated that there is considerable intraspecific variation within the foraging ecology and behaviour of kea and that this variation is linked with differences in morphology among the sexes and among different populations.

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and kept me smiling throughout (except, possibly, at 4.30am). Above and beyond, my friends: Sasha Roselli, Ian Warrington, Andrius Pašukonis, Laura Young and Raoul Schwing.

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This is for you, my love.

Preface

This thesis investigates intraspecific variation in the foraging ecology of kea, *Nestor notabilis*, and its link with morphological variation. I address this topic and the necessary background in five main data chapters. Each chapter is strongly linked with the others. All chapters have been written as stand-alone papers for publication. Consequently, there is a certain amount of unavoidable repetition between them in order that they can be read independently. Chapter 2 has been published in the *New Zealand Journal of Ecology* (Co-authors: Gyula Gajdon & Ximena Nelson) and Chapter 3 is In Press in *Methods in Ecology and Evolution* (Co-authors: Travis Horton & Ximena Nelson). Both have been formatted for presentation in this thesis, and the published articles are included as Appendices A&B. I carried out the fieldwork, statistical analysis and main part of the writing of both articles myself. For Chapter 2, GG and XN assisted with revising the manuscript for publication. For Chapter 3, TH carried out the sample analysis and TH and XN assisted with revising the manuscript for publication. The rest of the work in this thesis is predominantly my own. This research was carried out with a High Impact Collection Permit (WC-30391-FAU) and Low Impact Collection Permit (WC-30527-FLO) from the New Zealand Department of Conservation.

CHAPTER ONE

Introduction

Fledgling kea feeding on grass seeds in the high-altitude habitat. (*Photo: Brian McClatchy*).

The Spice of Life

Individual differences among species members form the raw material of evolution (Darwin 1859; Dall & Griffith 2014). Evolution can shape not only a species' morphology, but also its behaviour. For example, a spider's silk-producing spinnerets are of little use without any corresponding web-spinning behaviour. The spider's morphology affects the type of web it produces and the type of web affects the type of prey that the spider catches (Herberstein & Tso 2011). Changes in the availability of certain prey may, in turn, influence the type of web the spider produces through changes in morphology or behaviour (Sandoval 1994; Herberstein & Tso 2011).

That diet can have a direct influence on morphology can be seen in the trophic organ development of almost any animal. This is particularly obvious in some species, such as the giant anteater *Myrmecophaga tridactyla*, which has elongated jaws fused into a tube and an extremely long tongue that together form a highly effective, ant-eating apparatus (Naples 1999). Even subtle dietary differences can have significant morphological effects. Those sub-species of orangutan that rely more on bark and tough foods have deeper and wider jaws with a greater area, which allows them to chew harder foods more effectively than sub-species that eat softer food (Taylor 2006).

Intraspecific variation exists to some degree within all species; however, some species also have consistent differences among cohorts (e.g., between age classes, sexes, populations, polymorphs). Purple-throated carib hummingbird *Eulampis jugularis* males and females have very differently shaped bills, which match the anatomy of the *Heliconia* flowers preferred by each sex (Temeles et al. 2000). Darwin's ground finches *Geospiza fortis* have large and small bill morphs, which specialise on seeds of different size and hardness (De León et al. 2012). Spadefoot toad tadpoles *Spea multiplicata* have three different morphs that vary from eating mostly algae to being totally carnivorous (Martin & Pfennig 2010). One possible explanation for this variety is that individuals with different morphologies are better adapted to exploit different resources. This 'niche partitioning' among species members reduces intraspecific competition and can increase individual fitness as a result (Pfennig et al. 2007). However, in the case of differences among the sexes this 'ecological causation hypothesis' is hotly contested as it has been suggested that sexual selection is a

more parsimonious explanation (e.g., Parker & Pizzari 2015). Sexual selection – where attributes that increase the likelihood of reproduction are selected for – is a major driving force behind differences between males and females (Darwin 1874). Textbook examples of sexual selection include the peacock's tail, considered a result of female mate preference for males with the most flamboyant ornamentation (Darwin 1874); and the extreme size of male elephant seals *Mirounga* spp., (males are five to six times larger than females; Perrin et al. 2008), thought to result from pressure for increased size in order to compete to secure mating rights to a large harem of females. However, evidence that ecological factors can cause sexual dimorphism is growing. As mentioned previously, the hummingbird *E. jugularis* has sexual differences in bill shape that relate to differences in their feeding ecology (Temeles et al. 2000). A phylogenetic study into sexual dimorphism in hermit hummingbirds (sub-family: Phaethornithinae) shows that sexual dimorphism is related to differential plant use in many hummingbird species (Temeles et al. 2010). Studies of the co-operatively breeding green woodhoopoe *Phoeniculus purpureus*, in which the male's bill is 36% larger than the females, have revealed consistent sex differences in their foraging ecology (Radford & du Plessis 2003) and that reproductive success is not related to bill length, indicating that niche partitioning rather than sexual selection is the likely cause for sexual dimorphism in this species (Radford et al. 2004). Pinnipeds, or true seals (the clade to which elephant seals belong), have recently been shown to have evolved sexual size dimorphism *prior* to some developing a harem-based mating system, and the origin of sexual size dimorphism within this clade is more likely linked to niche partitioning among the sexes or sexual selection due to males forcing copulation on females (Krüger et al. 2014).

Where intraspecific variation among cohorts exists, considering each species member as ecologically interchangeable is misguided and can lead to mistaken assumptions regarding a species' niche space (Bolnick et al. 2003). Different age cohorts are another likely source of variation, particularly in species that have different morphs as they age (e.g., lepidoptera; many amphibians); or species in which learning plays a large role in foraging strategies or diet selection. For instance, Northwestern crows *Corvus caurinus* drop clams onto a hard surface to break open the shells. Juveniles sometimes drop the clams over water, catch them in the air before they land, or drop them from random heights, making them far more inefficient foragers than adults (Richardson & Verbeek

1987). Different populations may also have different foraging ecology as they adapt to exploit more profitable foods within their habitats (e.g., capuchin monkeys *Cebus capucinus*; Chapman & Fedigan 1990).

In this thesis I investigate intraspecific variation in diet, foraging behaviour and morphology in the kea, *Nestor notabilis*. Although kea have been called the "ultimate generalist" (Diamond & Bond 1999) they have a number of attributes that suggest that specialisation among cohorts may exist. Unusually for parrots, they are sexually dimorphic, particularly their bill, which is c. 13% longer in males (Bond et al. 1991). Kea have a very long juvenile period, attaining sexual maturity at four years (Kemp 2013), which is thought to allow them to acquire the vast amount of foraging experience necessary for independent survival in the high-altitude habitat (Diamond & Bond 1999). During this time they are granted leniency by adult kea, which even tolerate theft from youngsters (Diamond & Bond 1991). Finally, kea breed in two very different habitats – high-altitude montane regions (Fig. 1A) and lowland, temperate rainforest (Fig. 1B) – and their foraging strategies or morphologies may have adapted to better suit each environment.

Figure 1. The kea's high-altitude (A) and rainforest (B) habitats. Main picture (A) – Woolshed Hill in Hawdon Valley, Arthur's Pass (1,040 m), Insert (A) – an adult male kea feeding on tutu berries *Coriaria plumosa* at Death's Corner, Arthur's Pass; Main picture (B) – Okarito, Westland (50 m), Insert (B) an adult female kea eating a huhu grub *Prionoplus reticularis* in Okarito, Westland.

The Kea – A Notable Parrot

The kea is a large (c. 850g), omnivorous parrot endemic to the South Island of New Zealand. Often referred to as the "world's only alpine parrot" (Diamond & Bond 1999; Young et al. 2012), many kea live in the alpine zone of the Southern Alps dividing New Zealand's South Island. However, their native habitat includes subalpine scrubland, Southern beech forest, and lowland podocarp/broadleaf forest, in addition to the true alpine environment above the treeline (~1250m).

Kea are thought to have evolved from a forest-dwelling bird known as the proto-kākā (named after the only other extant *Nestor* species, the kākā *Nestor meridionalis*) approximately 5 MYA (million years ago; Wood et al. 2014). This timing coincides with the formation of the Southern Alps and the creation of the alpine habitat in New Zealand. The harsh, often changing nature of this new environment shaped the kea's evolution in a myriad of ways. Their bill became longer and thinner, changing into a shape suitable for almost any foraging purpose, including digging up roots, ripping bark apart in the hunt for invertebrates, or plucking fruit from alpine shrubs. The kea's behaviour also changed, and they became extremely neophilic and explorative, and thereby likely to discover any potential resource in their area (Diamond & Bond 1999; O'Hara et al. 2012). They also developed a remarkable level of intelligence and behavioural flexibility that has enabled them to learn how to search for and exploit available resources effectively (Diamond & Bond 1999).

The kea's exceptional cognitive abilities are currently the subject of much study (e.g., Auersperg et al. 2011; Gajdon et al. 2014) and in the lab they have demonstrated means-end understanding, social learning and second-order tool use (e.g. Auersperg et al. 2010; Huber & Gajdon 2006). Wild kea are known to be highly innovative, for example, by continuing to successfully steal food from rubbish bins, in spite of a variety of ever more complex anti-kea measures being added by the locals (G.K. Gajdon 2010 pers. comm.). Unfortunately the kea's innovative nature almost led to their destruction at the hands of humans. In the late 19th Century, farmers started noticing mysterious wounds on the backs of some sheep returning from high country runs. The wounds were caused by kea, some of which had discovered that by clinging to sheep's wool they could peck out the flesh and fat around the sheep's kidneys without being dislodged. Farmers were outraged, and in response the

government introduced a hefty bounty, of 10 shillings per kea beak (c. NZ$65 in today's money; Young et al. 2012). An estimated 150,000 kea were killed over the next c. 100 years, until the bounty was finally lifted in 1971 (Temple 1996). Today, kea are an endangered species (Robertson et al. 2013) with just 1,000 – 5,000 remaining in the wild (Anderson 1986).

Despite the unusual nature of the kea's foraging ecology, relatively little has been published on this topic. Early work provided descriptions of the kea's diet in the alpine and sub-alpine environment and revealed that kea eat a huge variety of plants - at least 100 different species (Jackson 1960; Clarke 1970; Campbell 1976) - and that they use a diversity of techniques to access valuable resources. For example, kea dig for roots, pluck leaves and fruit, crush flowers for nectar, and turn over rocks to uncover invertebrates. The kea's diet consists primarily of fruit and leaves, with estimates of the contribution of plant matter to the kea's diet ranging from 70% (Brejaart 1988) to 95% (Clarke 1970). These early studies also revealed that the kea's diet varies seasonally to some degree, as is common among temperate living species and among parrots in general (see, Magrath & Lill 1985; Moorhouse 1997; Greene 1998; Matuzak, et al. 2008). The only recent study of kea foraging showed them to be discerning rather than purely opportunistic foragers, selecting the fruits of preferred species even when others were more readily available, and revealed the crucial role played by kea as dispersers of native alpine plants (Young et al. 2012). However, kea are not confined to alpine and sub-alpine zones, but also inhabit lowland, temperate rainforest where both the vegetation and environment are vastly different. The only data on the kea's diet in this habitat came from a study describing the foods taken by a variety of forest birds in the area (O'Donnell & Dilks 1994). Here the kea's diet seems to consist mainly of nectar, with invertebrates playing a secondary role (O'Donnell & Dilks 1994). There were no observations of kea eating fruit and very few (4%) of leaf-eating (O'Donnell & Dilks 1994), which suggests that there may be a large difference between the diets of kea in the high-altitude and rainforest habitats. Although kea breed in the rainforest habitat, it remains unknown what degree of fidelity individuals have to one habitat or the other. Recent genetic work has shown that kea sampled within the rainforest are not a distinct sub-species, which suggests some degree of dispersal across habitats (Dussex et al. 2014). However, as breeding kea usually remain

within their home range of 1.5 km (Wilson 1990), it is likely that some adults, at least, live entirely within the confines of the rainforest habitat.

The paucity of studies on kea foraging behaviour is likely due to the difficulty of collecting observational data from a flying species in a precipitous mountain environment or dense temperate rainforest, rather than a lack of interesting questions that can be posed about the foraging ecology of such an unusual parrot. As alluded, kea are a difficult-to-follow species. Being capable of flying long distances to nearby mountain tops, they can transverse in less than a minute terrain that would take researchers half a day to cover. However, there are other options than field observations available to ecologists interested in diet, for instance, faecal sample analysis and stable isotope analysis. Faecal sample analysis has the advantages that faeces are easy to collect, and they reveal information about the diet of kea when they are not under direct observation. However, as food is passed through the kea's gut in c. 6 h (Young et al. 2012), they represent a very short time-span and many remains may be too digested to be reliably identified (for a discussion of the advantages and disadvantages of faecal analysis, see Putman 1984). Stable isotope analysis, discussed in detail below, is a modern addition to the ecologist's toolkit that allows researchers to draw inferences about diet over a longer time-frame. In this thesis I use these three methodologies to answer questions regarding the kea's foraging ecology and its link with morphology.

Stable Isotopes as a Tool for Ecologists

Isotopes are atoms that have the same number of protons and electrons but differ in their number of neutrons. Some isotopes are radioactive and decay, whereas others are stable and occur naturally alongside the typical elemental form. Carbon and nitrogen both have two stable isotopes - ^{12}C and ^{13}C, with the lighter isotope ^{12}C making up about 98.9% of all the carbon on Earth; and ^{14}N and ^{15}N with ^{14}N accounting for 99.6% of all nitrogen (Fry 2006). The difference in mass between the two isotopes of each element results in different levels of inertia. A mass spectrometer can detect this difference and thereby determine the ratio of heavy to light isotopes in a sample of material. Isotope

values are reported in parts per thousand (‰) as δX, the ratio of heavy to light isotope, relative to a standard:

$$\delta X = \left(\frac{\mathrm{R_{sample}}}{\mathrm{R_{standard}}} - 1\right) \times 1000$$

where X is either ^{13}C or ^{15}N, and R is either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. The standard for carbon is Vienna Pee Dee Belemnite and for nitrogen the standard is air. $\delta^{13}C$ or $\delta^{15}N$ are also referred to as the carbon or nitrogen stable isotope ratio, respectively.

The key discovery for ecologists came when DeNiro and Epstein (1978, 1981) demonstrated that the stable carbon and nitrogen isotope ratios of the food that an animal eats are reflected in their body tissues. Their work revealed that, typically (although not always, as I will demonstrate in Chapter 3/Greer et al. In Press), the carbon stable isotope ratios of the animal's diet are very similar to the carbon stable isotope ratios in their tissues, differing by only 0 - 1‰ (DeNiro & Epstein 1978). Different sources of carbon can have very different isotopic ratios. For example, marine algae are in the order of -19 to -24‰, terrestrial C3 plants (e.g., trees) are c. -28‰ and, due to their differing photosynthetic pathways, C4 plants (e.g., maize) are c. -13‰ (Fry 2006). The relative contribution of two isotopically different carbon sources to an animal's diet can therefore be determined by examining the resulting isotopic ratio of the animal's tissues (e.g., Phillips & Koch 2002). DeNiro and Epstein (1981) also discovered that stable nitrogen isotope ratios respond differently to carbon, and bio-magnify along the food-chain, such that an animal's tissues are c. 3‰ (later adjusted to 3.4‰ by Post 2002) heavier than its food. This provided researchers with a type of 'trophometer' to determine the trophic level at which an animal is feeding (Fry 2006). Ecologists have capitalised on this early work in order to investigate questions regarding an animal's dietary shifts (Phillips & Eldridge 2006), trophic level (Lemons et al. 2011), niche width (Layman et al. 2007), and food web structure (Hussey et al. 2014).

Recent advances in Bayesian statistics have significantly progressed the field of stable isotope ecology. Simple linear models can be used to solve for the contribution of different food sources to an

animal's tissues, but only as long as the number of sources is less than or equal to the number of isotopes plus one. However, using Bayesian likelihood estimations, the possible number of sources can be extended out much farther, although with a corresponding loss of precision (Phillips 2012). A number of these 'stable isotope mixing models' have been developed (e.g., Isosource (Phillips & Gregg 2003); SIAR (Parnell et al. 2010); MixSIAR (Stock & Semmens 2013)) and are freely available for use by ecologists in the form of an R statistical computing environment (R Development Core Team 2013) package. Where applicable, I use the mixing model MixSIAR (Stock & Semmens 2013) throughout this thesis, as it allows the uncertainty surrounding dietary isotope ratios; the differing concentration of carbon or nitrogen within food sources; and regional differences in food source isotopes ratios, to be incorporated into the model.

Different tissues can reflect an animal's diet over different time-periods because they differ in their metabolic turnover rates (Dalerum & Angerbjörn 2005). Tissues with high metabolic turnover e.g., liver and plasma, can be used to investigate short-term dietary intake over days, whereas bone collagen has a very slow turnover and reflects diet over years (Phillips & Eldridge 2006). Researchers can address changes in diet and niche width by sampling multiple tissues from an individual. Here I use blood and feather samples to investigate the diet and niche width of kea. Blood samples reflect diet over c. the last month (Dalerum & Angerbjörn 2005), whereas feathers, which are metabolically inert after synthesis, reflect diet at the time of growth – either the moult for birds over one year old or the diet when in the nest for younger kea. The data from both tissues are complementary as they provide duplicate measures when synthesised at the same time. This allowed me to investigate the kea's dietary niche over different time periods.

Thesis Outline

The overall objective of this thesis is to determine what intraspecific variation exists in the kea's diet and if dietary differences are linked to differences in kea morphology. In Chapter 2, I use a combination of field observations and faecal sample analysis to investigate the diet of kea within the high-altitude and rainforest habitats and to provide a detailed breakdown by sex, age and season of

foraging behaviour in the high-altitude habitat. In Chapter 3, I establish kea-specific stable isotope discrimination factors in order to ensure that I use the correct model parameters for the use of stable isotope mixing models throughout the remainder of the thesis. I also determine regression equations that allow me to directly compare the isotope ratios from blood and feather samples. In Chapter 4, I use stable isotopes to investigate the proportion of animal versus plant matter in the kea's diet in the high-altitude and rainforest habitat. I also examine possible morphological differences between kea found in either habitat that may be related to differing levels of invertebrate foraging. In Chapter 5, I examine if there is a correlation between a kea's morphological traits (bill length, head length and weight) and its degree of invertebrate foraging and I measure the strength of this relationship. Finally, in Chapter 6, I look at which environmental variables affect the kea's isotopic niche and how their niche varies across habitat, age and sex.

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CHAPTER TWO

Intraspecific variation in the foraging ecology of kea, the world's only mountain and rainforest-dwelling parrot.

Fledgling (left) and adult kea watching the researchers' activities (high-altitude habitat; *Photo: Andruis Pašukonis*

Greer, A. L., Gajdon, G. K., & Nelson, X. J. (2015). Intraspecific variation in the foraging ecology of kea, the world's only mountain-and rainforest-dwelling parrot. *New Zealand Journal of Ecology, 39*(2), http://newzealandecology.org/nzje/3232

Abstract

Intraspecific variation can have important knock-on effects on population dynamics and ecosystem processes. There are good indicators that intraspecific differences in the foraging ecology of kea parrots (*Nestor notabilis*) exist. Kea breed in two markedly different habitats (alpine and temperate rainforest), and have pronounced sexual size dimorphism of their upper bill, which may indicate niche partitioning between the sexes. Additionally, as a long-lived species, they can potentially acquire a vast amount of information regarding food sources within their environment, suggesting variation between age classes. We used field observations and faecal analysis to investigate the foraging ecology of kea in detail. We found evidence of invertebrate foraging significantly more frequently in temperate rainforest than in alpine regions, where kea foraged more frequently on fruit. In the alpine habitat, kea fed mainly on fruit during summer and autumn, changing primarily to leaves during winter and spring and increasing invertebrate consumption in springtime. Although there was no discernable impact of sex, we found that adult males foraged more on roots and invertebrates than immature kea, possibly because they were able to exploit a more varied diet through experience. Future research should investigate the relationship between invertebrate foraging and breeding ecology in kea.

Introduction

Foraging ecology is often described at species level. This implies that individuals are ecologically equivalent, possibly obscuring intraspecific variability (see Bolnick et al. 2003). There are many potential causes for intraspecific variation in foraging ecology. For example, living in different habitats may necessitate different diets (Belmaker et al. 2012), different age or sex classes may have different physiological needs (Navarro et al. 2010), and dominant individuals may be able to access more highly valued foods than their subordinates (Prop & Deerenberg 1991). Additionally, dietary choices may vary seasonally. Intraspecific variation can have large-scale impacts on population dynamics and ecosystem processes; consequently to properly understand the role played by a species within its ecosystem(s) it is important to know how their foraging ecology varies at different ecological levels (see Hughes et al. 2008). Here we present detailed foraging data on the kea (*Nestor notabilis*), an endangered (Robertson et al. 2013) parrot (Psittaciformes) endemic to the South Island of New Zealand for which circumstantial evidence suggests intraspecific variation at a variety of ecological levels.

Often referred to as the 'world's only alpine parrot' (Diamond & Bond 1999; Young et al. 2012), the majority of kea live in the alpine and subalpine zones of New Zealand's Southern Alps (700 – 2,000 m above sea level [a.s.l.]; Robertson et al. 2007) where their habitat comprises alpine grasslands, sub-alpine scrublands, southern beech forests (*Fuscospora* spp. and *Lophozonia menziesii*), bare rock and scree. The alpine climate is more extreme than in the rest of the country, having more extreme winds, lower temperatures and semi-permanent snow reaching down to ~1000 m during winter (NIWA 2014a). However, some kea breed almost at sea level in New Zealand's temperate rainforest (Jackson 1963). These hardwood/broadleaf forests are dominated by rimu (*Dacrydium cupressinum*), kāmahi (*Weinmannia racemosa*), Southern rātā (*Metrosideros umbellata*), and silver beech (*Lophozonia menziesii*). Snowfalls here are rare, and mean maximum daily temperatures vary by just 8°C across the year (NIWA 2014b). Breeding kea usually remain within 1.5 km of their nest (Wilson 1990) meaning that, at least for a portion of the year, many adults likely forage exclusively within the rainforest. Fledglings, however, disperse more widely, with individuals

tracked to both habitats (J. Amey, New Zealand Department of Conservation, pers. comm.) and recent work suggests that this population is not genetically isolated (Dussex 2014).

Kea have a highly generalist, omnivorous diet (Brejaart 1988). They explore their environment with innate curiosity and intelligence to exploit all potential food stuffs (Diamond & Bond 1999; Auersperg et al. 2011). Although they seem mainly herbivorous (estimates range from 70% [Brejaart 1988] to 95% [Clarke 1970]), foraging predominantly on fruits and leaves (Jackson 1960; Young et al. 2012), they are one of only two species of parrot (the other being the Antipodes Island Parakeet, *Cyanoramphus unicolor*; Greene 1999) that have been reported to hunt and kill other vertebrates (e.g., Hutton's shearwater *Puffinus huttoni* chicks and mice; Pullar 1996; Beggs & Mankelow 2002). The only study to investigate the kea's diet in the rainforest identified nectar/flowers, invertebrates and seeds as their main foods (O'Donnell & Dilks 1994). Notably, although the study was year-round, there were no instances of frugivory and few leaf-feeding observations (4%), suggesting a substantial difference in the foods taken by kea in the rainforest as compared to alpine zones. Fruit in this rainforest is scarce during spring and summer, whereas leaves and invertebrates could provide a more reliable year round source of food (O'Donnell & Dilks 1994).

Complex learned behaviours improve with experience and thus with age (Rosenzweig & Bennett 1996). The considerable breadth of the kea's diet and the often harsh nature of the alpine environment require kea to retain a great deal of information about potential food sources within their habitat. Typically, older birds are more successful or efficient foragers than their immature counterparts, but the effects of dominance and/or increasing bill size can be difficult to disentangle from those of age (Desrochers 1992; Riotte-Lambert & Weimerskirch 2013). Kea are an ideal species in which to study age effects because older birds are not necessarily more dominant (Tebbich et al. 1996; Diamond & Bond 1999). Also, as the kea's bill attains more than 96% of its adult size by the time individuals fledge (AG, Unpublished Data), age differences in bill size are unlikely to have a significant impact on their food handling capabilities. When Diamond and Bond (1991) investigated the foraging behaviour of kea at an open-air rubbish dump (now closed) they found that fledglings were the most inefficient foragers (time eating / time searching) and adults were the most capable of finding new foods. In a natural environment, the skills involved in digging up roots and extracting

invertebrates from wood and under rocks may match those displayed in uncovering food from garbage enabling adults to exploit their environment more effectively than immature kea.

The male kea's bill is much longer (13%) than the females, yet males are only ~5% larger in other linear measures of body size (Bond et al. 1991). Sexual size dimorphism (SSD) is often attributed to sexual selection; however, ecological causes, such as niche partitioning, have also been proposed, particularly when the trophic organ is dimorphic (Shine 1989), and have been convincingly demonstrated for some species (e.g., purple-throated carib hummingbirds *Eulampis jugularis*, Temeles et al. 2000; and house finches *Carpodacus mexicanus*, Badyaev & Hill 2000). It has been suggested that the bill SSD of kea and their only extant congener, the kākā (*Nestor meridionalis*), has an ecological cause, as both species are monogamous and non-territorial (Bond et al. 1991; Moorhouse et al. 1999; but see Székely 2004). Moorhouse et al. (1999) point specifically to the prolonged male provisioning of females and young in these species, and propose that their bill SSD enhances the males' provisioning power. Only male kākā excavate kānuka longhorn larvae (*Ochrocydus huttoni*) or crack hīnau (*Elaeocarpus dentatus*) seeds once they have hardened (Beggs & Wilson 1991; Moorhouse 1997). To date there is no evidence to support ecological causes for bill SSD in kea, at least in part because sexual differences in the diet or foraging behaviour of kea have not yet been investigated.

Here we examine the kea's diet and foraging behaviour in detail using a dataset from alpine and rainforest habitat along the Southern Alps. We predict that: (1) kea in the rainforest will forage less on fruit than those in alpine regions; (2) fruit foraging will decrease in winter and spring, at which time there will be a parallel increase in the amount of foraging on leaves; (3) adults will spend more time eating hard-to-find foods such as roots and invertebrates than immature kea; (4) male kea will be more efficient foragers than females and/ or will access resources which females cannot.

Methods

Study sites

Alpine study sites were located at Mount Arthur (41°13'S, 172°40'E; 1700m a.s.l.), Death's Corner (42°54'S, 171°34'E; 950 m a.s.l.), Hawdon Valley (42°57'S, 171°46'E; 1150 m a.s.l.), Craigieburn (43° 6'S, 171°42'E; 1300 m a.s.l.) and Red Tarns (43°44'S, 170° 5'E; 1050 m a.s.l.). Okarito and surrounds (43°13'S, 170°10'E; 50 m a.s.l.) represented the rainforest habitat. Study sites were chosen to span most of the kea's habitual altitudinal range. Figure 1 shows the location and types of data recorded at each study site. The season in which these data were collected and the age of sex of each kea sampled are detailed in Appendix 2.1.

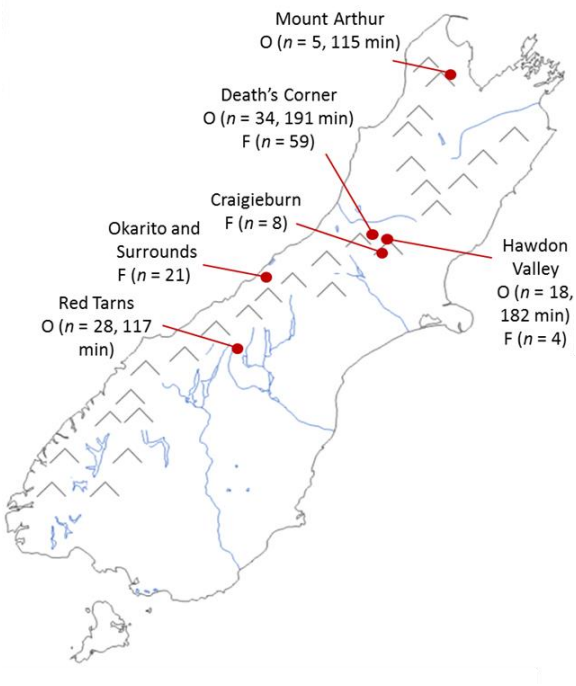

Figure 1. Map of New Zealand's South island, showing study site locations and numbers and types of data collected from each, O – foraging observations, minutes indicate total time of observations, F – faecal samples.

Foraging observations

Between October 2010 and July 2012 we filmed 85 kea foraging sessions > 1 min long (~600 min; Sony Handycam HDR-XR200VE) at alpine locations (Fig. 1). Foraging sessions began when a focal kea engaged in a foraging action and ended when no foraging action had occurred for > 1 min.

Foraging actions consisted of (1) feeding actions: eating fruit, leaves/leaf-buds, flowers, roots, stems, invertebrates/meat, other, unidentified and (2) searching actions: scouting (terrestrial locomotion between two foraging actions e.g., walking, hopping), digging, overturning rocks, ripping bark and nibbling. We preferentially recorded individuals already foraging. As we were interested in quantifying the kea's natural diet, we only recorded foraging on anthropogenic foods if a bird moved from eating natural to anthropogenic food within the same foraging session. Foraging observations < 1 min long and opportunistic observations of kea foraging outside of our study sites were included in the list of all foods kea were observed eating or which occurred in their faeces (Appendix 2.2), but were not analysed further.

Many observed kea were banded and individually identifiable. Where birds were not banded, age (by degree of yellow colouration on head and bill): nestling, fledgling, juvenile, sub-adult, adult; and sex (by sexual dimorphism of bill length) could often be distinguished (see Diamond & Bond 1999). We combined sub-adult observations (n = 2) with those of juveniles due to few observations and similar behaviour.

Video footage was analysed using JWatcher 1.0 (Blumstein et al., 2010). Additional foraging sessions begun by the same bird within 15 min of another were pooled. If a group was being recorded, up to, but not exceeding half of the birds were excluded from analysis. Individually identifiable birds or those distinguishable by age and/or sex were preferentially retained for analysis; otherwise excluded birds were chosen randomly. Statistical analyses were based on the proportion of time (in seconds) engaged in the behaviour while in sight of the observer.

We analysed observation data using one-way Analyses of Variance (ANOVAs) to investigate seasonal differences in foraging behaviour and 3 x 2 factorial ANOVAs to examine the impact of age (fledgling, juvenile, adult) and sex. We split our data analysis in this fashion, rather than using a 4 x 3 x 2 ANOVA design, because of low sample sizes in the autumn (n = 6) and winter (n = 5) seasons. Instead, we confined our age and sex analyses to summer (n = 56) and excluded the category 'adult female' due to low sample size (n = 1). All statistical analyses were conducted using a quasi-binomial distribution because our data were proportional and over-dispersed. The eleven behavioural categories on which ANOVAs were carried out were established *a priori* (Table 1) and do not include all

potential foraging actions. We used Benjamini and Hochberg's (1995) procedure ($\alpha = 0.05$) to control the false discovery rate (FDR) associated with multiple testing. We report FDR adjusted *p*-values as *q*-values throughout. All ANOVAs and *q*-values were calculated directly in R version 2.15.3 (R Development Core Team 2013), and over-dispersion was determined using the R package 'AER' (Kleiber & Zeileis 2008).

Faecal analysis

Faecal samples were collected when kea were handled for banding, when they were observed defecating, or when the samples were obviously very fresh (kea droppings are easily identifiable, see Young et al. 2012). Faecal analysis, while having its own limitations (see Putman 1984), can add to the list of known kea foods taken in different seasons and give an indication of the frequency with which a food is eaten so long as that food is reliably passed in an identifiable form (e.g., fruit seeds, cuticular remains of invertebrates). We collected 93 faecal samples, 55 from individually identifiable birds, 16 from unbanded birds of known age and/or sex, and 22 from unknown kea. Most (n = 71) samples were collected in the alpine habitat, with 22 samples collected in the rainforest. As nestlings are directly provisioned by adults these two age categories were combined into a single adult/nestling category. In one instance, faecal samples were collected from both an adult female and her nestling so we excluded the nestling sample in order to avoid pseudo-replication, leaving 21 rainforest samples. Each sample was poured into a petri dish overlaid on a 0.5 cm^2 grid and teased apart under a dissecting microscope. Contents were identified, where possible, to species level, using a combination of plant samples collected in the field and seed reference collections (Webb & Simpson 2001; Young 2012); or were grouped into broader categories, such as 'invertebrates' and 'woody material'. Woody material is likely ingested when kea rip apart wood in the search for invertebrates. We used Pearson's Chi-squares to investigate the effects of season, habitat (alpine, rainforest), age (fledgling, juvenile, adult/nestling) and sex on the occurrence of fruit seeds, fruit skin/pulp, invertebrate remains, and woody material within faecal samples. FDR *q*-values were used to determine statistical significance. All Chi-squares were conducted using SPSS Statistics 21 (IBM Corporation, NY 10589, USA).

Results

Foraging activity budget

Of their total foraging time, kea spent 65% feeding, and 35% searching for food. Kea ate fruit (47% of feeding time, *n* = 39), leaves/leaf-buds (27%, *n* = 28), invertebrates/meat (10%, *n* = 3), flowers (5%, *n* = 14), roots (5%, *n* = 11), stems (2%, *n* = 8), other foods (1%, *n* = 6) and, in 20 instances (3%), food that could not be identified. Scouting comprised 45% of searching time (*n* = 72), with digging 31% (*n* = 33), overturning rocks 17% (*n* = 21), ripping bark 5% (*n* = 1) and nibbling 2% (*n* = 21) comprising the remainder.

Habitat differences

Kea in alpine zones fed on over 30 species of plant, in addition to unidentified grasses and herbs, invertebrates, a common brushtail possum tail (*Trichosurus vulpecula)* and anthropogenic foods (listed in Appendix 2.2). Figure 2 illustrates the feeding time spent eating each food with > 1% of feeding time. A quarter of feeding time was spent eating just *Podocarpus nivalis* fruit or leaves/leaf-buds. Kea were not confined to eating native plants, also spending almost a quarter of their feeding time eating various introduced species. We divided the alpine daisy *Celmisia spectabilis* into two categories - 'in flower' (3%) and 'in seed' (3%) - because up to 97% of *Celmisia* spp. seedheads can host adult and larval seed predators (Molloy 1975) which may provide kea with an additional source of protein.

We recorded at least 27 plant species, invertebrates, moss and woody material in faecal samples collected in alpine regions (Appendix 2.2). The most commonly occurring species were: *Coprosma intertexta* (51%), *Coriaria sarmentosa* (11%), *Coprosma cheesmanii* (11%), *Podocarpus nivalis* (11%), *Astelia* spp. (10%), *Phyllocladus alpinus* (7%), *Gaultheria depressa* (7%), *Coprosma pseudocuneata* (6%). All other species were found in fewer than 5% of the samples.

At least twelve plant species, invertebrates, moss, lichen and woody material were found in faeces collected in the rainforest (Appendix 2.2). Seven of these plants (*Aciphylla* spp., *Coprosma intertexta, Dracophyllum* spp., *Gaultheria depressa, Lepidothamnus laxifolius, Phyllocladus alpinus,*

Podocarpus nivalis) were not known to be eaten by kea in the rainforest habitat. The most commonly occurring plants were: *Dracophyllum* spp. (16%), *Gaultheria depressa* (8%), and *Aciphylla* spp. (8%). All others were found in fewer than 5% of samples.

Faeces collected at alpine sites contained fruit seeds (73%; $\chi^2 = 6.70$, $q = 0.048$) more often than did those collected in the rainforest (43%; Fig. 3). Conversely, rainforest samples contained invertebrate remains (71% v. 25%; $\chi^2 = 14.96$, $q < 0.001$) more frequently and woody material (38% v. 15%; $\chi^2 = 5.05$, $q = 0.057$) marginally more. There was no difference between the two habitats in the occurrence of fruit skin/pulp (alpine: 75%; rainforest: 52%; $\chi^2 = 3.80$, $q = 0.102$).

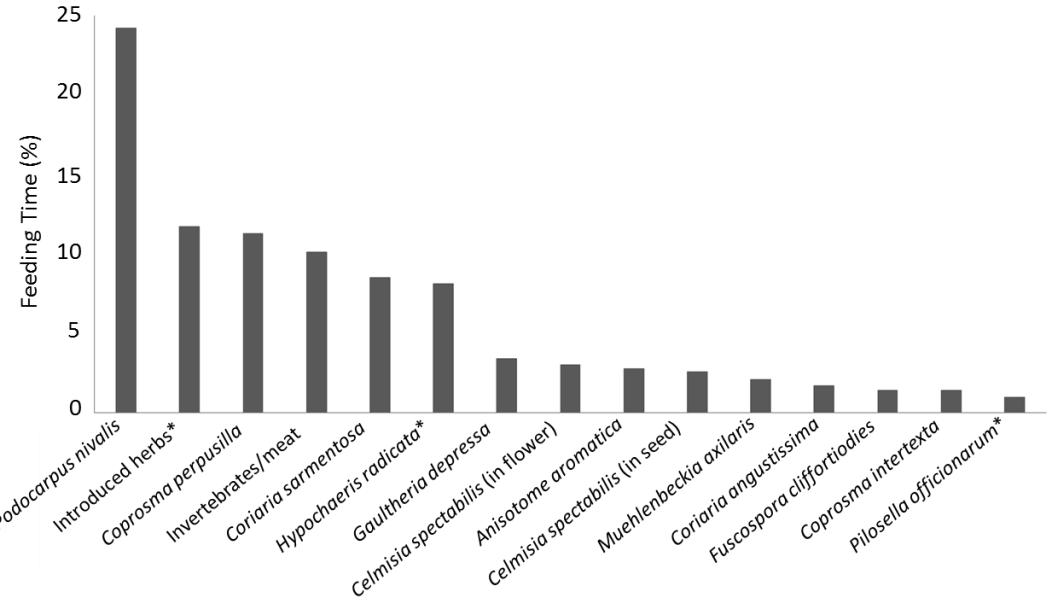

Figure 2. Foods on which kea in alpine regions spent >1% of their feeding time. *Introduced species.

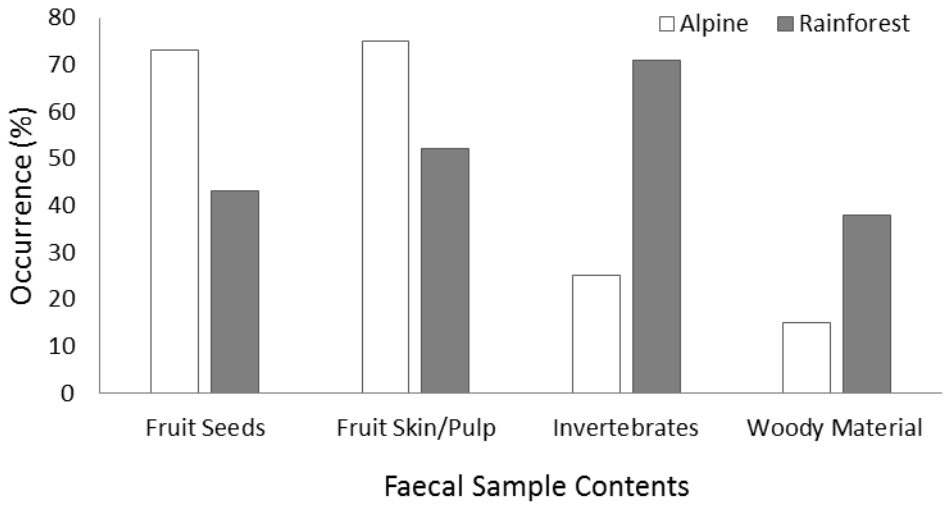

Figure 3. Frequency of occurrence of components related to fruit or invertebrate foraging in faecal samples from alpine and rainforest sites.

Table 1. Seasonal variation in the percentage of time in sight of the observer which was spent engaged in foraging actions. Efficiency = total feeding time / total searching time. *Reported as number of species and items eaten per minute. False discovery rate adjusted *p*-values are reported as *q*-values, d.f. = 3, 81.

	Seasonal Variation		**Spring (*n* = 18)**		**Summer (*n* = 56)**		**Autumn (*n* = 6)**		**Winter (*n* = 5)**	
	F	***q***	***x̄* %**	***SD***	***x̄* %**	***SD***	***x̄* %**	***SD***	***x̄* %**	***SD***
Feeding Actions										
Fruit	9.73	< 0.001	0	0	38.4	36.9	27.7	40.3	0	0
Flower	1.37	0.536	0.8	2.9	2.8	11.4	0	0	0	0
Leaves/Leaf-Buds	20.82	< 0.001	30.4	34.7	2.8	9.6	0.8	2.0	39.7	24.1
Roots	0.31	0.878	7.8	17.5	1.0	5.0	2.8	4.4	0	0
Stems	0.40	0.878	2.0	5.8	0.7	3.3	0.8	1.9	0	0
Searching Actions										
Digging	3.44	0.099	7.6	12.5	7.3	18.0	26.6	21.8	2.9	6.5
Overturning Rocks	0.52	0.853	3.0	12.2	4.9	15.2	3.0	7.2	0.4	0.9
Scouting	3.95	0.072	16.3	8.9	9.1	9.8	19.2	15.6	27.8	28.3
Efficiency	1.19	0.613	5.3	10.8	34.5	73.6	18.7	44.7	16.8	29.4
Number of Species*	1.72	0.403	0.4	0.5	0.4	0.4	0.7	0.7	0.6	0.4
Number of Items *	1.54	0.466	1.7	1.0	1.3	3.3	0.5	0.5	0.7	0.1

Seasonal differences

The time kea spent eating fruits and leaves/leaf-buds varied seasonally (Fig. 4). More time was spent eating fruit in summer and autumn and more time eating leaves/leaf-buds in winter and spring. There were no other significant seasonal differences in kea diet or behaviour, although the time spent scouting approached significance, with kea tending to move about least during the summer and most during the winter (Table 1). Faecal samples collected in spring contained invertebrates almost three times more frequently than those collected in other seasons ($\chi^2_3 = 24.49$, $q = <0.001$; Fig. 5). Fruit seeds ($\chi^2_3 = 9.40$, $q = 0.057$) and fruit skin/pulp ($\chi^2_3 = 10.33$, $q = 0.051$) tended to occur most frequently in samples collected in the autumn and least frequently in those collected during spring. The occurrence of woody material ($\chi^2_3 = 4.93$, $q = 0.236$) did not vary seasonally.

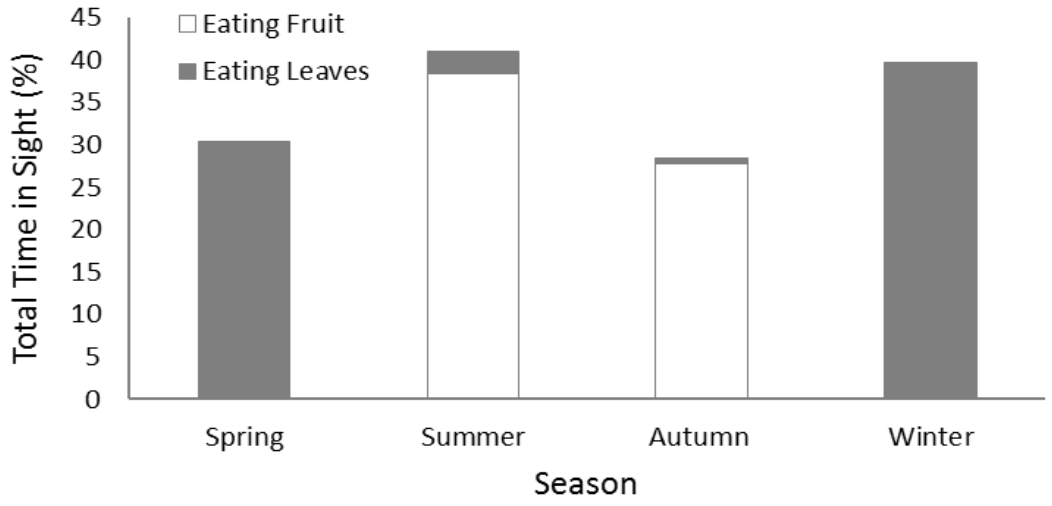

Figure 4. Seasonal variation in the time kea in alpine regions spent eating fruit and leaves/leaf-buds.

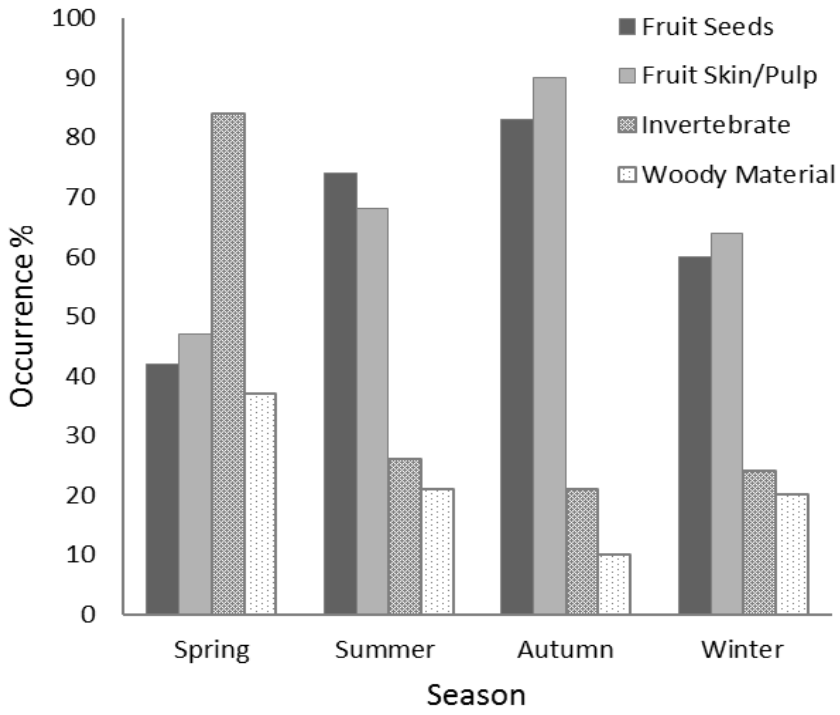

Figure 5. Seasonal occurrence of components related to fruit or invertebrate foraging in kea faecal samples.

Age and sex differences

Adult males spent more time eating roots than juveniles or fledglings, but there were no other age or sex differences in feeding actions (Table 2). When searching, fledglings spent less time scouting for food than juveniles or adult males (Table 2).

Invertebrate remains occurred more frequently in faecal samples from adults/nestlings than from any other age class ($\chi^2_2 = 8.78$, $q = 0.048$; Table 3). There were no further age or sex differences in faecal sample contents (Table 3).

Table 2. Age and sex differences in the percentage of time in sight of the observer which was spent engaged in foraging actions. Efficiency = total feeding time / total searching time. *Reported as number of species and items eaten per minute. False discovery rate adjusted *p*-values are reported as *q*-values, d.f. for age = 2, 32; sex = 1,31. All interactions were non-significant ($q > 0.05$).

Behaviour	**Age**		**Sex**		**Fledgling (*n* = 27)**		**Juvenile (*n* = 16)**		**Adult (*n* = 12)**		**Male (*n* = 28)**		**Female (*n* = 7)**	
	F	*q*	*F*	*q*	$\bar{x}$ %	*SD*	$\bar{x}$ %	*SD*	$\bar{x}$ %	*SD*	$\bar{x}$ %	*SD*	$\bar{x}$ %	*SD*
Feeding Actions														
Fruit	0.87	0.674	0.68	0.674	45.9	38.9	45.9	33.8	13.0	27.4	28.8	35.1	45.9	39.5
Flowers	3.86	0.132	2.48	0.320	0.9	4.6	0.1	0.5	10.5	22.3	4.5	15.2	3.7	8.9
Leaves/Leaf-Buds	3.34	0.180	0.06	0.878	1.8	5.0	0.5	1.4	7.7	18.7	4.7	13.0	2.5	4.3
Roots	6.45	0.041	0.01	0.935	0.1	0.8	0.1	0.6	4.0	10.4	1.8	6.9	0.6	1.5
Stems	2.62	0.267	7.01	0.072	0.2	0.8	1.7	5.7	0.6	2.2	0.4	1.6	3.9	8.5
Searching Actions														
Digging	0.19	0.878	2.73	0.300	6.1	17.7	7.7	19.3	9.1	17.8	6.1	13.2	2.0	2.3
Overturning Rocks	0.40	0.853	0.11	0.878	7.6	20.0	0.6	2.2	0.5	1.6	0.3	1.1	1.4	3.2
Scouting	10.59	< 0.001	3.65	0.218	6.0	8.2	11.8	12.0	12.4	8.7	10.1	8.2	14.5	15.8
Efficiency	1.13	0.618	0.27	0.853	44.7	73.5	41.1	94.2	5.4	7.0	29.0	74.3	40.7	67.7
Number of Species*	0.06	0.937	0.25	0.853	0.6	0.6	0.4	0.3	0.3	0.2	0.3	0.2	0.5	0.4
Number of Items*	0.76	0.717	0.71	0.674	3.1	6.6	0.8	1.0	0.8	0.6	0.9	0.8	1.4	3.6

Table 3. The percentage of kea faecal samples containing material related to fruit or invertebrate foraging by age (d.f. = 2) and sex (d.f. = 1). False discovery rate adjusted *p*-values are reported as *q*-values.

	Age		**Sex**		**Male** ***n*** **= 39**	**Female** ***n*** **= 19**	**Fledgling** ***n*** **= 19**	**Juvenile** ***n*** **= 21**	**Adult** ***n*** **= 30**
	χ^2	*q*	χ^2	*q*	%	%	%	%	%
Fruit Seeds	1.61	0.511	1.99	0.231	67	47	68	57	50
Fruit Skin/Pulp	5.17	0.133	0.01	0.944	64	63	84	57	53
Invertebrates	8.78	0.048	1.12	0.357	46	32	26	24	60
Woody Material	0.28	0.928	2.04	0.231	15	32	26	24	20

Discussion

We found that kea in alpine regions spent most time eating fruit, followed by leaves/leaf-buds, and animal matter. Reinforcing this finding, faeces collected in alpine areas contained evidence of fruit foraging almost three times as often as invertebrate foraging (leaf and other plant organ remains were too digested to be reliably quantified). In contrast, 75% of faecal samples collected in the rainforest contained evidence of invertebrate foraging. This suggests a large difference between the diets of kea in these habitats. While O'Donnell and Dilks' (1994) rainforest study confirmed kea feeding on invertebrates for only 13% of their observations, they also recorded a further 32% of 'probable invertebrate' observations. Thus invertebrate foraging likely accounted for almost half of their observations, versus just 4% of fruit and leaf feeding observations. A substantially increased amount of animal protein in the kea's diet could have a significant impact on their breeding ecology. Nestlings of frugivores which are also fed invertebrates may both grow and fledge more quickly (Roca 1994). New Zealand's yellow-crowned parakeet (*Cyanoramphus auriceps*) forages heavily on invertebrates and is thought to breed much earlier, and have a longer lasting breeding season, than the closely related red-crowned parakeet (*C. novaezelandiae*), which is predominantly herbivorous (Greene 1998).

In alpine areas, kea spent almost a quarter of their feeding time eating the fruits and leaves of *Podocarpus nivalis*, making this species by far the most handled food source. Various *Coprosma* spp.

were also among the most handled species and commonly occurred in faecal samples, thus our results agree with previous literature, which has noted *P. nivalis* and a variety of *Coprosma* species as being the mainstay of kea frugivory (Jackson 1960; Clarke 1970; Young et al. 2012). In addition, though *P. nivalis* is a true alpine plant and is not known to grow in the Westland rainforest (New Zealand Plant Conservation Network, accessed 05 June 2014), its remains were also found in a faecal sample collected in the rainforest, indicating that at least one (sub-adult, female) kea fed in both alpine and rainforest habitats during winter.

Large seasonal differences were noted in the kea's diet, such that fruit-feeding was most common during the summer and autumn. However, the presence of fruit seeds in winter and spring faecal samples revealed that kea were still eating some fruit throughout the year, taking advantage of both late-remaining berries, and late-fruiting species, such as *Coprosma intertexta*. Leaf/leaf-bud feeding increased substantially during the leaner months of winter and spring and invertebrates were eaten far more frequently during spring than any other season. Seasonal variations in diet have been found in other parrots, with increased invertebrate foraging noted as coincident with the breeding season (e.g. Smith & Moore 1991), or pre/post breeding season (e.g. Díaz & Peris 2011). For kea, increased invertebrate foraging coincides with the post-hatching, pre-fledging phase of chick development. This differs from kākā and the other member of the Strigopoidea family, the kākāpō (*Strigops habroptila*), which raise chicks during summer and only breed in years when trees are mast-fruiting or -seeding (Powlesland et al. 2009). This suggests that kea may maintain their annual spring nesting cycle by increasing the level of animal protein in their diet.

Adult kea spent more time eating roots than immature birds, and their faeces contained more invertebrates and woody material. These differences can be attributed to increased experience, as adult and immature kea have similar bill sizes and adults do not have preferential access to highly-prized foods (Tebbich et al. 1996; Diamond & Bond 1999). Roots and many invertebrates require extracting, meaning an extra step before the 'hidden' food can be obtained (King 1986). This additional complexity may take practice to master. Adult brown capuchin monkeys (*Cebus apella*) are more efficient extractive foragers than juveniles as they search more effectively (Gunst et al. 2010), whereas, failing to restrict searches to specific areas, juvenile wandering albatross (*Diomedea*

exulans) are the least successful foraging class (Riotte-Lambert & Weimerskirch, 2013). We found no difference in the time kea spent digging by age class, yet we only observed one instance of a fledgling actually eating roots and this bird was eating what remained in a hole already dug by another kea. Our results suggest that either the searching or excavating abilities of younger birds do not yet equal those of adults.

If ecological factors are the driving or maintaining force behind the kea's bill sexual size dimorphism (SSD), we would have expected males to differ from females in one of the following ways: be more efficient foragers; take more food items; exploit more species; or exploit a resource inaccessible to females, or for a longer period of time, or to a greater extent than females. We found no differences in the foraging ecology of male and female kea; however, we must offer two caveats to these results. Firstly, we were constrained by our observational data to analysing sex and age differences in the summer months only. Intraspecific differences in alpine regions may be at a minimum at this time of year due to an abundance of readily available berries. Any enhanced male provisioning ability may only be apparent during the colder months, when their longer bill could make it a more powerful or efficient digging/prying tool in hard, frozen ground, or in deep snow. This coincides with the period when females are incubating eggs or raising chicks and increased male foraging ability would be particularly advantageous. Secondly, we had insufficient summertime foraging observations of adult females to include in our analysis, which may have impacted on our ability to detect age and sex differences in the diet of kea, particularly as adult females form a unique category with the specific physiological requirement of egg-laying. However, our faecal sample analysis, which did not suffer from these limitations, also revealed no differences between males and female invertebrate or fruit foraging. Székely (2004), and Serrano-Meneses and Székely (2006) found that sexual selection rather than niche partitioning was the most likely explanation for SSD within largely monogamous and non-territorial seabird taxa. Here we found no evidence for an alternative explanation for kea.

In conclusion, there is a great degree of intraspecific variation in the foraging ecology of kea which seems mainly driven by season and habitat type, with age playing a more minor role. Future

research should investigate the potential impact of a diet substantially richer in animal protein on kea breeding ecology, particularly breeding season timing and length, and nestling growth rates.

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APPENDIX 2.1 Numbers of field observations and faecal samples of kea collected in each habitat by season, sex and age.

		Spring				**Summer**				**Autumn**				**Winter**			
Mount	*Observations*	Male	0	Fledgling	0	Male	2	Fledgling	1	Male	0	Fledgling	0	Male	0	Fledgling	0
Arthur	*Total = 5*	Female	0	Juvenile	0	Female	3	Juvenile	4	Female	0	Juvenile	0	Female	0	Juvenile	0
		Unknown	0	Sub-Adult	0	Unknown	0	Sub-Adult	0	Unknown	0	Sub-Adult	0	Unknown	0	Sub-Adult	0
				Adult	0			Adult	0			Adult	0			Adult	0
Death's	*Observations*	Male	5	Fledgling	0	Male	3	Fledgling	1	Male	3	Fledgling	2	Male	2	Fledgling	1
Corner/	*Total = 34*	Female	13	Juvenile	11	Female	0	Juvenile	3	Female	2	Juvenile	2	Female	1	Juvenile	1
Temple		Unknown	0	Sub-Adult	0	Unknown	2	Sub-Adult	0	Unknown	1	Sub-Adult	0	Unknown	2	Sub-Adult	0
Basin				Adult	7			Adult	1			Adult	2			Adult	3
	Faecal Samples	Male	2	Nestling	0	Male	8	Nestling	0	Male	4	Nestling		Male	7	Nestling	
	Total = 59	Female	0	Fledgling	0	Female	1	Fledgling	0	Female	4	Fledgling	8	Female	3	Fledgling	5
		Unknown	0	Juvenile	0	Unknown	2	Juvenile	7	Unknown	19	Juvenile	1	Unknown	9	Juvenile	4

				Sub-Adult	0			Sub-Adult	0			Sub-Adult	2			Sub-Adult	0
				Adult	2			Adult	2			Adult	2			Adult	4
				Unknown	0			Unknown	0			Unknown	14			Unknown	6
Hawdon	*Observations*	Male	0	Fledgling	0	Male	16	Fledgling	4	Male	0	Fledgling	0	Male	0	Fledgling	0
	Total = 18	Female	0	Juvenile	0	Female	2	Juvenile	3	Female	0	Juvenile	0	Female	0	Juvenile	0
		Unknown	0	Sub-Adult	0	Unknown	0	Sub-Adult	0	Unknown	0	Sub-Adult	0	Unknown	0	Sub-Adult	0
				Adult	0			Adult	11			Adult	0			Adult	0
	Faecal Samples	Male	0	Nestling	0	Male	4	Nestling	0	Male	0	Nestling	0	Male	0	Nestling	0
	Total = 4	Female	0	Fledgling	0	Female	0	Fledgling	0	Female	0	Fledgling	0	Female	0	Fledgling	0
				Juvenile	0			Juvenile	3			Juvenile	0			Juvenile	0
				Sub-Adult	0			Sub-Adult	0			Sub-Adult	0			Sub-Adult	0
				Adult	0			Adult	1			Adult	0			Adult	0
Craigieburn	*Faecal*	Male	0	Nestling	0	Male	0	Nestling	0	Male	0	Nestling	0	Male	3	Nestling	0

	Samples															
	Total = 8	Female	0	Fledgling	0	Female	0	Fledgling	0	Female	0	Fledgling	0	Female	1	Fledgling	4
		Unknown	3	Juvenile	3	Unknown	0	Juvenile	0	Unknown	0	Juvenile	0	Unknown	1	Juvenile	0
				Sub-Adult	0			Sub-Adult	0			Sub-Adult	0			Sub-Adult	0
				Adult	0			Adult	0			Adult	0			Adult	1
Okarito	*Faecal Samples*	Male	7	Nestling	7	Male	2	Nestling	3	Male	1	Nestling	0	Male	1	Nestling	0
	Total = 21	Female	4	Fledgling	0	Female	2	Fledgling	0	Female	1	Fledgling	2	Female	3	Fledgling	0
				Juvenile	0			Juvenile	0			Juvenile	0			Juvenile	0
				Sub-Adult	0			Sub-Adult	0			Sub-Adult	0			Sub-Adult	1
				Adult	4			Adult	1			Adult	0			Adult	3
Red Tarns	*Observations*	Male	0	Fledgling	0	Male	7	Fledgling	21	Male	0	Fledgling	0	Male	0	Fledgling	0
	Total = 28	Female	0	Juvenile	0	Female	3	Juvenile	4	Female	0	Juvenile	0	Female	0	Juvenile	0
		Unknown	0	Sub-Adult	0	Unknown	18	Sub-Adult	2	Unknown	0	Sub-Adult	0	Unknown	0	Sub-Adult	0
				Adult	0			Adult	1			Adult	0			Adult	0

APPENDIX 2.2 List of foods kea in both habitats were observed eating or that occurred in faecal samples. Key: *introduced species, †popular food (from observations, > 1% of feeding time), ‡commonly occurring (found in > 5% of faecal samples), #previously unknown to be eaten by kea in alpine locations, ~previously unknown to be eaten by kea in rainforest locations. Fl – Flower , Fr – Fruit, Le – Leaf/leaf bud, Ne – Nectar, Ro – Root, Se – Seed, St – Stem; A – Alpine, R – Rainforest; O – Observation, FS – Faecal Sample.

Species	**Spring (Sep, Oct, Nov)**	**Summer (Dec, Jan, Feb)**	**Autumn (Mar, Apr, May)**	**Winter (Jun, Jul, Aug)**	**Habitat**	**Source**
Achillea millefolium *	Le, St				A	O
Aciphylla spp. ‡ ~	Fr		Fr		A + R	FS
Agrostis capillaris * #	Se, St				A	O
Anisotome aromatica †		Ro			A	O
Anisotome haastii #		St			A	O
Anthropogenic Food †	Various				A	O
Astelia spp.			Fr	Fr	A	FS
Celmisia discolour		St			A	O
Celmisia spectabilis (alive) †		St, Ro, Fl			A	O
Celmisia spectabilis (dead) †		Fl, St			A	O
Celmisia traversii #		Fl, St			A	O
Coprosma cheesemanii ‡	Fr	Fr, Le		Fr	A	O + FS
Coprosma depressa		Fr		Fr	A	FS
Coprosma fowerakeri		Fr		Fr	A	O + FS
Coprosma intertexta †‡ ~		Fr	Fr	Fr	A + R	O + FS
Coprosma perpusilla †		Fr		Fr	A	O + FS
Coprosma pseudocuneata ‡			Fr	Fr	A	FS
Coprosma serrulata			Fr		A	FS
Coriaria angustissima † #	Fl, Le, St	Fr			A	O + FS
Coriaria sarmentosa †‡	Fl	Fr	Fr		A	O + FS

Crepis capillaris * #	Le				A	O
Dacrydium cupressinum		Fr			R	FS
Dracophyllum spp.~	Fr			Fr	R	FS
Fuscospora cliffortioides †	Fl	Le		Le	A	O
Gaultheria crassa #	Fl				A	O
Gaultheria depressa †‡ ~	Fr	Fr	Fr	Fr	A + R	O + FS
Gingidia montana	Ro	Ro			A	O
Grasses (Unidentified)	Se				A	O
Hypochaeris radicata *† #	Fl, Le, Ro, St				A	O
Introduced herb (unidentified) *†	Le				A	O
Invertebrate (unidentified) †	Invert				A	O
Lepidothamnus laxifolius # ~	Fr		Fr	Fr	A	FS
Leucogenes grandiceps #		Fl			A	O
Lotus pedunculatus * #	St		St		A	O
Luzula spp.	Se		Se		A	O
Muehlenbeckia axillaris †		Fl, Fr			A	O
Mycelis muralis *	Fl, Le				A	O
Nertera spp. #	Fr	Fr		Fr	A + R	FS
Pentachondra pumila		Fr			A	O
Phormium cookianum subsp. *cookianum*		Ne			A	O
Phyllocladus alpinus ‡ ~	Fr	Fr	Fr	Fr	A + R	FS
Pilosella officinarum† #	Le, Ro	Fl, St			A	O
Podocarpus nivalis †‡ ~		Fr, Le		Fr	A + R	O + FS
Prionoplus reticularis (Huhu beetle grub) ~	Invert				R	O
Pseudopanax spp.	Fr		Fr		A + R	FS
Rubus spp. #	Fr				R	FS
Rumex acetosella * #	Le				A	O
Senecio spp.	Fl, St				A	O

Trichosurus vulpecula (Common brushtail possum) † #	Decayed tail		A	O
Uncinia spp.		Se	R	FS
Wahlenbergia spp. #	Fl		A	O
Weinmannia racemosa #		Fr	A	FS

CHAPTER THREE

Simple ways to calculate stable isotope discrimination factors and convert between tissue types.

Taking a blood sample for stable carbon and nitrogen stable isotope analysis from an almost grown kea *Nestor notabilis* nestling (*Photo: Kea Conservation Trust*)

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Abstract

Traditional methods to determine stable isotope discrimination factors (Δ) between an animal's diet and tissue(s) are costly and time-consuming. Consequently, data are only available for relatively few species and are completely absent from some orders, including parrots (Order: psittaciformes). We present simple and cost-effective methodologies for establishing discrimination factors and converting between tissue types. We investigated $\Delta^{13}C_{diet\text{-}feather}$ and $\Delta^{15}N_{diet\text{-}feather}$ values for the kea parrot *Nestor notabilis* by comparing the isotope values from feathers of a population held under their regular conditions at a local zoo, with the $\delta^{13}C$ and $\delta^{15}N$ values from their weekly diet of >30 food items. We mathematically controlled for dietary elemental concentration, and the potential impacts of metabolic routing, the exclusive consumption of preferred foods, and the large-scale consumption of self-sourced plants and invertebrates; resulting in $\Delta^{13}C_{diet\text{-}feather}$ = 4.0‰ ± 0.0 and $\Delta^{15}N_{diet\text{-}feather}$ = 3.1‰ ± 0.2. We also determined regression equations for predicting feather $\delta^{13}C$ and $\delta^{15}N$ values from whole blood values by sampling simultaneously grown feathers and blood from wild kea nestlings. These are the first feather-blood discrimination equations determined for terrestrial birds in the wild. Our $\delta^{13}C$ feather-blood discrimination equation was similar to an equation developed for use across marine birds; however, the $\delta^{15}N$ feather-blood discrimination equation for marine birds consistently underestimated kea feather $\delta^{15}N$ values. These methodologies, while developed for use in birds, can easily be applied to other animal classes given the appropriate selection of tissues.

Introduction

Natural abundance stable isotope ratios, in particular those of carbon (^{13}C / ^{12}C, represented by $\delta^{13}C$) and nitrogen (^{15}N / ^{14}N, or $\delta^{15}N$), have a broad range of ecological applications. The $\delta^{13}C$ and $\delta^{15}N$ values obtained from an animal's tissue reflect those of its diet, and can be used to investigate dietary shifts (Phillips & Eldridge 2006), the diet of difficult-to-track species (Borrell et al. 2013), niche width (Layman et al. 2007), food web structure (Hussey et al. 2014), and migration routes (Hobson 1999).

Sampling tissues with differing metabolic turnover rates enables researchers to examine diet over days (plasma, liver), weeks/months (blood, muscle) or years (bone collagen; Dalerum & Angerbjörn 2005). Tissues which can be sampled non-destructively (e.g., blood, fur and muscle), can be sampled repeatedly, and are suitable when investigating rare species. For avian ecologists, blood and feathers are increasingly becoming the "tissues of choice" (Bearhop et al. 2002). Blood reflects a bird's diet over the previous two to six weeks depending on the species (Hobson & Clarke 1993; Hobson & Bairlein 2003), whereas feather is made of keratin which remains metabolically inert after synthesis, and reflects diet at the time of feather growth, perhaps months earlier (Hobson & Clarke 1992). This temporal disjunct makes simultaneous sampling of blood and feathers particularly useful, as it allows investigations of diet over two separate time periods. For example, dual-tissue sampling from a nesting female allows her breeding (blood) and moulting (feather) diets to be compared.

When food is incorporated into animal tissue, light and heavy isotopes react differently due to differences in mass and bond strength. Nitrogen atom bonds are formed and broken during amino acid synthesis which leaves the product amino acids enriched in ^{15}N (Chikaraishi et al. 2009). Thus, fewer ^{15}N atoms are excreted resulting in ^{15}N enrichment in consumer tissues across progressively higher trophic levels of a food-chain (DeNiro & Epstein 1981). The difference between the dietary isotope value and the resulting tissue value is referred to as the discrimination factor (denoted by Δ). Discrimination factors vary both between species and within, depending on factors such as tissue type (Cherel et al. 2014); dietary protein quality (Robbins et al., 2010); and the consumer's growth rate (Martínez del Rio et al. 2005). Dietary protein may also be preferentially routed into the synthesis of

proteinaceous tissues, such as feathers and blood (Voigt et al. 2008), disproportionately affecting $\Delta^{13}C$ and $\Delta^{15}N$ values. Some metabolic routing of dietary protein into tissue synthesis certainly occurs because essential amino acids can only be obtained from dietary protein. Moreover, because it is metabolically more efficient to incorporate non-essential dietary amino acids directly from the diet than to create new ones, additional routing is highly likely. Yet few studies attempting to establish accurate discrimination factors take metabolic routing into account (but see Podlesak & McWilliams 2006; Kurle et al. 2014).

Discrimination factors are typically determined by 'constant diet experiments' (e.g., Bearhop et al. 2002; Kurle et al. 2013) where an animal is fed a controlled diet of very few (usually no more than two or three) food items for a period of months. However, these experiments are costly, time-consuming and may not be ethical, depending on the species concerned and their natural feeding behaviour. Consequently, discrimination factors have been established for relatively few species. Values from a related species or generic values from reviews (e.g., $\Delta^{15}N$ = 3.4‰ per trophic level, $\Delta^{13}C$ = 0‰ to 1‰ per trophic level; Post 2002) are commonly substituted. However, even within birds, $\Delta^{13}C$ and $\Delta^{15}N$ values range widely (-1.5‰ to 4.3‰ per trophic level; and 0.2‰ to 5.6‰ per trophic level, respectively; Hobson & Clarke 1992; Pearson et al. 2003; Kempster et al. 2007; Federer et al. 2012), making this practice questionable (Caut et al. 2009). Certainly, because Δ is tissue dependent (Wolf et al. 2009), it is inappropriate to apply generic values to all tissues equally. The accuracy of discrimination factors is of central importance to isotope ecologists as they are used to calculate trophic position and relationships (Post 2002); and also form an integral part of the equations used in stable isotope mixing models where a discrepancy of just 1‰ can greatly alter the estimated contributions of dietary sources (Bond & Diamond 2011). Parrots (Order: Psittaciformes) are among the orders for which discrimination factors have not yet been established. Here, we devised a methodology to determine stable carbon and nitrogen isotope discrimination factors for kea *Nestor notabilis*, an endangered parrot for which constant diet experiments are inappropriate due to their extremely varied natural diet and high captive enrichment requirements (Gyula K. Gajdon, pers. comm.).

An animal's various tissues differ in their diet-to-tissue discrimination factors (DeNiro & Epstein 1981). Therefore, $\delta^{13}C$ and $\delta^{15}N$ values from blood and feathers can only be directly compared if these differences in discrimination factors are accounted for (Dalerum & Angerbjörn 2005). Traditionally, tissue differences have been established in constant diet experiments at the same time as diet-to-tissue discrimination factors. However, it is also possible to establish tissue-specific differences in discrimination by sampling tissues synthesised at the same time. For example, blood and currently growing feathers, which can easily be sampled from either moulting birds, or nestlings attaining their fledgling plumage, will have been produced over a similar time scale (Quillfeldt et al. 2008). Therefore the difference in discrimination between feathers and blood can be investigated and species-specific regression equations calculated to allow direct comparisons between tissues. To date, these methods have only been applied by marine bird ecologists, who have recorded differences in $\Delta^{13}C$ and $\Delta^{15}N$ values between blood and feather from >30 species (see Cherel et al. 2014); whereas, for terrestrial birds, these differences have been entirely established during constant diet experiments and are available for a mere six species (Hobson & Clarke 1992; Hobson & Bairlein 2003; Pearson et al. 2003; Kempster et al. 2007; Kurle et al. 2013). Here we present the first data on the differences between $\Delta_{\text{diet-feather}}$ and $\Delta_{\text{diet-blood}}$ values for a parrot, and the first use of this methodology for terrestrial birds. We also investigate the utility of regression equations derived for use in predominantly carnivorous, marine bird taxa (Cherel et al. 2014) to convert between the stable isotope values from blood and feathers from an omnivorous parrot.

Methods

Determination of discrimination factors for feathers

We obtained 18 kea feathers for carbon and nitrogen stable isotope analysis from a population held at an open-range zoo, Orana Wildlife Park, Christchurch, New Zealand. The kea is an endangered parrot (Robertson et al. 2013), that inhabits the mountains and rainforests of New Zealand's South Island. They are omnivorous, with a diverse, predominantly plant-based diet (Greer et al. 2015). Feathers were collected during a one week period in February, 2013 from an aviary

housing five adults. The zookeepers provided a diet sheet detailing the types and quantities of foods fed to the birds per day, along with a sample of listed food items. However, initially an incomplete list was supplied and, although subsequently updated, we did not receive samples of all the food items fed to the kea. From a total of 33 food items, 9 were missing so we substituted available literature values, covering 98% of the keas' diet. The raw data for each food item are presented in Appendix 3.1. Listed food quantities were mostly in grams (wet weights) but where a subjective quantity was used (e.g., one large banana), an example of the item was weighed to the nearest gram. Wet:dry weights were obtained by drying a sample of each food to constant mass in a 60°C oven. We used the dry weights of food fed per day in all calculations. Zookeepers were consulted to establish kea food preferences and each food was marked as 'preferred' or 'non-preferred'. Zoo-keeping protocols require all types of food to be spread throughout the enclosure to prevent monopolisation of resources by dominant individuals. All birds were held on this diet for more than six months prior to sample collection, during which time a moult had occurred.

Isotope values are reported in parts per thousand (‰) and standard definitions for isotopic compositions (δ^{13}C and δ^{15}N) are used throughout (see Coplen 2011).

Δ^{13}C and Δ^{15}N values were calculated as follows:

Firstly, the proportion (p) of each food item (m) in the total daily diet was calculated as

$$p_m = \frac{\text{dry weight}_m}{\text{dry weight}_{total}} \qquad \text{(Equation 1)}$$

However, foods can vary widely in their per cent carbon or nitrogen concentrations (%C or %N, respectively). Unless elemental concentration is accounted for, foods with high %C or %N can exert an undue influence on the calculated discrimination factor (Phillips & Koch 2002). Therefore, we incorporated %C and %N into our equations to account for the differing elemental concentrations of every food item (following Martínez del Rio & Wolf 2005). The contribution (E) made to the total pool of element *X* by each food item was calculated as

$$\mathrm{E}X_{\mathrm{m}} = \frac{\%X_{\mathrm{m}}}{\%X_{\mathrm{total}}} \quad \text{(Equation 2)}$$

Then dietary δ^{13}C and δ^{15}N values (weighed by elemental concentration) were calculated using mass balance as

$$\delta^{y}X_{\mathrm{diet}} = \sum_{\mathrm{m}=1}^{\mathrm{n}} \delta^{y}X_{\mathrm{m}} \left(\frac{\mathrm{p}_{\mathrm{m}}\mathrm{E}X_{\mathrm{m}}}{\sum_{\mathrm{m}=1}^{\mathrm{n}} (\mathrm{p}_{\mathrm{m}}\mathrm{E}X_{\mathrm{m}})}\right) \quad \text{(Equation 3)}$$

where, n is the number of food items.

Finally, discrimination factors were calculated as

$$\Delta^{y}X = \text{mean } \delta^{y}X_{\mathrm{feather}} - \delta^{y}X_{\mathrm{diet}} \quad \text{(Equation 4)}$$

In order to investigate the potential impact of metabolic routing we also calculated stable carbon and nitrogen discrimination factors assuming 100% metabolic routing of protein to tissue. The contribution of each food item to the pool of total dietary protein (O) was calculated as:

$$\mathrm{O}_{\mathrm{m}} = \frac{\%\mathrm{O}_{\mathrm{m}}}{\%\mathrm{O}_{\mathrm{total}}} \quad \text{(Equation 5)}$$

New dietary δ^{13}C and δ^{15}N values which assumed complete routing of dietary protein to tissue synthesis were then calculated using mass balance as:

$$\delta^{y}X_{\mathrm{routing}} = \sum_{\mathrm{m}=1}^{\mathrm{n}} \delta^{y}X_{\mathrm{m}} \left(\frac{\mathrm{p}_{\mathrm{m}}\mathrm{E}X_{\mathrm{m}}\mathrm{O}_{\mathrm{m}}}{\sum_{\mathrm{m}=1}^{\mathrm{n}} (\mathrm{p}_{\mathrm{m}}EX_{\mathrm{m}}\mathrm{O}_{\mathrm{m}})}\right) \quad \text{(Equation 6)}$$

To account for any potential impact from kea ingesting additional plants and animals from within their enclosure, we also modelled a scenario where kea incorporated 10% alpine plants and 10% invertebrates into their diet (plants: n = 84; δ^{13}C = -28.92‰ ± 2.39 and δ^{15}N = -4.56‰ ± 3.68 and invertebrates: n = 19; δ^{13}C = -25.76‰ ± 3.16; δ^{15}N = 2.13‰ ± 3.10; raw data available in Appendix 3.2). In addition, we modelled kea eating only the more preferred dietary items, because our data were based on the quantity of foods provisioned as opposed to ingested. These two conditions represent extreme limits to the discrimination factors as consultations with zookeepers indicated that kea devoted very little time to sourcing their own food within the enclosure and that most food was eaten at each feed. Final discrimination factors were calculated using the no routing and full routing of protein conditions only. Means are reported ± standard deviations throughout. All calculations were carried out in Microsoft Excel 2010 (Microsoft Corporation, Washington, USA).

Differences between the $\delta^{13}C$ and $\delta^{15}N$ values from kea blood and feathers

Blood and feather samples were collected simultaneously from 19 wild kea nestlings (seven females and 12 males from 14 different nests) in the final stages of fledgling feather growth, in Oct – Dec, 2011 and Oct, 2012. All nestlings were sourced from nests located in Westland National Park on the South Island of New Zealand (43°13'S, 170°10'E). Kea incubate their eggs for 22-24 days and chicks remain in the nest for a further three months before fledging (Kemp 2013). Egg yolk provides a source of nutrients to birds for the first few days after hatching (Moran 2007) and could influence the δ^{13}C and δ^{15}N values of tissues synthesised during that period. However, as the turnover of blood for medium-sized birds is estimated at c. six weeks (American crow *Corvus bruchyrhynchos*; Hobson & Clarke 1993) and primary feather growth begins only three to four weeks prior to fledging (Renton 2002), yolk δ^{13}C and δ^{15}N values are an unlikely source of bias here.

The top 2 cm of a 1st primary (P1) and 10th primary (P10) feather of each bird were clipped and stored in a sealed plastic bag until processing. Approximately 0.3 cc of blood was drawn from the brachial wing vein of each bird and a few drops were stored in 70% ethanol for later analysis. Storage in 70% ethanol does not affect tissue δ^{15}N values and is thought to have a negligible effect on δ^{13}C

values (Halley et al. 2008); however, Bugoni et al. (2008) found a significant effect on the δ^{13}C value of blood stored in absolute ethanol. To ensure that no preservation artefacts were introduced by the long-term storage of blood in 70% ethanol, we also took blood samples from nine fledgling kea outside Arthur's Pass Village (42°54'S, 171°34'E) and split each sample between 70% ethanol and an empty vial which was frozen at -20°C for up to one week and then stored at -80°C until analysis (always ≤9 months).

We averaged the δ^{13}C and δ^{15}N values from the P1 and P10 feathers to give a single feather for each nestling. Pairwise t-tests were then used to determine if blood and feather δ^{13}C and δ^{15}N values differed from one another and if the method of blood sample storage affected stable isotope values. We used linear regressions to test if δ^{13}C or δ^{15}N values from blood could be reliably used to predict feather values. Tissue $\Delta^{y}X$ value differences were calculated by $\Delta^{y}X_{\text{feather}} - \Delta^{y}X_{\text{blood}}$. Means are reported ± 1 standard deviation throughout. All analyses were conducted using SPSS Statistics 21 (I*BM* Corporation, NY 10589, USA).

Exclusions

We lost data from both of one nestling's feather samples and from one frozen blood sample due to a mass spectrometer technical malfunction so we excluded all data from these birds from statistical analyses. One nestling (J2) had a higher $\delta^{15}N_{blood}$ than $\delta^{15}N_{feather}$ value. This is the opposite pattern to that found in all constant diet studies (except Hobson & Clarke 1992), therefore we conclude that J2's pattern is anomalous and indicates a change in diet at a crucial time. The slower metabolic turnover of blood (c. six weeks) than fledgling feather growth (c. three weeks) means that a substantial shift from higher to lower trophic level in the diet fed to J2 between the start of blood synthesis and feather growth could result in its feathers having a lower δ^{15}N value than its blood. J2 also had the largest tissue difference in δ^{13}C (3.3‰), further suggesting a change in diet. Consequently, in order to increase the applicability of our regression equations, we excluded data from J2. However, this case highlights the need for caution when applying this methodology, particularly in opportunistic species which may take advantage of temporary bonanzas (e.g., increases

in invertebrate numbers or occasional vertebrate carcasses) to supplement their diet and that of their young.

Sample Preparation

Feathers soaked in 2:1 chloroform:methanol solution for 24 h were rinsed twice in fresh solution and air dried in a fume cupboard for 48 h. The top 1 cm of the inner feather vane was removed and finely clipped. Blood and food samples were dried to constant mass in a 60°C oven and ground to talc powder consistency in a ball mill (Retsch MM2000, Hahn, Germany). Samples were homogenised and weighed out on an ultra-microbalance (accurate to 0.1 µg; Mettler-Toledo UMX2, Greifensee, Switzerland) to 0.5 - 0.7 mg for kea tissue and 3.5 - 5 mg for food and inserted into individual 4 x 6 mm tin capsules for mass spectrometer analysis. In three cases, the food type was a mixture (e.g., insectivore mix or seed mix), so we prepared repeated samples. We did not extract lipids because recent work suggests that carbohydrates and lipids are also used for tissue synthesis and thus a portion of lipid in the diet is desirable to provide the full suite of dietary macro-molecules (Newsome et al. 2014). Data on the protein content of each food item were sourced from the US National Nutrient Database (2014).

Mass Spectrometry

Samples were analysed for $\delta^{13}C$, $\delta^{15}N$, %C and %N using a Costech Elemental Combustion System (ECS) 4010 (Costech Analytical Technologies, California, USA) connected to a Delta V Plus Isotope Ratio Mass Spectrometer (IRMS; Thermo Fischer Scientific, Massachusetts, USA) via a Finnigan Conflo III (Thermo Fischer Scientific, Massachusetts, USA). All samples were loaded into a ZeroBlank autosampler with an isolation valve (Costech Analytical Technologies, California, USA) and were individually combusted at 1050°C under a continuous flow (c. 110 ml/min) of ultra-high purity helium (>99.999%). Molecular N_2 and CO_2 were separated using a gas chromatography column housed in the ECS and held at a static 45°C. IRMS fast peak jumps were calibrated at least daily, and reference gas linearity tests were performed at the start of every other analytical sequence. Internal

precision (the standard deviation across ten reference gas analyses i.e., zero-enrichment test) was determined prior to every analytical sequence and was always <±0.06‰ for both $\delta^{13}C$ and $\delta^{15}N$. Data were normalised to Vienna PeeDee Belemnite for $\delta^{13}C$ and Air for $\delta^{15}N$ using a stretch-and-shift 2-point normalisation based on replicate analyses of certified reference materials within individual analytical sequences. External precision (the standard deviation of replicate analyses of certified reference materials and internal lab-check standards over the course of the study) was <±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Results

Determination of discrimination factors for feathers

The food items which comprised the diet of kea at the zoo varied widely in their $\delta^{13}C$ (mean = -24.83‰ ± 6.66; range = -33.20‰ to -11.15‰) and $\delta^{15}N$ values (mean = 3.97‰ ± 3.18; range = -1.19‰ to 15.07‰); their %N (mean = 1.09% ± 7.95; range = 0.17% to 9.40%); and their protein content (mean = 6.15% ± 10.20; range = 0.3% to 52%; Appendix 3.1). In contrast, the kea feathers collected from the enclosure varied little in either their $\delta^{13}C$ (mean = -23.28‰ ± 0.36; range = -23.75‰ to -22.5‰) or $\delta^{15}N$ (mean = 7.32‰ ± 0.45; range = 6.63‰ to 8.23‰) values. The raw data for these 18 feathers are available in Appendix 3.3.

The $\Delta^{13}C_{diet\text{-}feather}$ values were consistently ~4‰ regardless of the model conditions, ranging by only ≤0.3‰ (Table 1). The $\Delta^{15}N_{diet\text{-}feather}$ values varied somewhat more widely in the extreme conditions (2.78‰ to 4.00‰; Table 1). However, when we based our calculations on the full diet provided to kea there was a variation of just 0.4‰ (No routing: $\Delta^{15}N_{diet\text{-}feather}$ = 2.90‰; complete routing: $\Delta^{15}N_{diet\text{-}feather}$ = 3.30‰). We suggest that future stable isotope studies on kea adopt $\Delta^{13}C_{diet\text{-}feather}$ = 4.00‰ ± 0.03; $\Delta^{15}N_{diet\text{-}feather}$ = 3.10‰ ± 0.20.

Table 1: $\delta^{13}C_{diet}$ and $\delta^{15}N_{diet}$, and $\Delta^{13}C_{diet\text{-}feather}$ and $\Delta^{15}N_{diet\text{-}feather}$ values calculated for captive kea for each model condition: no routing of protein, complete routing of protein, the inclusion of 10% plant and 10% invertebrates in their diet, and the ingestion of preferred foods only. All models take the elemental concentration of food items into account. $\Delta^{13}C$ and $\Delta^{15}N$ values were calculated using $\delta^{13}C_{feather}$ = -23.28‰ and $\delta^{15}N_{feather}$ = 7.32‰.

	Diet δ^{13}C (‰)	**Diet δ^{15}N (‰)**	**Δ^{13}C (‰)**	**Δ^{15}N (‰)**
No routing of protein	-27.25	4.42	3.97	2.90
Complete routing of protein	-27.30	4.01	4.02	3.30
10% plant, 10% invertebrate	-27.27	3.37	3.98	4.00
Preferred foods only	-27.00	4.53	3.72	2.78

Table 2. Mean, standard deviation (SD) and range of δ^{13}C, δ^{15}N and C:N values for 1st Primary (P1) and 10th Primary (P10) feathers, and blood sampled simultaneously from wild-caught kea nestlings (n = 18).

	δ^{13}C (‰)		**δ^{15}N (‰)**		**C:N**	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
P1 Feather	-21.03 ± 2.04	-26.48 to -19.19	2.88 ± 1.75	0.84 to 7.74	3.22 ± 0.08	3.07 to 3.36
P10 Feather	-21.14 ± 1.94	-26.14 to -19.22	2.96 ± 1.67	1.25 to 7.51	3.30 ± 0.10	3.09 to 3.52
Blood	-22.97 ± 1.93	-27.95 to -20.62	2.11 ± 1.87	0.30 to 7.03	3.36 ± 0.05	3.24 to 3.45
Feather*	-21.08 ± 1.99	-26.31 to -19.31	2.92 ± 1.71	1.04 to 7.63	3.26 ± 0.07	3.08 to 3.39

*denotes a single value for feather obtained by averaging the P1 and P10 values for each nestling.

Differences between the $\delta^{13}C$ and $\delta^{15}N$ values from kea blood and feathers

Across kea nestlings, the stable carbon and nitrogen isotopic compositions were widely disparate, ranging by c. 7‰ for δ^{13}C and δ^{15}N within-tissue values (Table 2), indicating different dietary sources. Within individuals, feathers were significantly higher than blood for both their stable carbon ($\delta^{13}C_{feather}$ = -21.08‰ ± 1.99; $\delta^{13}C_{blood}$ = -22.97 ± 1.93; t_{17} = 9.90, p <0.001) and nitrogen ($\delta^{15}N_{feather}$ = 2.92‰ ± 1.71; $\delta^{15}N_{blood}$ = 2.11‰ ± 1.87; t_{17} = 7.62, p <0.001) isotope compositions. Feathers and blood were also significantly different in their C:N ratios (t_{17} = 5.35, p <0.001; Table 2). Individual nestling blood and feather stable isotope values and C:N ratios are available in Appendix

3.4. There were no differences between frozen blood samples and those stored in 70% ethanol in their $\delta^{13}C$ (t_7 = 0.24, p = 0.816; mean difference = 0.02‰ ± 0.19) or $\delta^{15}N$ values (t_7 = -2.18, p = 0.066; mean difference = -0.44‰ ± 0.58).

Regression equations

We calculated linear regression equations to predict $\delta^{13}C_{feather}$ from $\delta^{13}C_{blood}$ values; and $\delta^{15}N_{feather}$ from $\delta^{15}N_{blood}$ values. The values from feathers and blood were positively related and both regression equations explain over 90% of the variance ($\delta^{13}C_{feather}$ = 0.973 (±0.289) $\delta^{13}C_{blood}$ + 1.133 (±6.613), R^2 = 0.93, $F_{1,15}$ = 61.69, p <0.001; $\delta^{15}N_{feather}$ = 0.920 (±0.186) $\delta^{15}N_{blood}$ + 1.041 (±0.515), R^2 = 0.97, $F_{1,15}$ = 417.84, p <0.001; Fig. 1). The difference between actual and predicted feather values for $\delta^{13}C$ was small (<0.5‰) in 13 cases, moderate (<1.0‰) in three cases, with one large (>1.0%) difference. For $\delta^{15}N$ there was a small difference in 15 cases, and a moderate difference in two cases. The slope of our regression equation for predicting kea $\delta^{13}C_{feather}$ from $\delta^{13}C_{blood}$ values was not significantly different from that of the corresponding equation calculated for use across marine birds (t_{48} = 0.01, p = 0.989; $\delta^{13}C_{marine\text{-}bird\ feather}$ = 0.972 (±0.020) $\delta^{13}C_{marine\text{-}bird\ blood}$ + 0.962 (±0.414); Cherel et al. 2014; Fig. 1); however the slopes of the regression equations derived to predict kea and marine bird $\delta^{15}N_{feather}$ from $\delta^{15}N_{blood}$ values were significantly different (t_{48} = 2.05, p = 0.046; $\delta^{15}N_{marine\text{-}bird\ feather}$ = 1.014 (±0.056) $\delta^{15}N_{marine\text{-}bird\ blood}$ + 0.447 (±0.665); Cherel et al. 2014; Fig. 1).

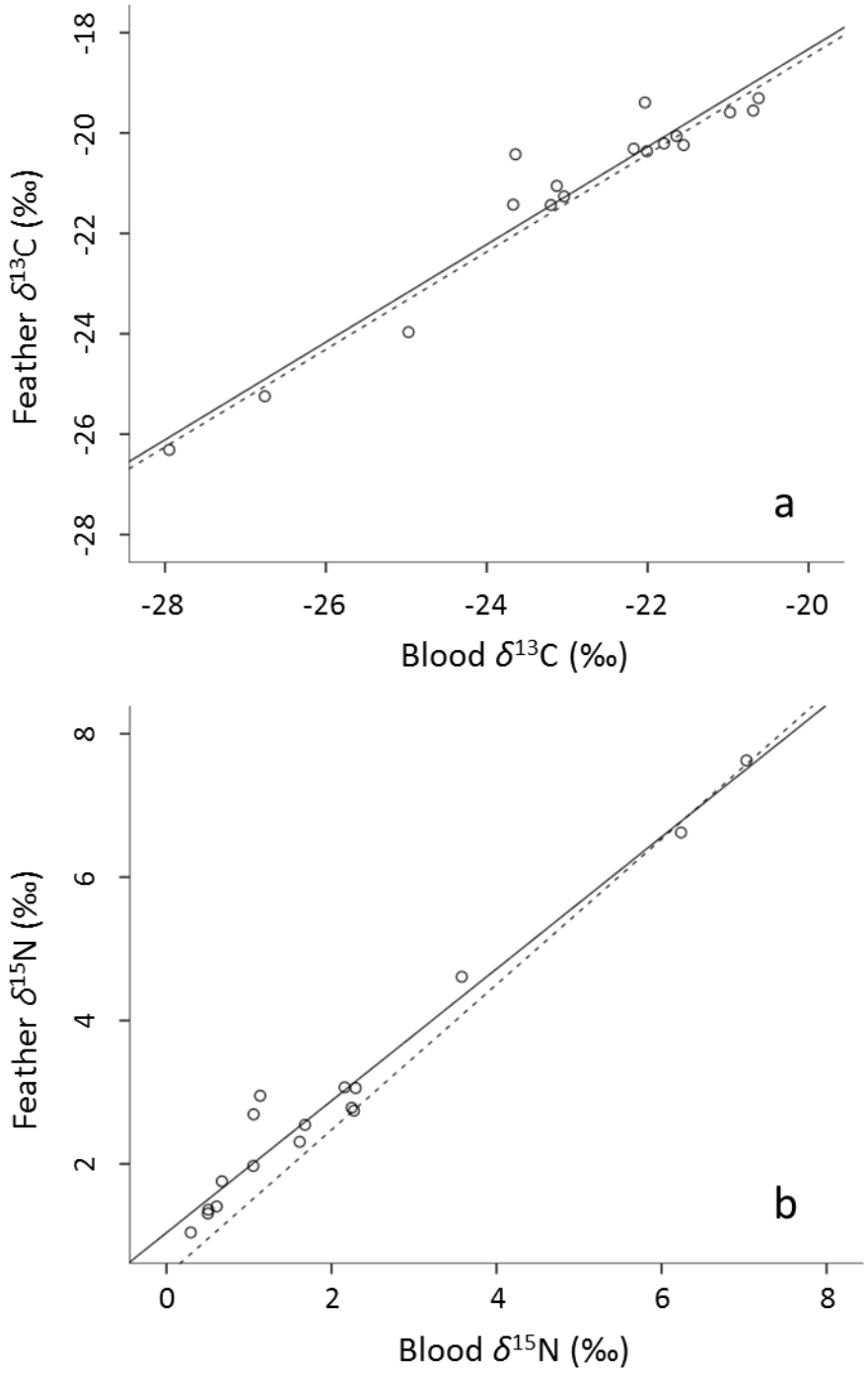

Figure 1. The relationships between the δ^{13}C (a) and δ^{15}N (b) values from parrot blood and feathers. Open circles represent individual kea nestlings. Solid lines indicate the regression equations calculated here; dashed lines indicate the corresponding equations derived for marine birds (Cherel et al. 2014).

Discussion

Here we report the first diet-to-tissue discrimination factors for a parrot; and the first equations for predicting feather carbon and nitrogen stable isotope values from blood values to be determined using wild terrestrial birds. When studying species or tissues for which discrimination factors are as yet unknown, the application of these methodologies has the potential to greatly improve the accuracy of dietary mixing models outputs and trophic levels comparisons.

Determination of discrimination factors for feathers

The $\Delta^{13}C$ values we obtained for kea feathers were highly consistent (c. 4‰), varying ≤0.3‰ even under the most extreme model conditions. A stable carbon isotope discrimination factor of 4‰ falls within the higher range of reported values (Caut et al. 2009; Cherel et al. 2014), which can, in part, be explained by the choice of tissue. Feathers typically have more positive $\delta^{13}C$ and $\delta^{15}N$ values than blood or muscle (Caut et al. 2009) and converting $\delta^{13}C_{feather}$ to $\delta^{13}C_{blood}$ values using the kea-specific regression equation calculated here reduces $\Delta^{13}C$ to 2.96‰. However, this value still exceeds the commonly assumed 0 - 1‰ value (Post 2002), further emphasising the need for species-specific discrimination factors to be determined before incorporating them into highly-sensitive Bayesian mixing models (Bond & Diamond 2011). $\Delta^{15}N_{diet\text{-}feather}$ values differed by 1.2‰, the highest and lowest values being obtained when we assumed that kea took 20% of their diet from plants and animals within their enclosure or ate only preferred foods. However, both of these model conditions represent extreme limits and $\Delta^{15}N_{diet\text{-}feather}$ values from the complete diet fed to kea varied by only 0.4‰. The stable nitrogen isotope discrimination factor of 3.1‰ obtained here for kea is close to the frequently adopted value of 3.4‰ per trophic level (Post 2002).

The unusually high stable carbon isotope discrimination factor reported here may be the consequence of the kea's extremely high basal metabolic rate (BMR; 37% higher than expected from their body mass; McNab & Salisbury 1995). Given that respiration and BMR are positively related (Mansell & MacDonald 1990), this suggests that kea also have a higher rate of CO_2 production. The $\delta^{13}C$ value of an animal's exhaled breath is typically lower than its dietary $\delta^{13}C$ value; therefore

respiration is a likely cause of diet-to-tissue ^{13}C enrichment and a physiologically similar animal which respires more should have an increased stable carbon isotope discrimination factor (DeNiro & Epstein 1978). Published $\delta^{13}C_{feather}$ values are only available for two other parrot species (red-crowned parakeet *Cyanoramphus novaezelandiae,* Hawke & Holdaway 2009; cape parrot *Poicephalus robustus*; Symes & Woodborne 2009). In both studies, the parrots were found to have unexpectedly positive $\delta^{13}C_{feather}$ values when compared to the surrounding vegetation. It is noteworthy that the BMR of the red-crowned parakeet is also elevated, at 112% of the expected rate (McNab & Salisbury 1995) and it has been suggested that parrots generally may have higher BMRs than other species (McNab 2012), although Montgomery et al. (2012) found no evidence of this when comparing parrots and quails (Order: Galliformes). Future research targeting parrot $\Delta^{13}C_{diet\text{-}tissue}$ values, and their comparison to discrimination factors determined for other birds across a range of BMRs and metabolic pathways is urgently needed.

Differences between the $\delta^{13}C$ and $\delta^{15}N$ values from kea blood and feathers

$\delta^{13}C$ and $\delta^{15}N$ values from kea feathers were significantly higher than those from their blood. With the exception of $\delta^{15}N$ for one nestling, these differences were highly consistent, allowing the derivation of regression equations to confidently predict feather $\delta^{13}C$ and $\delta^{15}N$ values from blood values within kea.

Our equation for $\delta^{13}C$ was remarkably similar to one derived for use across marine bird taxa (Cherel et al. 2014), suggesting that $\delta^{13}C$ differences between feathers and blood may be highly consistent across bird taxa in general. The differences between $\delta^{13}C$ values from blood and feathers are likely the result of the tissues' differing biochemical compositions (Wolf et al. 2009). Lipid $\delta^{13}C$ values are lower than protein values and lipids are present in greater quantities in kea blood than feathers (verified here by blood's significantly higher C:N values – an inversely-related, proxy for lipid content; Post et al. 2007). Different amino acids also vary in their isotopic ratios so the differing amino acid composition of these tissues could also contribute to these $\delta^{13}C$ differences (Wolf et al. 2009; Cherel et al. 2014), seemingly to the same degree in kea as for marine birds.

Applying the marine bird equation for predicting $\delta^{15}N_{feather}$ from $\delta^{15}N_{blood}$ values (Cherel et al. 2014) to kea tissues was less successful, resulting in mostly (76%) moderate or large errors and the consistent underestimation of $\delta^{15}N_{feather}$ values at lower levels of $\delta^{15}N_{blood}$ (<2‰). This may be due to a difference between kea and marine birds in their metabolic routing of protein. Feather production relies particularly heavily on cysteine, a semi-essential sulphur amino acid which can be only ingested directly from food or synthesised from the essential amino acid methionine (Murphy et al. 1990). Both cysteine and methionine are present in greater quantities in high protein foods. Omnivorous birds, such as kea, may not obtain enough cysteine or methionine for feather production from the plant portion of their diet, and may route most animal protein consumed into feather production rather than into the creation of other tissues. On the other hand, predominantly carnivorous birds i.e., marine birds, may not need to route animal protein specifically for feather production. Increases in dietary animal protein content has been experimentally demonstrated to be positively related to $\delta^{15}N$ (Pearson et al. 2003), so if more animal protein is being routed to feather production over blood in kea but not in marine birds, this would explain why the marine bird equation (Cherel et al. 2014) underestimated kea $\delta^{15}N_{feather}$ values. However, there is evidence to suggest that a contrary effect occurs, whereby increases in protein *quality* lead to a reduction in $\delta^{15}N$ and a lively debate surrounds this issue (e.g., Caut et al. 2009; Perga & Grey 2010; Kurle et al. 2013).

An alternative explanation is that because kea obtain less cysteine from their diet than marine birds do, they have to synthesise more of it from methionine. This would increase the number of metabolic transanimations, the isotopic fractionations involved would increase the $\delta^{15}N$ of the product amino acid (Chikaraishi et al. 2009), and consequently the $\delta^{15}N$ of the feathers, leading to the observed trend. We note, however, that little is known about the isotopic composition of cysteine and methionine in feathers, as they were not measured in the only published study on feather amino acid isotopes (Lorrain et al. 2009). We propose that the degree of difference between blood and feather $\delta^{15}N$ values may be more closely related to the bird's degree of carnivory than the ecosystem (marine or terrestrial) they inhabit.

Conclusion

These methodologies have considerable potential to improve upon the large-scale adoption of discrimination values from review articles, especially considering that these may be based on different tissues or sometimes vastly different animal taxa. Indeed, a database of discrimination factors for many species across orders may reveal trends that are not yet visible through the current paucity of data. Although we conducted this work using an avian species, these methodologies can be easily adapted to other classes, including mammals and reptiles. To determine $\Delta_{\text{diet-tissue}}$ values directly only the tissue selected must be changed. To convert between tissues, shaved mammalian hair can be used instead of feathers, with the length of hair selected matched to the amount grown during the synthesis of blood, or for reptiles, new scales grown in preparation for moulting can be sampled and compared with blood (depending on tissue turnover rates). We hope that researchers will apply these methodologies before relying on generic discrimination factors or making cross-tissue comparisons.

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APPENDIX 3.1 Raw data for isotopic ratios, weights, protein content and elemental concentrations of food items fed to kea. P denotes preferred food item, N non-preferred, U unknown; * literature values, # food items with no literature values available.

Name	$\delta^{13}C$	$\delta^{15}N$	Wet Weight (g)	Dry Weight (g)	Protein (%)	%C	%N	Preferred	References
Apple (*Malus domestica*)	-26.01	3.66	241	36.19	0.3	39.78	0.17	P	
Banana (*Musa* spp.)	-27.66	2.75	69	15.54	1.09	38.81	1.13	P	
Beetroot (*Beta vulgaris*)	-28.65	10.22	73	7.64	1.61	38.42	3.12	P	
Bread (White)	-30.87	2.02	16	9.51	10.66	43.47	2.44	P	
Brown sugar#	NA	NA	2	NA	NA	NA	NA	U	
Carrot (*Daucus carota*)	-32.62	2.77	176	16.88	0.93	41.09	1.31	N	
Date# (*Pheonix dactlifera*)	NA	NA	1	NA	NA	NA	NA	P	
Potato (*Solanum tuberosum*)	-33.20	2.30	23	4.48	1.71	42.27	1.22	N	
Pumpkin (*Cucurbita pepo*)	-30.04	5.04	14	1.29	1	44.08	1.58	N	
Corn (*Zea mays*)	-15.44	1.89	14	2.83	9.42	47.10	2.78	P	
Egg	-24.50	5.22	56	16.62	12.58	57.69	9.40	P	
Grape (*Vitis vinifera*)	-14.79	2.69	49	10.54	0.81	41.88	0.41	P	

Honey#	NA	NA	2	NA	NA	NA	NA	U	
Insectivore mix (Wombaroo)	-26.73	5.74	18	8.18	52	47.97	7.44	P	
Jam	-26.89	4.05	4	2.72	0.37	41.10	0.99	P	
Kiwifruit (*Actinidia spp.*)	-30.27	3.91	19	3.12	1.14	41.64	0.84	N	
Kumara* (*Ipomoea batatas*)	-27.13	2.35	166	38.87	1.37	41.52	0.61	P	Okada & Kumura (1986); Yonebayashi et al. (2014)
Melon (*Cucumis melo*)	-25.77	15.07	19	2.45	1.1	39.28	1.48	N	
Nut/fruit mix	-15.66	1.66	12	5.25	10	49.72	2.34	P	
Oats* (*Avena sativa*)	-26.70	3.00	18	13.22	13.15	44	2.27	N	Wolter et al. (1982); Yevdokimov et al. (2007); Choi et al. (2012); Mahroof (2013)
Orange (*Citrus sinensis*)	-27.06	5.89	40	6.09	0.8	41.45	1.25	N	
Parsnip (*Pastinaca sativa*)	-30.05	2.88	12	2.06	1.2	43.36	0.58	N	
Pasta*	-28.02	2.99	5	4.55	7.46	43.5	2.4	N	Pampana et al. (2007); Yousfi et al. (2013)
Peanut butter	Missing	0.63	7	7.13	22.21	Missing	4.68	P	

Pear (*Pyrus* spp.)	-29.53	3.56	144	23.23	0.36	41.18	0.30	P	
Peas (*Pisum sativum*) and corn	-25.12	2.61	24	5.02	7.32	44.77	3.80	P	
Pineapple (*Ananas comosus*)	-11.83	0.91	23	3.87	0.54	41.20	0.38	N	
Rice*	-28.50	2.40	6	2.28	2.69	43.5	0.45	N	Yoneyama et al. (2001); Gealy & Fischer (2010)
Seed mix (parrot; Top Flight)	-29.29	4.10	79	70.99	11.9	50.45	2.36	P	
Silverbeet (*Beta vulgaris*)	-27.30	3.60	67	7.73	2.2	38.42	3.12	P	
Tomato (*Solanum lycopersicum*)	-30.29	-1.19	5	0.27	0.88	46.58	2.73	N	
Vanilla complan#	NA	NA	1	NA	NA	NA	NA	U	
Yam* (*Dioscorea* spp.)	-28.09	2.74	42	5.80	1.53	41.3	0.92	P	Cornet et al. (2007); Kinaston et al. (2014)

Appendix 3.1 References

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APPENDIX 3.2 The stable carbon and nitrogen isotope ratios from wild sourced alpine plants and invertebrates, South Island, New Zealand.

Sample Type	$\delta^{13}C$	$\delta^{15}N$
Anisotome aromatica	-30.97	-8.59
Anisotome aromatica	-29.97	-9.63
Anisotome aromatica	-30.32	-9.13
Anisotome aromatica	-29.91	-8.88
Celmisia discolor	-28.94	-4.55
Celmisia discolor	-28.49	-6.83
Celmisia discolor	-28.00	-6.59
Celmisia discolor	-27.21	-7.03
Celmisia discolor	-26.70	-6.27
Celmisia spectabilis	-26.63	-9.95
Celmisia spectabilis	-26.26	-9.73
Celmisia spectabilis	-26.71	-7.02
Celmisia spectabilis	-27.14	-8.27
Celmisia spectabilis	-27.65	-8.35
Celmisia spectabilis	-27.36	-8.82
Chionochloa conspicua	-25.30	-5.91
Chionochloa spp	-25.49	-2.34
Coprosma cheesemanii	-30.96	-5.09
Coprosma cheesemanii	-30.74	-5.95
Coprosma cheesemanii	-30.31	-7.87
Coprosma cheesemanii	-28.74	-2.19
Coprosma cheesemanii	-27.93	-3.37
Coprosma depressa	-29.37	-8.71
Coprosma intertexta	-30.01	0.94

Coprosma intertexta	-29.74	-0.80
Coprosma intertexta	-29.47	-3.93
Coriaria angustissima	-26.58	-0.95
Coriaria plumosa	-27.79	-0.13
Coriaria plumosa	-27.48	-0.31
Coriaria sarmentosa	-27.48	-0.60
Coriaria sarmentosa	-26.77	-0.14
Coriaria sarmentosa	-26.98	-0.92
Coriaria sarmentosa	-26.31	-0.19
Coriaria sarmentosa	-26.44	-1.53
Coriaria sarmentosa	-24.68	0.06
Coriaria sarmentosa	-24.69	-0.77
Gaultheria crassa	-27.76	-3.16
Gaultheria depressa	-29.72	-3.92
Gaultheria depressa	-28.98	-7.09
Gaultheria depressa	-28.04	-0.91
Gentianella corymbifera	-30.43	-6.93
Gentianella corymbifera	-29.72	-2.80
Gentianella corymbifera	-28.95	-3.21
Gentianella corymbifera	-29.40	-2.65
Gentianella corymbifera	-30.56	-6.50
Gentianella corymbifera	-29.77	-9.70
Lepidothamnus laxifolius	-28.38	-7.84
Lepidothamnus laxifolius	-27.82	-6.90
Muehlenbeckia axillaris	-29.27	3.80
Muehlenbeckia axillaris	-28.92	0.92
Mycelis muralis	-31.80	-0.09

Mycelis muralis	-34.75	1.24
Mycelis muralis	-33.57	1.74
Fuscospora cliffortioides	-35.77	-4.33
Fuscospora cliffortioides	-30.99	-3.35
Fuscospora cliffortioides	-36.03	-3.39
Fuscospora cliffortioides	-32.90	-5.74
Fuscospora cliffortioides	-31.36	-6.82
Fuscospora cliffortioides	-28.85	-5.89
Fuscospora cliffortioides	-26.78	-2.37
Fuscospora cliffortioides	-32.56	-4.97
Pentachondra pumila	-30.33	-8.89
Pentachondra pumila	-28.63	-9.94
Pentachondra pumila	-27.29	-9.71
Pentachondra pumila	-26.90	-5.44
Phyllocladus alpinus	-31.96	-3.21
Phyllocladus alpinus	-31.27	-4.10
Phyllocladus alpinus	-31.05	-4.02
Phyllocladus alpinus	-27.05	-5.10
Phyllocladus alpinus	-31.25	-1.55
Podocarpus nivalis	-24.13	-5.02
Podocarpus nivalis	-27.07	-5.07
Podocarpus nivalis	-27.62	-4.98
Podocarpus nivalis	-26.84	-1.94
Podocarpus nivalis	-27.65	-1.81
Podocarpus nivalis	-26.91	-8.60
Podocarpus nivalis	-27.18	-8.34
Metrosideros umbellata	-25.27	9.22

Taraxacum spp.	-31.54	-6.50
Taraxacum spp.	-31.72	-6.65
Taraxacum spp.	-29.94	-5.84
Taraxacum spp.	-30.82	-8.29
Taraxacum spp.	-29.54	-8.53
Taraxacum spp.	-29.10	-7.39
Invertebrate	-29.13	-2.07
Invertebrate	-28.56	-2.40
Invertebrate	-26.41	-0.53
Invertebrate	-25.68	5.75
Invertebrate	-22.87	-0.06
Invertebrate	-25.19	-2.33
Invertebrate	-25.05	-3.80
Invertebrate	-26.95	2.80
Invertebrate	-32.35	3.39
Invertebrate	-23.27	2.54
Invertebrate	-23.22	2.09
Invertebrate	-25.82	3.54
Invertebrate	-22.67	6.76
Invertebrate	-31.37	3.39
Invertebrate	-21.45	4.87
Invertebrate	-29.82	5.53
Invertebrate	-25.56	6.16
Invertebrate	-22.10	1.46
Invertebrate	-21.94	3.36

APPENDIX 3.3 δ^{13}C, δ^{15}N and C:N values for 1st Primary (P1) and 10th Primary (P10) feathers and blood from all nestlings sampled.* denotes the only nestling with blood more enriched in δ^{15}N than feathers whose values were excluded from regression equations; # both P1 and P10 data were lost due to an auto-sampler malfunction, so this bird was excluded from all analyses.

Kea	P1 δ^{13}C (‰)	P1 δ^{15}N (‰)	P10 δ^{13}C (‰)	P10 δ^{15}N (‰)	Blood δ^{13}C (‰)	Blood δ^{15}N (‰)	P1 C:N	P10 C:N	Blood C:N
BooBoo	-19.59	2.92	-19.59	2.99	-20.98	1.14	3.21	3.27	3.32
Elvin	-20.83	3.17	-21.28	2.97	-23.13	2.16	3.22	3.33	3.45
Frankenstein#	N/A	N/A	N/A	N/A	-23.62	2.66	N/A	N/A	3.32
Hamburglar	-19.94	0.84	-20.47	1.25	-21.80	0.30	3.25	3.41	3.32
J2*	-19.19	2.76	-19.49	2.68	-23.67	3.01	3.24	3.31	3.39
Kaitlyn	-25.45	6.63	-25.04	6.61	-26.76	6.24	3.36	3.32	3.27
Kiekie	-20.15	1.29	-20.58	1.32	-22.01	0.50	3.13	3.33	3.39
Matai	-20.33	1.40	-20.29	1.41	-22.17	0.61	3.30	3.32	3.39
McNugget	-20.22	1.23	-19.90	1.49	-21.64	0.51	3.29	3.22	3.39
Miro	-19.50	2.53	-19.28	2.56	-22.03	1.68	3.07	3.09	3.36
Mordor	-21.35	2.68	-21.52	2.89	-23.20	2.24	3.26	3.52	3.45
Precious	-21.03	2.75	-21.49	3.36	-23.04	2.29	3.24	3.33	3.40
Q	-23.80	4.67	-24.13	4.55	-24.97	3.58	3.09	3.34	3.33
Salsa	-20.20	1.92	-20.65	2.02	-23.64	1.05	3.27	3.33	3.38
Sansa	-20.21	2.26	-20.26	2.36	-21.55	1.61	3.23	3.29	3.35
Swedish Chef	-26.48	7.74	-26.14	7.51	-27.95	7.03	3.28	3.21	3.41
Tea	-19.42	1.82	-19.68	1.69	-20.69	0.67	3.11	3.43	3.31
Temple	-21.40	2.72	-21.45	2.76	-23.67	2.27	3.15	3.15	3.36
Yogi	-19.39	2.54	-19.22	2.84	-20.62	1.06	3.23	3.22	3.24

APPENDIX 3.4 Stable carbon and nitrogen isotope values from feathers collected from an enclosure housing five adult kea in a local zoo in Christchurch, New Zealand.

	$\delta^{13}C$	$\delta^{15}N$
Feather 1	-23.74	6.88
Feather 2	-23.68	7.88
Feather 3	-23.24	7.11
Feather 4	-23.30	7.69
Feather 5	-23.62	8.23
Feather 6	-23.38	6.93
Feather 7	-23.69	6.83
Feather 8	-23.75	7.38
Feather 9	-23.11	8.03
Feather 10	-22.90	6.99
Feather 11	-23.38	7.44
Feather 12	-23.28	7.16
Feather 13	-22.87	7.43
Feather 14	-22.50	6.63
Feather 15	-23.49	6.93
Feather 16	-23.12	7.40
Feather 17	-22.77	7.01
Feather 18	-23.23	7.73

CHAPTER FOUR

Evidence for the ecological causation of bill dimorphism in an omnivorous parrot

Two immature kea, displaying male-biased sexual size dimorphism: female (top), male (*Photos: Amanda Greer*)

Abstract

Intraspecific specialisation is an important ecological variable linked to morphological and behavioural adaptations, which can have knock-on effects on a species' niche breadth and extinction risk. In general, specialisation increases with environmental variability e.g. additional habitat types; and generalisation increases with temporal variability. Kea *Nestor notabilis* live primarily along New Zealand's Southern Alps and have a highly varied, omnivorous diet thought to be an adaptation to the extreme temporal variability of their montane habitat. However, kea also inhabit temperate rainforest, suggesting within-species dietary specialisation and the possibility of habitat-specific, morphological adaptations. I ran Bayesian, stable isotope mixing models based on dual isotopes (carbon and nitrogen) to estimate the kea's specialisation on animal versus plant foods. I analysed both feather and blood tissue, finding similar results for each. I found a major difference between the diets of kea within the high-altitude and rainforest habitats, such that in high-altitude regions the kea's diet was primarily plant-based, whereas in the rainforest habitat it was mainly animal-based. Morphometric measurements (bill length, head length and weight) revealed that kea from the rainforest also had significantly longer bills and heads than those is high-altitude regions, but were of similar weight. In the rainforest, males ate significantly more animal foods than females, possibly due to the longer bill of male kea. My data suggest that ecological specialisation on invertebrates caused or maintains the sexual size dimorphism of the kea's bill.

Introduction

Historically considered a species characteristic, ecological specialisation (the adaptation of an organism to a subset of its possible environments; Poisot et al. 2011) can operate at all biological levels, including population, age/sex class, or even individual (Araújo et al. 2011). Intraspecific specialisation is an important ecological process that can affect behaviour, morphology and genetic variability and influence population and community dynamics (see Bolnick et al. 2011). Specialisation can even drive sympatric speciation, whereby a new species evolves despite no barriers to gene flow between populations, through phenotypic polymorphism and assortative mating (Darwin 1859; Maynard Smith 1966; Via 2001). More common, however, are cases where there is a continuous or discrete change in some phenotypic character under genetic influence, which is adaptive for the specialist. These changes increase intraspecific variability, which has knock-on effects such as decreasing intraspecific competition, vulnerability to environmental change, and extinction risk; and increasing species niche breadth, productivity and population growth rate (see Wennersten & Forsman 2012).

Categorisations of generalist or specialist are largely dependent on the ecological scale at which they are considered. At species level, the more varieties of habitat occupied, the greater the degree of intraspecific specialisation (Fox & Morrow 1981). This is due to restrictions on the availability of different resources within each habitat, leading to the development of local dietary, behavioural and/or morphological adaptations (Fox & Morrow 1981; Poisot et al. 2011). In any species within which specialisation occurs, the assumption that each member is ecologically interchangeable is misguided (Bolnick et al. 2003) and may lead to erroneous suppositions regarding the species' requirements. However, temporal variability in a species' environment promotes generalisation, because members must cope with changing environmental conditions during their lifetimes (Fox & Morrow 1981; Poisot et al. 2011). Therefore, it is entirely possible for a species to be composed of a number of populations highly specialised on their own habitats, but whose individual members can be considered generalists (Bolnick et al. 2003).

The endangered kea *Nestor notabilis*, the world's only true mountain parrot, inhabits New Zealand's Southern Alps, a habitat with wide temporal extremes in both weather conditions and food availability. As expected, kea are renowned for being opportunistic generalists (Diamond & Bond 1999), having an extremely varied diet of over 100 plant and animal species (Chapter 2/Greer et al. 2015). Although their core diet is frugivorous, kea routinely eat leaves, roots, nectar, seeds and invertebrates (Clarke 1970; Young et al. 2012; Chapter 2/Greer et al. 2015) and will gorge themselves at the remains of a kill left by hunters (Schwing 2010). However, although kea are strongly associated with the mountains, some also live at sea level in a vastly different habitat: broadleaf/podocarp rainforest. While here seasonal variation in weather conditions and food abundance is less extreme, strong temporal variations are still evident because podocarps fruit and flower irregularly, typically having three to five year intervals between good crops (O'Donnell & Dilks 1994). In such an environment, the predominantly frugivorous strategy of kea in high-altitude regions may not be sustainable and survival here may require specialising on different food types. There are suggestions to this effect, with an observational study in the rainforest finding no instances of kea foraging on fruit, but 13% on invertebrates and a further 32% on 'probable invertebrate' (O'Donnell & Dilks 1994) and faecal sample analysis finding invertebrate remains in 71% of samples collected within the rainforest (Chapter 2/Greer et al. 2015). However, it remains unknown if kea living in the rainforest have a predominantly animal-based diet, and if so, if they have morphological adaptations that facilitate invertebrate foraging.

While generalisation/specialisation theory predicts population level specialisation in kea, kea morphology also suggests specialisation among sex classes. Unusually for parrots, kea are sexually dimorphic in body size, with males c. 5% greater in all body measurements except the bill, which is c. 13% longer (Bond et al. 1991). The additional sexual size dimorphism (SSD) of the male's trophic organ (bill) may reduce intersexual competition for food; or increase male foraging ability, enabling them to provide food for themselves, their nest-bound females, and young during winter and spring (Moorhouse et al. 1999). The only direct investigation into sexual specialisation in kea found no evidence of such, although comparisons were restricted to summer when sex differences are likely minimised due to the ready availability of fruit (Chapter2/Greer et al. 2015). Other possibilities are

that the additional SSD of the male kea bill is a result of sexual selection for longer bills (although this seems unlikely as kea are monogamous and non-territorial; see Shine 1989), or be a left-over ancestral trait with no current adaptive significance. The kākā *Nestor meridionalis*, the only other extant member of the *Nestor* family, displays similar bill SSD, suggesting inheritance from a common ancestor; however, in kākā, sex differences in foraging behaviour have been found such that males can access food sources that females cannot, cracking seeds with hardened shells and excavating grubs from hard, live wood (Moorhouse 1997). I consider it likely that the male kea's increased bill SSD is similarly adaptive and that there is sexual specialisation in kea foraging that has not yet been identified.

Here I investigate the kea's dietary specialisation and its relationship to bill morphology using a combination of physical measurements and blood and feather carbon and nitrogen stable isotope values. Stable isotope analysis is an extremely useful tool to distinguish between dietary sources with high degrees of isotopic distinctiveness (e.g., C3 v. C4 plants, or plant v. animal sources). The ratios of the stable isotopes ^{13}C and ^{15}N in an animal's tissue reflect those of the diet consumed. Dietary mixing models can then estimate the contribution of each source to the total diet. The results from such models correlate strongly with those obtained from stomach contents (Araújo et al. 2007) making stable isotope analysis a particularly useful technique for dietary investigations in difficult-to-follow species, such as the kea.

Methods

Study Species

Kea are a large (c. 850 g) parrot endemic to New Zealand's South Island. They are highly explorative and neophillic and will investigate any novel feature in their environment. Their bill has been likened to a 'swiss army knife' as it seems adaptable for almost any foraging purpose, including digging, scraping, plucking, and gleaning (Diamond & Bond 1999). Kea breed annually, typically laying two or three eggs per clutch in winter (Moorhouse et al. 1999; Diamond & Bond 1999). Eggs take a little over three weeks to hatch and chicks a further 13 weeks to fledge (Jackson 1963). New

fledglings appear and become more common starting in December (ALG pers. obs.). Fledglings retain the feathers they grew in the nest until the next summer when they undergo their first post-fledging moult and moult annually from then on. The structure of kea wing moult is highly variable and their moulting season is protracted, lasting from January to May (Davis 2001).

Study Sites

High-altitude Sites: In Arthur's Pass National Park (42°57'S, 171°46'E; 500 – 1,600 m a.s.l.), Craigieburn Forest Park (43°6'S, 171°42'E; c. 1,300 m a.s.l.), and Mount Cook/Aoraki National Park (43°44' S, 170°6'E, 850 – 1,600 m a.s.l) kea are most commonly found at 700 to 2,000 m a.s.l. where they feed in the open alpine grasslands, the sub-alpine scrub or the almost monoculture southern beech forests (*Fuscospora* spp.) that blanket the lower elevations. The climate here is more extreme than in the rest of the country, being characterised by higher winds, lower temperatures and stronger seasonal variation. Semi-permanent snow reaches down to ~1000m during winter (NIWA, 2014a).

Rainforest Site: In Westland (43°13'S, 170°10'E), kea live at sea level (c. 50 m a.s.l.). The broadleaf/podocarp mix of vegetation here is dominated by rimu *Dacrydium cupressinum*, kāmahi *Weinmannia racemosa* and southern rātā *Metrosideros umbellata*. In contrast with the montane variability, here mean maximum daily temperatures vary by just 8°C across the year and snow is rare (NIWA 2014b).

Morphological Data

Recent genetic work shows the population of kea inhabiting the Westland temperate rainforest to be most closely related to the kea occupying the high-altitude regions of the central South Island (Dussex et al. 2014). To compare the morphology of kea inhabiting the rainforest with the most closely related high-altitude populations, I analysed data collected from kea in two central high-altitude locations: Arthur's Pass NP and surrounds (n = 89) and Mount Cook/Aoraki National Park (n = 34); and from kea (n = 90) in Westland rainforest between February, 2007 and October, 2012. Bill length (from the base of the cere to the tip of the bill) and head length (the base of the skull to the top

of the cere) were measured using a vernier callipers to the nearest mm; weight was measured to the nearest 5g using a 5kg spring balance. Kea were sexed and aged based on a combination of behaviour, appearance and measurements and all kea were in their second summer of life or older. I conducted a multivariate analysis of co-variance (MANCOVA) in SPSS v. 21 (IBM Corporation, NY 10589, USA) in order to determine if the kea's bill and head lengths varied among high-altitude and rainforest habitats. I included the co-variates (1) weight, in order to correct bill and head length allometrically against an overall measure of body size (see Bond et al. 1991); and (2) season, as bill length varies seasonally in some species (Davis 1954). Male and females were analysed separately because kea display sexual size dimorphism.

Tissue Samples

Blood and/or feather samples were collected from 144 kea between Dec, 2010 and Oct, 2012. The top 2 cm of a 1st primary (P1) and 10th primary (P10) feather of each bird were clipped and stored in a sealed plastic bag until processing. These feathers were chosen to provide a span of the moult season and their isotopic ratios were later averaged to give a single feather δ^{13}C or δ^{15}N value for each kea. Approximately 0.3 cc of blood was drawn from the brachial wing vein and a few drops were stored in 70% ethanol, which does not affect δ^{15}N or δ^{13}C (Chapter 3; Greer et al. In Press). Tissue samples were collected from kea in the high-altitude regions of Arthur's Pass National Park (feather *n* = 72; blood *n* = 44) and nearby Craigieburn Forest Park (feather *n* = 3; blood *n* = 5) and from kea in Westland rainforest (feather *n* = 64; blood *n* = 26).

Diet Samples

Samples of plants (high-altitude *n* = 84; rainforest *n* = 38) and animals (high-altitude *n* = 22; rainforest *n* = 7) either known to be eaten by kea or considered likely kea food were collected from the same study sites as kea tissue samples (complete list in Appendix 4.1). Because only a small number of the animal samples were sourced within the rainforest habitat, I used a *K* - nearest neighbours test to investigate if there was a significant difference between the animal samples

collected from both habitats (the *K* - nearest neighbours test treats $\delta^{13}C$ and $\delta^{15}N$ values as spatial data; see Rosing et al. 1998 for the application of this test within stable isotope ecology). As there was no significant difference (p >0.05) in animal samples collected from both sites, I clumped these into a single 'animal' source (Table 1). I used region-specific plant sources in all mixing model calculations.

Table 1. Isotopic values of dietary sources. *Denotes habitat-specific animal samples combined into the single source 'animal'.

	$\delta^{13}C$ ‰		**$\delta^{15}N$ ‰**	
	Mean ± SD	Range	Mean ± SD	Range
Plant – High-altitude (*n* = 84)	-28.92 ± 2.39	-36.03 to -24.13	-4.56 ± 3.68	-9.95 to 9.22
Plant – Rainforest (*n* = 38)	-29.72 ± 2.11	-33.86 to -25.20	-2.54 ± 2.64	-8.11 to 5.05
Animal – high-altitude (*n* = 22)*	-25.84 ± 3.02	-32.35 to -21.45	2.31 ± 3.00	-3.80 to 6.76
Animal – rainforest (*n* = 7)*	- 25.71 ± 4.24	- 32.65 to -18.65	-0.34 ± 1.47	-2.97 to 1.40
Animal (*n* = 29)	-25.81 ± 3.27	-32.65 to -18.65	1.67 ± 2.92	-3.80 to 6.76

Sample Preparation

Feathers soaked in 2:1 chloroform:methanol solution for 24 h were rinsed twice in fresh solution and air dried in a fume cupboard for 48 h. The top 1 cm of the inner feather vane was removed and finely clipped. Blood, plant and animal samples were dried to constant mass in a 60°C oven. Blood and animal samples were pulverised with a mortar and pestle and plant samples were ground to talc powder consistency in a ball mill (Retsch MM2000, Hahn, Germany). All samples were homogenised and weighed out on an ultra-microbalance (accurate to 0.1 µg; Mettler-Toledo UMX2, Greifensee, Switzerland) to 0.5 - 0.7 mg for kea tissue and animal samples and 3.5 - 5 mg for plant samples, and inserted into individual 4 x 6 mm tin capsules for mass spectrometer analysis. I did not extract lipids from kea tissue or diet sources because recent work suggests that carbohydrates and lipids are also used for tissue synthesis and thus a portion of dietary lipid is desirable to provide the full suite of dietary macro-molecules (Newsome et al. 2014).

Mass Spectrometry

Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ using the mass spectrometry protocols laid out in Chapter 3 (Greer et al. In Press). A subset of samples were also analysed for %C and %N to provide data on elemental concentration.

Isotope values are reported in parts per thousand (‰) as δX, the ratio of heavy to light isotope, relative to the appropriate standard:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where X is either ^{13}C or ^{15}N, and R is either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

Mixing Model

I used the Bayesian mixing-model, MixSIAR (Version 2.1; Stock & Semmens 2013), to calculate the contribution of plant and animal matter to kea feather and blood tissue. MixSIAR has the advantages of being able to take uncertainty in dietary source values and varying elemental concentrations of sources into account, which is essential for accurate dietary proportioning in omnivores. Plants and animals typically have very different nitrogen concentrations that could skew results if unaccounted for. Using a subset of the dietary sources, I calculated %C (plant: n = 14; mean = 48.99 ± 5.15; animal: n = 9; mean = 50.45 ± 6.52) and %N (plant: n = 14; mean = 1.10 ± 0.28; animal: n = 9; mean = 9.09 ± 3.21) and included these as in my models as concentration-dependent data. Model parameters were set to three Markov chains with a length of 100,000 and a burn-in of 50,000. Results from the Heidelberger-Welch and Geweke diagnostic tests were inspected to ensure that the model had successfully converged on the posterior distribution. In all instances, the results of MixSIAR are reported as means ± standard deviations, along with Bayesian 95% credibility intervals (CI) given as ranges from 2.5% to 97.5%. Mixing model results are plotted as the proportion of each dietary source against the scaled posterior density. Posterior densities have been scaled in order to make the contribution of each dietary source easier to see. The resulting curves are visually equivalent

to a probability distribution in that 95% of the area under the curve equates to 95% probability. MixSIAR was run using R 2.15.3 (R Development Core Team, 2013).

Stable carbon and nitrogen isotope ratios of feather tissue reflect those of the bird's diet at the time of feather production because keratin is metabolically inert after synthesis. Therefore, sampling feather tissue from a juvenile, sub-adult or adult at any time of the year will provide data on their diet during the moult, whereas sampling feather from a nestling or fledgling will reflect the diet provisioned in the nest when they grew the feather. However, whole blood is a metabolically active tissue and its stable isotope ratios reflect diet over c. the last month (Dalerum & Angerbjörn 2005).

I included all kea in the mixing model when comparing the high-altitude and rainforest populations. When comparing male and female diets I excluded any tissues synthesised while a bird was being provisioned with food (feathers and bloods from nestling and fledgling kea, and bloods collected from nesting adult females). To compare moulting diet with the diet provisioned to nestlings, I compared feathers from nestlings and fledglings with those from adults. I had insufficient blood samples to investigate sex differences within rainforest kea using blood tissue. Seasonal analysis of blood samples was restricted to blood collected throughout the year for this purpose from specific locations within Arthur's Pass National Park (n = 26) and Craigieburn Forest Park (n = 5).

I ran multivariate analyses of variance (MANOVAs) with $\delta^{13}C$ and $\delta^{15}N$ as dependent variables to test for statistical significance between groups ($\alpha = 0.05$).

Discrimination Factors

I incorporated kea specific diet-to-feather discrimination factors for carbon ($\Delta^{13}C = 4.00‰ \pm 0.03$) and nitrogen ($\Delta^{15}N = 3.10‰ \pm 0.20$) as determined in Chapter 3 (Greer et al. In Press) into the mixing model. Blood isotope ratios were converted into feather isotope ratios using the kea-specific equations: $\delta^{13}C_{feather} = 0.973\ (\delta^{13}C_{blood}) + 1.122$ and $\delta^{15}N_{feather} = 0.920\ (\delta^{15}N_{blood}) + 1.041$ derived in Chapter 3 (Greer et al. In Press) and feather discrimination factors used throughout. The digestibility of food sources can also affect discrimination factors but no data are available on the differential digestibility of plant and animal matter in kea. Therefore, I followed current best practice

recommendations for the use of mixing models (Phillips et al. 2014) and extended the uncertainty around the discrimination factors by 0.3‰ to attribute this to noise, giving final $\Delta_{\text{diet-feather}}{}^{13}\text{C} = 4.00‰ \pm 0.33$ and $\Delta_{\text{diet-feather}}{}^{15}\text{N} = 3.10‰ \pm 0.50$, which were used in all mixing model calculations.

Results

Morphology

There was a significant effect of habitat on the bill and head lengths of both male (bill: $F(1, 126) = 60.74$ $p < 0.001$; head: $F(1, 126) = 16.21$, $p < 0.001$) and female kea (bill: $F(1, 78) = 69.21$ $p = 0.002$; head: $F(1, 78) = 58.14$, $p < 0.001$). The bill and heads of both sexes were longer from kea sampled in the rainforest habitat (Table 2). Weight co-varied significantly with bill and head lengths in males (bill: $F(1, 126) = 24.24$; $p < 0.001$; head: $F(1, 126) = 6.26$; $p = 0.014$) ; and with head length in females ($F(1, 78) = 28.55$; $p = 0.004$); however season did not co-vary with either bill or head length in males (bill: $F(1, 126) = 0.195$, $p = 0.830$; head: $F(1, 126) = 5.95$; $p = 0.200$) or females ($F(1, 78) = 0.222$, $p = 0.857$; $F(1, 78) = 0.011$, $p = 0.952$). When the means of bill and head length were adjusted for weight, male kea had bills which were 6.3% longer in the rainforest habitat (high-altitude: mean 47.8 ± 2.1; rainforest: mean 50.8 ± 2.1) and heads which were 2.0% longer in the rainforest habitat (high-altitude: mean 65.6 ± 1.9; rainforest: mean 66.9 ± 1.9). Female kea had bills which were 4.3% longer in the rainforest habitat (high-altitude: mean 42.3 ± 2.6; rainforest: mean 44.1 ± 2.6), and heads which were 2.8% longer (high-altitude: mean 61.5 ± 1.8; rainforest: mean 63.2 ± 1.8).

Table 2. Morphological measurements of kea sampled within the high-altitude and rainforest habitats.

		Bill length (mm)		**Head length (mm)**		**Weight (g)**	
	n	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Males							
High-altitude	80	48.0 ± 2.0	41.9 to 52.4	65.6 ± 1.8	61.3 to 73.7	903 ± 79	700 to 1100
Rainforest	50	50.6 ± 2.6	43.9 to 59.0	66.8 ± 2.2	62.0 to 73.0	865 ± 75	668 to 1010
Females							
High-altitude	43	42.3 ± 2.9	37.7 to 48.6	61.6 ± 1.6	58.0 to 64.5	783 ± 81	580 to 960
Rainforest	39	44.1 ± 2.2	40.8 to 49.2	63.2 ± 2.1	59.0 to 67.8	781 ± 85	638 to 1100

High-altitude and Rainforest Diets

Mixing models that compared the overall diets of kea in high-altitude and rainforest habitat estimated very different proportions of animal matter in the diets of the two populations (Fig. 2). Kea in high-altitude regions had a predominantly plant-based diet (feather: mean = 64% ± 7 plant, CI = 50 to 78%; blood: mean = 61% ± 10 plant, CI = 42 to 83%); whereas those from the rainforest had a predominantly animal-based diet (feather: mean = 74% ± 7 animal, CI = 60 to 100%; blood: mean = 81% ± 11 animal, CI = 61 to 100%). These differences were significant for both $\delta^{13}C$ and $\delta^{15}N$ values (Table 3).

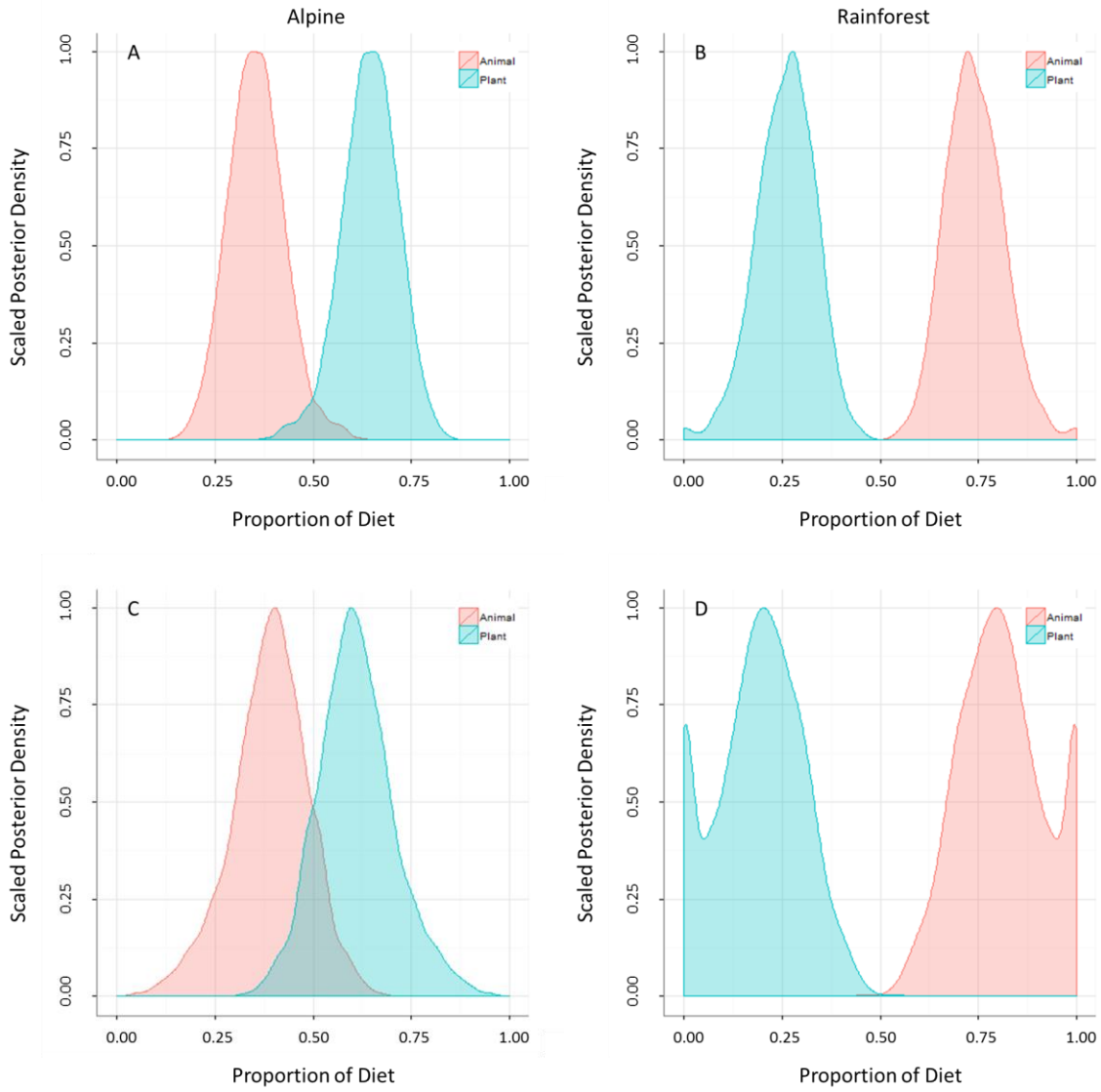

Figure 2. Proportions of plant (blue) and animal (pink) matter eaten by kea in high-altitude (a, c) and rainforest (b, d) habitat based on feather (a, b) and blood (c, d) samples.

Table 3. Proportions of animal matter consumed by compared groups of kea, as calculated by MixSIAR. Proportion of plant matter = 1 – animal matter. Provisioned diet and moulting diet refer to the food provisioned to nestlings, and eaten by adult kea during the moult, respectively. Provisioning season = Oct, Nov, Dec; Moulting season = Jan, Feb, Mar; Resting season = Apr, May, Jun; Laying season = Jul, Aug, Sep.

	High-altitude ‰						**Rainforest ‰**					
	Feather			**Blood**			**Feather**			**Blood**		
	n	**Mean**	**95% CI**	***n***	**Mean**	**95% CI**	***n***	**Mean**	**95% CI**	***n***	**Mean**	**95% CI**
Overall	72	0.36 ± 0.07	0.22 to 0.50	44	0.39 ± 0.10	0.17 to 0.58	64	0.74 ± 0.07	0.60 to 0.89	26	0.81 ± 0.11	0.61 to 1.00
Male	23	0.31 ± 0.10	0.13 to 0.51	11	0.33 ± 0.13	0.11 to 0.60	16	0.79 ± 0.11	0.60 to 1.00	NA	NA	NA
Female	16	0.37 ± 0.12	0.14 to 0.61	9	0.32 ± 0.15	0.05 to 0.64	16	0.48 ± 0.13	0.22 to 0.70	NA	NA	NA
Provisioned Diet	31	0.33 ± 0.08	0.19 to 0.50	NA	NA	NA	23	0.85 ± 0.10	0.66 to 1.00	NA	NA	NA
Moulting Diet	13	0.14 ± 0.12	0.00 to 0.45	NA	NA	NA	26	0.68 ± 0.09	0.52 to 0.86	NA	NA	NA
Provisioning Season	NA	NA	NA	6	0.31 ± 0.15	0.08 to 0.65	NA	NA	NA	NA	NA	NA
Moulting Season	NA	NA	NA	9	0.25 ± 0.13	0.05 to 0.53	NA	NA	NA	NA	NA	NA
Resting Season	NA	NA	NA	7	0.13 ± 0.13	0.00 to 0.45	NA	NA	NA	NA	NA	NA
Laying Season	NA	NA	NA	11	0.11 ± 0.12	0.00 to 0.41	NA	NA	NA	NA	NA	NA

Table 4. δ^{13}C & δ^{15}N values and results of MANOVAs for each compared group. All blood isotopic ratios have been converted to feather ratio equivalents to enable direct comparisons. **P* ≤ .05, ***P* ≤ .01, ****P* ≤ .001. Habitat is denoted by H – high-altitude, R – rainforest. Provisioned diet and moulting diet refer to the food provisioned to nestlings, and eaten by adult kea during the moult, respectively. Provisioning season = Oct, Nov, Dec; Moulting season = Jan, Feb, Mar; Resting season = Apr, May, Jun; Laying season = Jul, Aug, Sep.

	Feather				**Blood**				**Feather**		**Blood**	
	δ^{13}C ‰		**δ^{15}N ‰**		**δ^{13}C ‰**		**δ^{15}N ‰**		**δ^{13}C ‰**	**δ^{15}N ‰**	**δ^{13}C ‰**	**δ^{15}N ‰**
Comparison	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**MANOVA**			
Overall (H)	-23.44 ± 1.03	-25.36 to -20.23	2.10 ± 2.53	-2.23 to 7.43	-23.03 ± .72	-24.59 to -21.71	.59 ± 1.78	-2.14 to 5.75	$F(1,134) = 54.52$, $p < 0.001$ ***	$F(1,134) = 4.14$, $p = 0.044$ *	$F(1,68) = 21.03$, $p < 0.001$ ***	$F(1,68) = 39.09$, $p < 0.001$ ***
Overall (R)	-21.52 ± 1.92	-26.31 to -18.98	2.87 ± 1.75	-.37 to 7.63	-21.49 ± 2.03	-26.07 to -18.94	3.42 ± 1.92	1.31 to 7.88				

	Feather				Blood				Feather		Blood	
	δ^{13}C ‰		δ^{15}N ‰		δ^{13}C ‰		δ^{15}N ‰		δ^{13}C ‰	δ^{15}N ‰	δ^{13}C ‰	δ^{15}N ‰
Comparison	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**MANOVA**			
Male (H)	-23.46 ± .77	-25.11 to -22.40	1.30 ± 2.32	-1.85 to 6.45	-23.35 ± 0.60	-24.36 to -22.25	1.70 ± 2.10	-1.45 to 5.75	$F(1,37) = 3.55$, $p = 0.067$	$F(1,37) = 0.02$, $p = 0.903$	$F(1,18) = 1.01$, $p = 0.330$	$F(1,18) = 1.03$, $p = 0.320$
Female (H)	-22.97 ± .86	-25.36 to -21.79	1.22 ± 1.97	-2.23 to 4.64	-23.03 ± .82	-24.01 to -21.71	.93 ± .92	-.83 to 2.02				
Male (R)	-20.77 ± 1.36	-24.01 to -18.98	3.23 ± .74	2.03 to 4.86	NA	NA	NA	NA	$F(1,30) = 17.64$, p <0.001 ***	$F(1,30) = 3.01$, $p = 0.093$	NA	NA
Female (R)	-22.78 ± 1.35	-25.72 to -21.18	2.36 ± 1.86	-.37 to 7.06	NA	NA	NA	NA				
Provisioned diet (H)	-23.72 ± 1.19	-25.31 to -20.23	3.22 ± 2.56	-1.97 to 7.43	NA	NA	NA	NA	$F(1,42) = 6.45$, $p = 0.015$ *	$F(1,42) = 18.58$, p <0.001 ***	NA	NA
Moulting diet (H)	-22.85 ± 0.55	-23.59 to -21.79	-.01 ± 1.24	-1.64 to 2.55	NA	NA	NA	NA				
Provisioned diet (R)	-21.51 ± 2.23	-26.31 to -19.34	2.97 ± 2.20	0.68 to 7.63	NA	NA	NA	NA	$F(1,47) = .40$, $p = 0.528$	$F(1,47) = .75$, $p = 0.391$	NA	NA
Moulting diet (R)	-21.86 ± 1.56	-25.72 to -18.98	2.53 ± 1.24	-0.37 to 4.86	NA	NA	NA	NA				
Provisioning Season (H)	NA	NA	NA	NA	-23.93 ± .51	-24.59 to -23.23	2.90 ± 1.97	.89 to 5.75	NA	NA	$F(3,29) = 11.31$, p <0.001 ***	$F(3,29) = 11.27$, p <0.001 ***
Moulting Season (H)	NA	NA	NA	NA	-23.44 ± .29	-24.01 to -23.05	1.32 ± .71	-.17 to 2.02	NA	NA		
Resting Season (H)	NA	NA	NA	NA	-22.84 ± .18	-23.13 to -22.65	-.40 ± 1.63	-2.14 to 2.92				
Laying Season (H)	NA	NA	NA	NA	-22.67 ± .66	-24.27 to -22.06	-.82 ± 1.30	-2.05 to 2.48				

Male and Female Diets

In the high-altitude habitat, male and female kea had very similar proportions of plant and animal matter in their diet as evidenced by both feather and blood tissues (Table 3). However, in the rainforest, male kea (mean = 79% ± 11, CI = 60 to 100%) had a significantly greater contribution of animal matter to their diet than females (feather: mean = 48% ± 13, CI = 22 to 70%; Fig. 3)

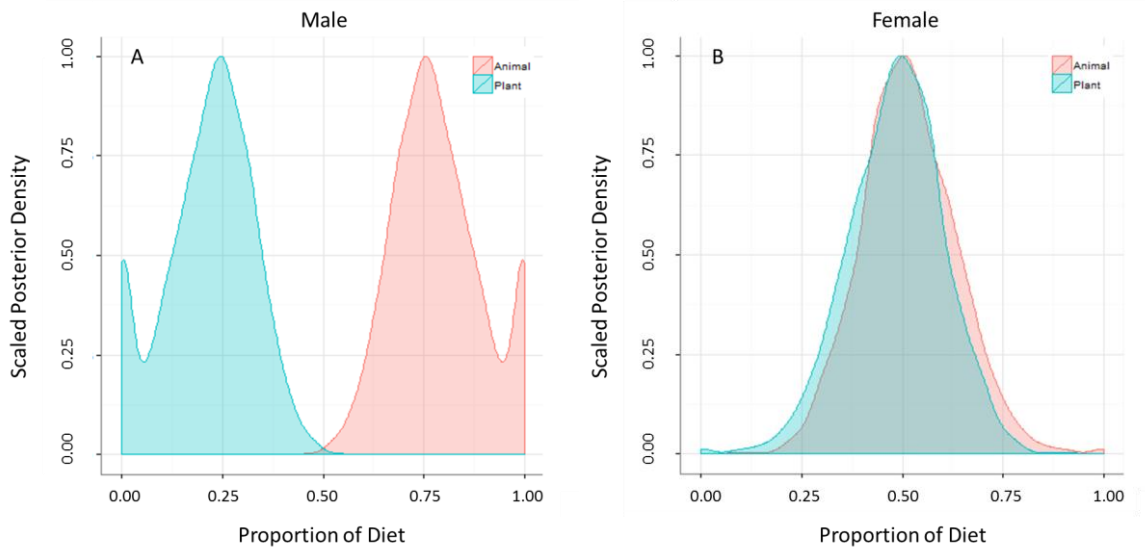

Figure 3. Contributions of plant (blue) and animal (pink) matter to the feather tissue of (a) male and (b) female kea sampled within the rainforest habitat.

Seasonal Variation

The diet that kea in the high-altitude habitat provisioned to their nestlings was higher in proportion of animal matter (mean = 33% ± 8, CI = 19 to 50%) than the diet eaten by the adults during the moult (mean = 14% ± 12, CI = 0 to 45%). There was no difference in the provisioned and moulting diets of kea within the rainforest (Table 4). Blood sample analysis allowed me to investigate dietary changes in the diet of kea in high-altitude locations throughout the year. I sub-divided the year into four seasons – provisioning, moulting, resting and laying, which are a month out of step with New Zealand's spring, summer, autumn and winter, but that hold greater relevance for the kea's yearly cycle of activity (Table 4). Yearly variation in $\delta^{13}C$ ($F(3,29) = 11.31$) and $\delta^{15}N$ ($F(3,29) = 11.27$) were highly significant ($p < 0.001$). Post-hoc comparison tests (Tukey's HSD; $\alpha = 0.05$) revealed that this difference was mainly due to a greater proportion of animal matter being eaten

during the provisioning season (c. spring) than in the resting (c. autumn) or laying seasons (c. winter; $p < 0.005$), but the diet eaten while moulting (c. summer) also consisted of significantly more animal matter ($p < 0.05$) than that eaten during the laying season.

Discussion

These results show that the diets of kea in high-altitude and rainforest habitat are dramatically different and suggest that the population of kea living in the rainforest be considered primarily insectivorous. Estimates of the average contribution of animal matter to the kea's diet in high-altitude locations ranged from 11 to 39%, whereas in the rainforest they ranged from 48 to 85%. In addition, kea in the rainforest had an increased bill length relative to their high-altitude counterparts (male 6.3% longer; female 4.3%, adjusted for body weight), suggesting that the longer bill is a specific adaptation to living in the rainforest due to increased specialisation on invertebrates. An overall increased bill length may improve the power or efficiency with which kea can rip off bark, extract grubs from live wood and demolish decaying wood, as found in male kākā (Moorhouse 1997). Kea in the rainforest also had a longer head than those from the high-altitude habitat, however to a much lesser degree (male 2.0% longer; female 2.8%) and this may be a result of genetic correlation between bill and head length (Lande 1984). Kea that live in the Westland rainforest at first glance seem to be geographically isolated from the rest of the population by the Southern Alps, yet genetic work has confirmed that they are not a distinct sub-species (Dussex et al. 2014). Kea are capable of crossing the alps by following valleys and flying over lower peaks (Temple 1996) and at least some rainforest kea also feed in the high-altitude habitat (Chapter 2/Greer et al. 2015). Nevertheless, the Southern Alps still form a significant physical barrier that must reduce free gene flow to some degree, thus facilitating the evolution of local morphological and behavioural specialisations.

I found major differences in the diet of male and female kea in the rainforest habitat such that males ate half again (79%) more animal matter than females (48%). I did not find evidence for sex differences within the high-altitude habitat; however, I only compared moulted feathers (grown mainly during summer) and it is possible that in this population sex differences only become apparent

in the harsher months when tissue samples and feeding observations of mature birds are extremely difficult to obtain. The increased consumption of animal matter by male kea within the rainforest provides the first direct evidence that differences in ecology may be the cause or maintaining force of increased bill SSD in kea (Moorhouse et al. 1999). The increased consumption of animal matter by kea in the rainforest in general and the co-incident increase in the bill length of this population further support this hypothesis. SSD is typically attributed to sexual selection rather than ecological causes (Temeles et al. 2000). However, evidence is mounting that ecological factors can drive sexual size dimorphisms in foraging relevant attributes such as bill morphology (Nebel & Thompson, 2011; Radford & du Plessis 2004; Temeles et al. 2000) and body mass (Cook et al. 2013). The extinct hūia *Heteralocha acutirostris* had a famously dimorphic bill not attributable to sexual selection (Moorhouse 1996); and body mass, which is positively related to dive depth and duration, has been shown to vary greatly with sex and micro-geographic location in Kerguelen shags *Phalacrocorax verrucosus* (Cook et al. 2013). Kea join the handful of species for which there is strong evidence to suggest ecological factors play a causative or maintaining force for dimorphism at both population and sexual levels (e.g., Badyaev et al. 2000).

Within the high-altitude habitat, seasonal differences were found in the contribution of animal matter to the kea's diet. Feathers grown by chicks revealed the nestlings' diet to be c. 19% richer in animal content than the diet eaten by adults during the moult. While no data are available on seasonal invertebrate abundance in the high-altitude zones, data from the beech forests show little seasonal variation (Clout & Gaze 1984; Murphy & Kelly 2003). Therefore, superficially, it seems that kea in high-altitude locations are preferentially searching out animal sources to feed their developing chicks. This is a common strategy among birds and the additional nitrogen provided by a diet rich in animal protein can lead to shorter fledging times, and greater growth rates (Riehl & Adelson 2008). However, analysis of blood samples collected from a variety of kea, rather than just breeding adults, showed that they were also consuming an increased proportion of animal matter at this time (c. spring). Therefore, it seems that the high-altitude kea population as a whole increases their animal foraging in spring, which may be related to increased invertebrate activity in the warmer temperatures (O'Donnell 2000) and a shortage of other foods. Blood sampled during the main hunting season (March - June) revealed

that opportunistic scavenging from deer carcasses plays little role in the kea's overall consumption of animal matter.

The association of increased bill length in kea with increased invertebrate foraging across different habitat types and across sex strongly suggests that ecological factors play a causative or maintaining role in the SSD of this species. Future research should investigate the strength of this relationship (Chapter 5) and if further differences exist between the ecology of kea from different habitats, particularly in relation to the timing of their breeding season, chick growth and fledging success rates. I strongly suggest extending the recommendation of Dussex et al. (2014) to focus conservation efforts on kea populations in the north and south of the South Island, to also include the Westland population of rainforest kea in order to preserve the greatest degree of intra-specific variability in this endangered parrot.

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APPENDIX 4.1

Table 1. List of animal matter samples that were included as dietary sources in each mixing model, and the habitat from which they were collected.

Animal Matter	Habitat
Ant	Rainforest
Ants	Rainforest
Arachnid	High-Altitude
Arachnid	High-Altitude
Arachnid (Opilionid)	High-Altitude
Beetle	High-Altitude
Beetle	High-Altitude
Caterpillar	Rainforest
Cockroaches	High-Altitude
Cockroaches	Rainforest
Deer	High-Altitude
Deer	High-Altitude
Insect Eggs	High-Altitude
Deer	High-Altitude
Insect Grub	High-Altitude
Insect Grub	High-Altitude
Insect Grub	High-Altitude
Insect Grub	High-Altitude
Insect Grub	Rainforest
Insect Grub	Rainforest
Larva	High-Altitude
Millipede	High-Altitude
Millipede	High-Altitude
Pseudoscorpion	High-Altitude
Pupa	High-Altitude
Scale Insects	High-Altitude
Termites	Rainforest
Worm	High-Altitude
Worm	High-Altitude

Table 2. List of plant matter samples that were included as dietary sources in the appropriate mixing models, and the habitat from which they were collected.

Species	Plant Organ	Habitat
Anisotome aromatica	Leaf	High-Altitude
Anisotome aromatica	Root	High-Altitude
Anisotome aromatica	Leaf	High-Altitude
Anisotome aromatica	Root	High-Altitude
Aristotelia serrata	Flower	Rainforest
Asplenium bulbiferum	Leaf	Rainforest
Astelia fragrans	Flower	Rainforest
Bulbophyllum pygmaeum	Fruit & seeds	Rainforest
Cardamine debilis	Leaf & flower	Rainforest
Celmisia discolor	Leaf	High-Altitude
Celmisia discolor	Root	High-Altitude
Celmisia discolor	Pollen	High-Altitude
Celmisia discolor	Leaf	High-Altitude
Celmisia discolor	Root	High-Altitude
Celmisia spectabilis	Leaf	High-Altitude
Celmisia spectabilis	Root	High-Altitude
Celmisia spectabilis	Pollen	High-Altitude
Celmisia spectabilis	Pollen	High-Altitude
Celmisia spectabilis	Stem	High-Altitude
Celmisia spectabilis	Leaf	High-Altitude
Chionochloa conspicua	Stem	High-Altitude
***Chionochloa* spp**	Stem & root	High-Altitude
Coprosma cheesemanii	Leaf	High-Altitude
Coprosma cheesemanii	Fruit & seeds	High-Altitude
Coprosma cheesemanii	Leaf	High-Altitude
Coprosma cheesemanii	Leaf	High-Altitude
Coprosma cheesemanii	Fruit & seeds	High-Altitude
Coprosma depressa	Fruit & seeds	High-Altitude
Coprosma intertexta	Leaf	High-Altitude
Coprosma intertexta	Leaf	High-Altitude
Coprosma intertexta	Fruit & seeds	High-Altitude
Coprosma propinqua	Leaf	Rainforest
Coriaria angustissima	Fruit	High-Altitude

Coriaria arborea	Flower	Rainforest
Coriaria arborea	Leaf	Rainforest
Coriaria plumosa	Leaf	High-Altitude
Coriaria plumosa	Fruit & seeds	High-Altitude
Coriaria sarmentosa	Leaf	High-Altitude
Coriaria sarmentosa	Fruit & seeds	High-Altitude
Coriaria sarmentosa	Leaf	High-Altitude
Coriaria sarmentosa	Fruit & seeds	High-Altitude
Coriaria sarmentosa	Leaf	High-Altitude
Coriaria sarmentosa	Fruit & seeds	High-Altitude
Coriaria sarmentosa	Fruit	High-Altitude
Cyathea smithii	Shoots	Rainforest
Dacrycarpus dacrydioides	Leaf	Rainforest
Earina autumnalis	Root	Rainforest
Fuchsia excorticata	Flower	Rainforest
Fuscospora cliffortioides	Leaf	High-Altitude
Fuscospora cliffortioides	Leaf	High-Altitude
Fuscospora cliffortioides	Leaf	High-Altitude
Fuscospora cliffortioides	Leaf	High-Altitude
Fuscospora cliffortioides	Leaf	High-Altitude
Fuscospora cliffortioides	Leaf	High-Altitude
Fuscospora cliffortioides	Leaf	High-Altitude
Fuscospora cliffortioides	Leaf	High-Altitude
Gahnia rigida	Seeds	Rainforest
Gaultheria crassa	Flower	High-Altitude
Gaultheria depressa	Fruit & seeds	High-Altitude
Gaultheria depressa	Fruit & seeds	High-Altitude
Gaultheria depressa	Fruit & seeds	High-Altitude
Gentianella corymbifera	Root	High-Altitude
Gentianella corymbifera	Flower	High-Altitude
Gentianella corymbifera	Stem	High-Altitude
Gentianella corymbifera	Root	High-Altitude
Gentianella corymbifera	Flower	High-Altitude
Gentianella corymbifera	Stem	High-Altitude
Gleichenia dicarpa	Shoots	Rainforest
Halocarpus biformis	Leaf	Rainforest

Halocarpus biformis	Cones	Rainforest
Histiopteris incisa	Shoots	Rainforest
Hypochaeris radicata	Leaf	Rainforest
Hypochaeris radicata	Root	Rainforest
Hypolepis millefolium	Leaf	Rainforest
Lepidothamnus laxifolius	Seeds	High-Altitude
Lepidothamnus laxifolius	Seeds	High-Altitude
Metrosideros fulgens	Flower	Rainforest
Metrosideros perforata	Flower capsule	Rainforest
Metrosideros umbellata	Flower	High-Altitude
Metrosideros umbellata	Flower	Rainforest
Muehlenbeckia axillaris	Fruit	High-Altitude
Muehlenbeckia axillaris	Fruit & seeds	High-Altitude
Mycelis muralis	Leaf	High-Altitude
Mycelis muralis	Leaf	High-Altitude
Mycelis muralis	Flower	High-Altitude
Myrsine australis	Leaf	Rainforest
Nertera depressa	Leaf & flower	Rainforest
Pentachondra pumila	Fruit	High-Altitude
Pentachondra pumila	Fruit & seeds	High-Altitude
Pentachondra pumila	Fruit & seeds	High-Altitude
Pentachondra pumila	Fruit & seeds	High-Altitude
Phyllocladus alpinus	Leaf	High-Altitude
Phyllocladus alpinus	Leaf	High-Altitude
Phyllocladus alpinus	Leaf	High-Altitude
Phyllocladus alpinus	Leaf	High-Altitude
Phyllocladus alpinus	Leaf	High-Altitude
Phyllocladus alpinus	Leaf	Rainforest
Phyllocladus alpinus	Cones	Rainforest
Pittosporum cornifolium	Flower	Rainforest
Podocarpus laetus	Leaf	Rainforest
Podocarpus nivalis	Fruit	High-Altitude
Podocarpus nivalis	Pollen	High-Altitude
Podocarpus nivalis	Leaf	High-Altitude
Podocarpus nivalis	Fruit & seeds	High-Altitude
Podocarpus nivalis	Seeds	High-Altitude

Podocarpus nivalis	Fruit & seeds	High-Altitude
Podocarpus nivalis	Seeds	High-Altitude
Podocarpus totara	Leaf	Rainforest
Podocarpus totara	Cones	Rainforest
Prumnopitys ferruginea	Leaf	Rainforest
Prumnopitys ferruginea	Leaf	Rainforest
Ripogonum scandens	Shoot	Rainforest
Rubus cissoides	Flower	Rainforest
Schefflera digitata	Leaf	Rainforest
***Senecio* spp.**	Leaf	Rainforest
***Taraxacum* spp.**	Flower	High-Altitude
***Taraxacum* spp.**	Leaf	High-Altitude
***Taraxacum* spp.**	Root	High-Altitude
***Taraxacum* spp.**	Leaf	High-Altitude
***Taraxacum* spp.**	Root	High-Altitude
***Taraxacum* spp.**	Flower	High-Altitude
Weinmannia racemosa	Flower	Rainforest

CHAPTER FIVE

Birds of a feather: Kea with similar bill and head lengths have more similar diets than those that differ

Two young kea take a break from foraging in order to play (summertime, Mount Cook. *Photo: Andruis Pašukonis*)

Abstract

Polymorphisms within a species may result from niche separation due to high levels of intraspecific competition. However, this makes the implicit assumption that those individuals that are phenotypically alike have more similar dietary niches than those that are phenotypically dissimilar. Here I tested this this supposition using kea *Nestor notabilis*, an omnivorous parrot that has pronounced male-biased, sexual dimorphism of the bill (c. 13%) and a habitat difference in bill and head size. Specifically, I investigated how much variance in kea diet is explained by morphology. To measure the strength of the relationship, I compared the pairwise morphological distance (bill length, head length and weight) with the pairwise Euclidean distance between stable carbon and nitrogen isotope ratios, and with the pairwise difference in the proportion of animal matter in each individual kea's diet - as estimated by a stable isotope mixing model (MixSIAR). There were strong relationships between dietary dissimilarity and difference in bill and head lengths within adult kea, which were due to differences among the sexes and differences across habitat. Kea with similar weights did not have more similar diets than those of dissimilar weights. At an individual level, no relationship was evident; however, this may be a result of having an insufficient sample size to detect a typically weak effect.

Introduction

Within all species, individual members exhibit some degree of phenotypic variation upon which natural selection can act (Darwin 1859). Selection is said to be stabilising when the average phenotype, being well adapted to obtain its primary food source, is favoured, while less efficient, extreme phenotypes are selected against (Fig. 1a). However, intense intraspecific competition or changed environmental conditions can instead lead to disruptive selection (Pfennig et al. 2007; Svanbäck & Persson 2009). Under disruptive selection more extreme morphs have higher fitness, as they experience less competition than more 'average' conspecifics or are better suited to the new environment (Bolnick & Paull 2009; Svanbäck & Persson 2009; Fig. 1b). Disruptive selection can have a strong diversifying effect on both a species' niche width and its morphology, sometimes leading to sexual, age, and discrete polymorphisms, and even driving sympatric speciation if sufficient opportunity for niche expansion exists (Maynard Smith 1962; Bolnick 2004; Barluenga et al. 2006; Bolnick & Fitzpatrick 2007).

While sexual dimorphisms are often attributed to sexual selection (Olsen et al. 2013; Parker & Pizzari 2015), there is increasing evidence that in some species, sexual dimorphism evolved as a result of disruptive selection to reduce intraspecific competition. Many bird species, including the humming bird *Eulampis jugularis* (Temeles et al. 2000), Darwin's finches *Certhidea* spp. (Grant & Grant 2003), and green woodhoopoes *Phoeniculus purpureus* (Radford & du Plessis 2004), have sexual bill dimorphisms that result in the division of food resources among the sexes and reduce intraspecific competition. However, underlying the premise that disruptive selection can drive polymorphisms due to intense intraspecific competition, is the implicit assumption that resource competition among phenotypically similar individuals is stronger than among phenotypically dissimilar individuals, here referred to as the 'resource competition hypothesis' (Rougharden 1972; Ackermann & Doebli 2004; Bolnick & Paull 2009). For certain hummingbirds, where the sexes have bill shapes that correspond to the morphologies of different species of flowers that they probe for nectar (e.g., *Eulampis jugularis;* Temeles et al. 2000), it is self-evident that competition for food among the sexes will be minimal because each sex drinks from different species of plant, supporting the resource competition

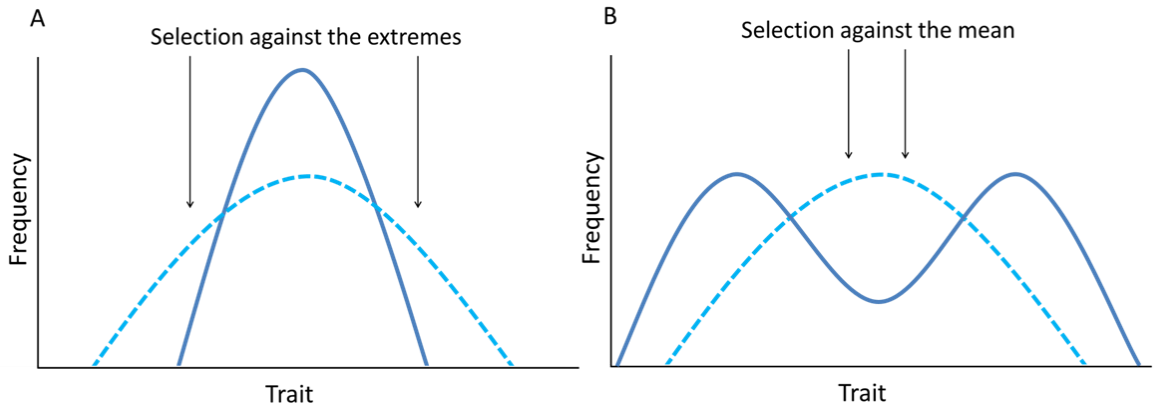

Figure 1. Stabilising selection (A) where the extreme phenotypes are selected against, and disruptive selection (B) where the average phenotype is selected against. Dashed lines represent the original populations, solid lines represent each population after selection.

hypothesis. However, it is less obvious whether, within the sexes, individual birds with similar morphologies will compete more strongly with one another.

In general, evidence to support the resource competition hypothesis at an individual level is mixed. In support, Svanbäck and Bolnick (2007) found an overall correlation between diet and morphology in the three-spined stickleback *Gasterosteus aculeatus*, which increased with the level of intraspecific competition. However, when Bolnick and Paull (2009) developed a methodology to precisely calculate the dietary overlap among each pair of fishes as a function of their morphological distance from each other, they found the relationship, although significant, was remarkably weak, suggesting that behavioural differences actually played a larger role than morphological ones in shaping individual dietary differences. Martin and Pfennig (2010) found a relationship between the frequency of occurrence of the three polymorphs of the spadefood toad tadpole *Spea multiplicata* and the degree of intraspecific competition they experienced. Stabilising selection favoured the intermediate tadpole morph in ponds where intraspecific competition was low; however, under conditions of high intraspecific competition, the extreme morphs occurred more frequently (Martin & Pfennig 2010). In digger wasp species (Crabronidae), wasps with similar body sizes had more similar diets, but only when nests were highly clumped and size-related limitations to prey selection existed (Polidori et al. 2012). No correlation between diet and morphology was found within four species of Brazilian frog (Leptodactylidae; Araújo et al. 2009), or in two species of annual fishes, *Austrolebias*

minuano and *Cynopoecilus fulgens* (Keppeler et al. 2015). Overall, the results of these studies suggest that while phenotypically similar individuals are somewhat likely to have more similar diets, under typical conditions the strength of this effect may be weak. However, the relationship may become more prominent under conditions of intense intraspecific competition or resource depletion.

Here, I investigate if kea *Nestor notabilis* with similar morphologies have similar diets. Kea are a large, omnivorous species of parrot (Psittaciformes), with a very broad diet (Greer et al. 2015) and a high degree of behavioural flexibility (Auersperg et al. 2010). Kea are an interesting species in which to investigate this link as, unusually for parrots, they are sexually dimorphic (Bond et al. 1991) and this dimorphism has been attributed to ecological factors, such as enhanced male provisioning ability (Moorhouse et al. 1999). Additionally, kea live in two very different habitats: high-altitude/montane and temperate rainforest. Those inhabiting temperate rainforest consume more animal matter and have longer bills and heads than kea inhabiting nearby montane regions, directly suggesting a link between invertebrate foraging and bill length in this species (Chapter 4). Here I investigate the strength of the relationship between diet and morphology in kea, and if it holds at an individual level. To investigate these relationships I use kea feather stable carbon and nitrogen isotope data ratios as a proxy for diet (see Bolnick & Paull 2009) and also investigate the link between increased invertebrate foraging and bill length by using estimates of the contribution of animal matter to the diet of individual kea obtained from a stable isotope mixing model.

Methods

Study species and sample collection

Kea are endemic to New Zealand's South Island where they primarily inhabit the alpine and sub-alpine zone (700 – 2,000 m a.s.l.) along the Southern Alps; however, there is also a breeding population in a stretch of lowland temperate rainforest along the West Coast (c. 50 m a.s.l.; Robertson et al. 2007). These two habitats differ greatly from one another in terms of their flora and fauna (see Chapter 2/Greer et al. 2015 for more information) and kea in each exploit different plant species and differ in the quantity of animal matter that they consume (Chapter 2/Greer et al. 2015; Chapter 4). Kea

from the rainforest habitat have longer bills than their high-altitude counterparts (5.2% for males and 4.0% for females) and consume a diet richer in animal protein (Chapter 4).

In addition to these geographic differences in morphology and behaviour, kea are sexually dimorphic, with males c. 5% larger than females in linear measures of body size except for their bill length, which is c. 13% longer (Bond et al. 1991). As males do all of the provisioning during the chick-rearing season (winter to spring) when many food sources are buried under snow or frozen in the ground, this additional length has been proposed as an adaptation to enable males to access food at this time (Moorhouse et al. 1999). A longer bill could aid in excavating invertebrates from trees, or digging out food from under snow.

Between Dec, 2010 and Oct, 2012 I collected morphological data and feather samples from 72 kea, 40 in high-altitude habitat: Arthur's Pass National Park (42°57'S, 171°46'E; 500 – 1,600 m a.s.l., 32 in rainforest habitat: Westland National Park (43°13' S, 170°10' E; 50 m a.s.l.) in their second summer of life or older. Bill length (from the base of the cere to the tip of the bill) and head length (the base of the skull to the top of the cere) were measured to the nearest mm using a vernier callipers; weight was measured to the nearest 5 g using a 5 kg spring balance. Morphological differences were investigated using independent samples t-tests in SPSS v. 21 (IBM Corporation, NY 10589, USA).

Kea were sexed based on a combination of behaviour and measurements (Bond et al. 1991). Kea were divided into two age classes: juvenile, if they still had yellow colouration around their head, bill or eye ring, and adult, if these areas had fully darkened (Diamond & Bond 1999). A 2 cm feather sample was clipped off the top of a first primary (P1) and tenth primary feather (P10) and stored in a sealed plastic bag until processing. Kea moult from January to May (Davis 2001) and all feather samples collected here represent this time period. At this time, while adult kea remain largely solitary or in pairs, juvenile kea band together into large, mobile flocks, possibly experiencing higher levels of intraspecific competition as a consequence (Jackson 1960).

Mass Spectrometry

Feather samples were prepared for mass spectrometry as described in Chapter 4. Samples were analysed for δ^{13}C and δ^{15}N using the mass spectrometry protocols laid out in Greer et al. (In Press/Chapter 3). The isotopic ratios of both feather samples were then averaged, resulting in a single feather value for each individual bird that represents its diet throughout the moulting season.

Isotope values are reported in parts per thousand (‰) as δX, the ratio of heavy to light isotope, relative to the appropriate standard:

$$\delta X = \left(\frac{\mathrm{R_{sample}}}{\mathrm{R_{standard}}} - 1\right) \times 1000$$

where X is either ^{13}C or ^{15}N, and R is either ^{13}C /^{12}C or ^{15}N /^{14}N, respectively.

Data Analysis

I analysed the isotopic ratios obtained for each individual kea in a stable isotope dietary mixing model, MixSIAR (Version 2.1; Stock & Semmens 2013), which provides an estimate of the proportion of animal and plant sources in the diet of each individual bird (e.g., Newsome et al. 2007). MixSIAR analyses were conducted following the protocols laid out in Chapter 4. Model parameters were set as follows. Isotope ratios for dietary sources: High-altitude habitat – plant: n = 84, δ^{13}C = -28.92‰ ± 2.39, δ^{15}N = -4.56‰ ± 3.68; animal: n = 29, δ^{13}C = -25.81‰ ± 3.27, δ^{15}N = 1.67‰ ± 3.27. Rainforest habitat – plant: n = 38, δ^{13}C = -29.72‰ ± 2.11, δ^{15}N = -2.54‰ ± 2.64; animal: n = 29, δ^{13}C = -25.81‰ ± 3.27, δ^{15}N = 1.67‰ ± 3.27 (sample types are detailed in Appendix 4.1). Stable carbon and nitrogen discrimination factors were Δ^{13}C = 4.00‰ ± 0.33, Δ^{15}N = 3.10‰ ± 0.5. Elemental concentration was set at plant %C = 0.49, %N = 0.01, animal %C = 0.50, %N = 0.09.

I then calculated the difference in the proportion of animal matter consumed for each pair of kea. This resulted in a pairwise diet dissimilarity matrix of n^2 cross-comparisons of each individual with every other individual, where n is the sample size (e.g., adult kea n = 39). This matrix has a diagonal of 1's where each individual is correlated with itself. Additionally, pairwise morphological

distances were calculated separately for each trait (bill length, head length and weight) and the morphological distance matrix was correlated with the pairwise diet dissimilarity matrix. According to the resource competition hypothesis, I expected a positive correlation, indicating that morphologically dissimilar individuals have dissimilar diets. Statistical significance was determined using 9,999 Mantel test replications. Where significant results were obtained, I partitioned the dataset by habitat and by sex and re-ran the analyses separately to test whether the resource competition hypothesis was supported at the within-cohort, individual level.

I further correlated pairwise morphological distance with the pairwise Euclidean distance between each individual kea's stable carbon and nitrogen isotope ratios. Consistent with the resource competition hypothesis, I expected a positive correlation because pairwise isotopic distance and morphological distance are both measures of dissimilarity. Matrix correlations and Mantel test replicates were conducted as above. Results of correlations are reported as Pearson's *r*. All analyses were run using the R package 'RInSp' (Zaccarelli et al. 2013).

Although the pairwise diet dissimilarity and pairwise isotopic distance are related to one another, they are not equivalent measurements and so their results can be expected to differ to some degree. Here pairwise diet dissimilarity is a measure of how different kea are to one another solely in the proportions of animal versus plant matter in their diet. While this accounts for isotopic variability among different food source, differences in plant or animal species ingested are not examined. However, Euclidean isotopic distance varies depending on factors other than just the trophic level of the consumer, for example, different plant species vary in their stable isotope ratios, photosynthetic plant organs typically differ in their δ^{13}C ratios to non-photosynthetic organs in the same plant, and terrestrial carbon has lower δ^{13}C values than marine carbon. Therefore comparing Euclidean isotopic distance with morphology could potentially reveal dietary differences obscured by the diet dissimilarity metric used here. I used these complementary sources of data here in order to compensate for the weaknesses of each alone.

Results

Morphological and dietary differences across sex and habitat

Male kea from both high-altitude and rainforest habitats had longer bills (high-altitude: t_{36} = 5.39, p <0.001; rainforest: t_{30} = 10.47, p <0.001) and heads (high-altitude: t_{35} = 6.39, p <0.001; rainforest: t_{30} = 3.18, p = 0.003) and were heavier (high-altitude: t_{35} = 5.39, p <0.001; rainforest: t_{29} = 2.05, p = 0.049) than their female counterparts (Table 1). Males sampled in the rainforest habitat had significantly longer bills (t_{36} = 3. 97, p <0.001) and heads (t_{36} = 2.71, p = 0.013) than males from high-altitudes. However, while females in the rainforest also had longer heads (t_{29} = 4.28, p <0.001), their bills were no longer than those of female kea in high-altitude regions (t_{30} = 1.29, p = 0.212; Table 1). Within sex, weights remained consistent across both habitats (male: t_{35} = 0.66, p = 0.516; female: t_{29} = 1.52, p = 0.139; Table 1).

Both males (t_{36} = 9.55, p <0.001) and females (t_{30} = 2.96, p = 0.006) sampled in the rainforest habitat ate more animal matter than their high-altitude counterparts (Table 1). Within the rainforest, males ate more animal matter than females (t_{30} = 5.62, p <0.001), but in the high-altitude habitat there was no difference among the sexes in the amount of animal matter consumed (t_{30} = 1.32, p = 0.195; Table 1).

Relationship between dietary and morphological dissimilarity

For adult kea there were highly significant, positive correlations between their pairwise diet dissimilarity and their pairwise bill (r = 0.19, p = 0.001) and head (r = 0.25, p < 0.001) length dissimilarities, revealing that adult kea with divergent morphologies had dissimilar proportions of animal matter in their diet (Figs. 2A & 2D). This relationship also held true for pairwise isotopic distance - adult kea with dissimilar bill (r = 0.185, p = 0.002) and head lengths (r = 0.23, p = 0.006) also had dissimilar isotopic signatures (Figs. 3A & 3D). Kea of dissimilar weights did not differ in their diet or isotopic distance (r = -0.04, p = 0.274; r = 0.04, p = 0.258, respectively; Table 2) so this trait was not analysed further. For juvenile kea, there was no significant relationship between any

measured morphological trait and dietary or isotopic dissimilarity, therefore their data were not analysed further (r = -0.07, p = 0.152; r = -0.02, p = 0.446, respectively; Table 2).

Pairwise bill length dissimilarity was highly correlated with pairwise diet dissimilarity (r = 0.41, p < 0.001) and isotopic distance (r = 0.28, p <0.001; Figs. 2B & 3B) within kea sampled in the rainforest habitat. This relationship between dietary divergence and difference in bill length was strong, explaining 17% of the variation in diet. In real terms, for kea within the rainforest, this amounted to an increase of 6% in the proportion of animal matter consumed per 1 cm increase in bill length. Partitioning the rainforest dataset by sex revealed that this difference was due to morphological and dietary differences between males and females. Within the sexes there was no individual-level relationship between dissimilarity in bill length and either dietary or isotopic dissimilarity (males: r = 0.02, p = 0.347; females: r = -0.06, p = 0.448; Table 2). Although not as strong, there was also a significant correlation between head length dissimilarity and dietary dissimilarity within the rainforest habitat, which was also attributable to sex differences (r = 0.15, p = 0.034). There was no relationship between bill or head length and dietary or isotopic dissimilarity in the high-altitude habitat (r = -0.06, p = 0.616; r = 0.19, p = 0.125, respectively; Table 2).

Within adult males there was a significant correlation between pairwise head length dissimilarity and pairwise diet (r = 0.29, p = 0.003) and isotopic dissimilarity (r = 0.23, p = 0.012; Figs. 2F & 3F), and marginally significant, positive correlations with bill length dissimilarity (diet dissimilarity: r = 0.15, p = 0.054; isotopic distance: r = 0.14, p = 0.081; Figs. 2C & 3C). These correlations did not hold when the high-altitude and rainforest habitats were investigated separately (Table 2), revealing that the correlations were due to differences in the morphology and diet of kea across habitat. Within adult males, an increase in bill length of 1 cm resulted in an increase of 9% in the proportion of animal versus plant matter in the kea's diet (Table 2). There was no relationship between morphology and diet or isotope ratios for adult female kea.

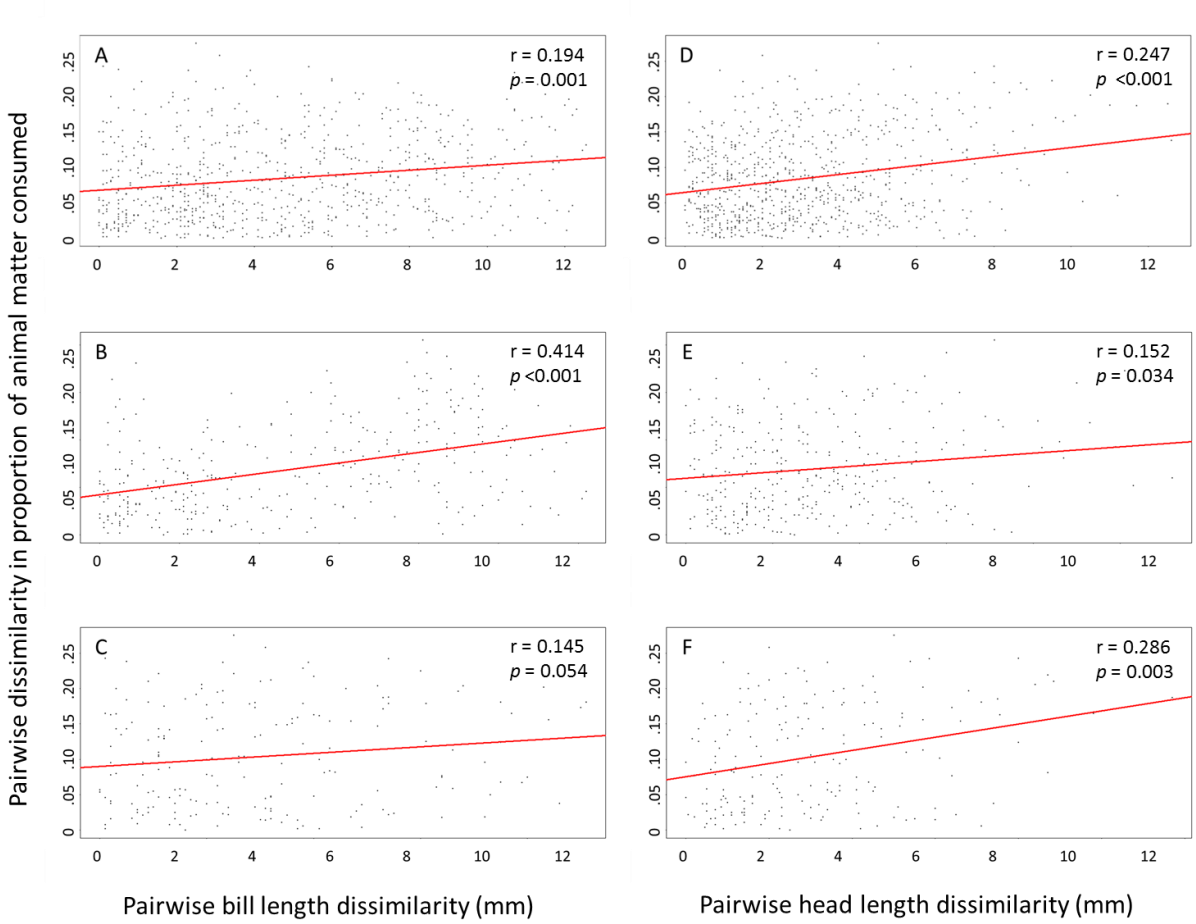

Figure 2. Correlation (coefficient denoted by *r*) between the pairwise dissimilarity in the proportion of animal matter in the diet of adult kea and the pairwise dissimilarity in their bill lengths (A, B & C) and head lengths (D, E & F). A & D illustrate data from the overall population, B & E from kea within the rainforest habitat only, C & F from males only. Statistical significance was determined by 9,999 Mantel-test replications.

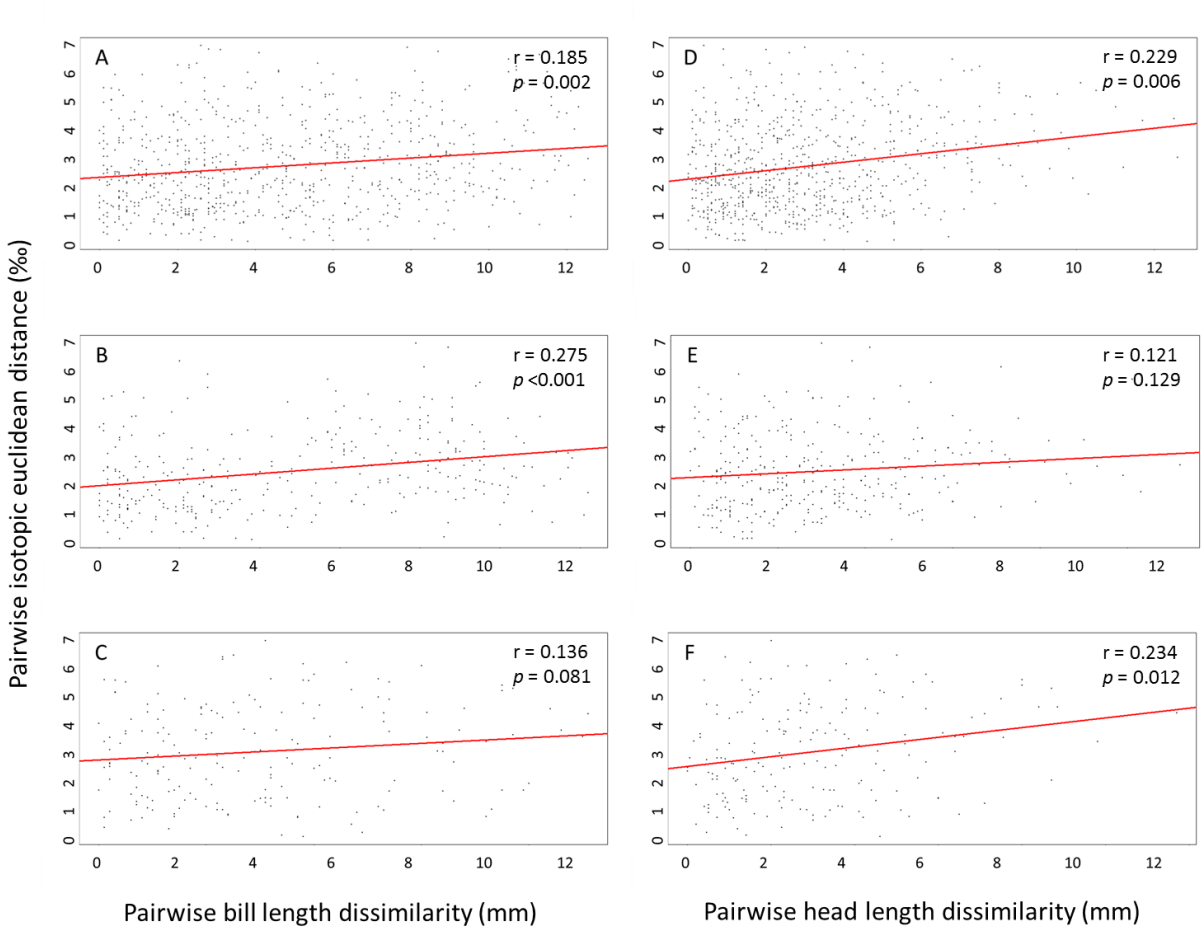

Figure 3. Correlation (coefficient denoted by *r*) between the pairwise isotopic Euclidean distance between feather samples from adult kea and the pairwise dissimilarity in their bill lengths (A, B & C) and head lengths (D, E & F). A & D illustrate data from the overall population, B & E from kea within the rainforest habitat only, C & F from males only. Statistical significance was determined by 9,999 Mantel-test replications.

Table 1. Morphological traits, mixing-model estimated proportions of animal matter in diet, and stable carbon and nitrogen isotope values for each group of kea.

Habitat	**Age**	**Sex**	***n***	**Bill length (mm)**		**Head length (mm)**		**Weight (g)**		**Animal matter**		**δ^{13}C**		**δ^{13}N**	
				$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
High-altitude	Adult	Male	8	48.46	2.21	64.61	1.74	915	105	0.42	0.04	-23.12	0.39	0.27	1.27
		Female	5	43.25	3.13	61.98	1.50	800	33	0.43	0.02	-22.41	0.41	-0.45	0.90
	Juvenile	Male	14	48.13	1.26	65.51	0.97	892	50	0.43	0.05	-23.73	0.79	1.68	2.48
		Female	11	42.28	2.83	62.01	1.57	766	56	0.47	0.06	-23.22	0.84	1.98	1.76
Rainforest	Adult	Male	12	51.04	2.26	67.98	2.12	915	64	0.58	0.05	-20.97	1.43	3.35	0.62
		Female	14	43.73	1.54	64.68	1.70	829	93	0.50	0.04	-22.62	1.15	1.84	1.16
	Juvenile	Male	4	49.88	1.19	64.34	0.19	788	13	0.61	0.02	-20.17	0.61	2.88	0.87
		Female	2	43.45	1.55	64.20	1.90	750	40	0.53	0.04	-23.89	1.71	6.05	1.01

Table 2. Correlations between pairwise morphological distance and either diet dissimilarity or isotopic distance, *p*-values are based on 9,999 Mantel Test replicates, *denotes p-value of <0.05, ** ≤0.01, ≤0.001, § marginally significant <0.1.

					Diet dissimilarity vs. morphological distance				**Isotope distance vs. morphological distance**			
Trait	**Habitat**	**Age**	**Sex**	***n***	**Intercept**	**slope**	***r***	***p***	**Intercept**	**slope**	***r***	***p***
Bill length (mm)	Both	Adult	Both	39	0.068	0.004	0.194	0.001***	2.358	0.084	0.185	0.002**
	Both	Juvenile	Both	33	0.087	-0.000	-0.019	0.439	3.676	-0.055	-0.095	0.097§
	High-altitude	Adult	Both	13	0.042	-0.001	-0.055	0.616	1.582	0.028	0.099	0.194
	Rainforest	Adult	Both	26	0.043	0.006	0.414	<0.001***	1.986	0.104	0.275	<0.001***
	Rainforest	Adult	Male	12	0.052	0.000	0.021	0.347	2.186	-0.073	-0.149	0.285
	Rainforest	Adult	Female	14	1.405	-0.050	-0.062	0.448	2.008	-0.003	-0.003	0.563
	Both	Adult	Male	20	0.090	0.005	0.145	0.054§	2.743	0.095	0.136	0.081§
	Both	Adult	Female	19	1.273	-0.066	-0.120	0.803	2.275	-0.023	-0.029	0.448
	High-altitude	Adult	Male	8	0.432	0.023	0.139	0.230	1.986	-0.077	-0.159	0.266
Head length (mm)	Both	Adult	Both	39	0.065	0.006	0.247	<0.001***	2.296	0.148	0.229	0.006**
	Both	Juvenile	Both	32	0.083	0.001	0.050	0.274	3.459	0.017	0.023	0.362
	High-altitude	Adult	Both	13	0.031	0.003	0.194	0.125	1.464	0.050	0.185	0.119
	Rainforest	Adult	Both	26	0.060	0.003	0.152	0.039*	2.269	0.074	0.121	0.129
	Rainforest	Adult	Male	12	0.057	-0.001	-0.041	0.533	2.234	-0.090	-0.151	0.267
	Rainforest	Adult	Female	14	1.228	0.041	0.052	0.312	1.825	0.084	0.086	0.260
	Both	Adult	Male	20	0.076	0.009	0.286	0.003**	2.522	0.168	0.234	0.012*
	Both	Adult	Female	19	1.246	-0.056	-0.095	0.734	2.274	-0.023	-0.027	0.485
	High-altitude	Adult	Male	8	0.512	-0.007	-0.035	0.451	1.553	0.103	0.167	0.250
Weight (g)	Both	Adult	Both	38	8.783	-2.389	-0.040	0.274	2.693	0.001	0.042	0.258
	Both	Juvenile	Both	32	8.020	6.677	0.072	0.152	3.554	-0.000	-0.016	0.446

Discussion

Even though kea have an extremely broad diet and relatively subtle morphological differences between individuals, it is clear that bill and head morphology is related to diet in this species. Applying a novel approach, I used both the proportion of animal matter consumed and the Euclidean isotopic distance to demonstrate a correlation between morphology and diet. Both data sources revealed extremely similar patterns, which suggests that the relationship between diet and morphology in kea is mainly driven by differences in the trophic level at which they are feeding. If differences in the types or organs of plants consumed were the driving force, one would expect different results from each data source. In the rainforest, the longer bills and heads of male versus female kea corresponded with a greater proportion of animal matter in their diet; as did the longer bills and heads of male kea inhabiting the rainforest versus high-altitude habitat. Having a longer bill and head appears then to confer some advantage to kea when sourcing invertebrates, and may make it easier to excavate invertebrates from trees, extract them from under rocks, or dig them out of the ground.

The relationship between morphological and dietary similarity was remarkably strong in kea, particularly given the behavioural flexibility and influence of learning in this highly intelligent dietary generalist (Auersperg et al. 2010; Chapter 2/Greer et al. 2015). Within the rainforest, sex differences in bill morphology explained 17% of the variance in the proportion of animal matter in their diet; and within adult males, habitat explained 8% of the variance. For comparison, in a study of the relationship between diet and morphology in digger wasps morphological differences explained c. 4% of the variation in diet (Polidori et al. 2012). In their study of sticklebacks - a species in which polymorphisms, differences in diet selection and disruptive selection (Bolnick & Lau 2008) have all been well documented - Bolnick and Paull (2009) noted that the relationship between diet and morphology was surprisingly weak, and explained only 1 or 2% of the variation in diet. Given the well documented niche variation among stickleback polymorphs (Bolnick & Paull 2009), this effect size must still be sufficient to cause resource partitioning among different stickleback morphs. Our results therefore provide clear evidence that the relationship between morphology and diet in kea is strong enough that increased reliance on invertebrate foraging may have resulted in the larger bills

and heads of kea inhabiting the temperate rainforest habitat (Chapter 4); and that resource partitioning among the sexes is a likely maintaining force for the disproportionate sexual dimorphism of the kea's bill (Moorhouse et al. 1999; Chapter 4).

Although juvenile kea experience an increased level of intraspecific competition as a result of congregating into large flocks, I found no link between morphology and dietary divergence in juvenile birds. This is likely because juveniles are more opportunistic than adults in their dietary choices, being more inclined to eat what is readily available rather than seek out particular resources. Compared with adults, immature kea both move about less during foraging, indicating a greater degree of opportunism, and eat less invertebrates and roots, indicating that their extractive foraging skills are still developing (Chapter 2/Greer et al. 2015). Learning plays a significant role in the development of extractive foraging techniques. Juvenile capuchin monkeys *Cebus apella* are much less effective at foraging on larvae encased in bamboo stalks than adults because it takes a lot of experience to learn to select appropriate bamboo stalks (Gunst et al. 2010). The under-developed extractive foraging skills of juvenile kea may explain why there was no discernable relationship between morphology and diet - some young kea with highly suitable morphologies may not yet have become proficient invertebrate foragers, while others with less ideal bill and head lengths may have learned to extract prey more effectively.

That I did not find a significant relationship between diet and morphology at an individual level is perhaps not surprising given the low sample sizes within cohorts in this study (range: n = 8 to 14) and the extremely weak effect sizes noted for this relationship within other species (Araújo et al. 2009; Bolnick and Paull 2009; Agashe and Bolnick 2010; De León et al. 2012; Polidori et al. 2012). In Bolnick and Paull's (2009) study of sticklebacks, sample sizes were far greater: n = 101 for males and 163 for females; yet, within males, no statistically significant correlations were found, a result that the authors attributed to the lower sample size of the male cohort. Darwin's finches are a famous adaptive radiation of species that have different bill shapes and sizes depending on their primary diet (De León et al. 2012). Genetic work suggests that the two different morphs of the medium ground finch *Geospiza fortis* are currently diverging from one another in sympatry due to disruptive selection (De León et al. 2010). When De León and colleagues (2012) investigated the relationship between

morphology and diet selection in 96 individual *G. fortis* they found that although all trends were in the expected direction, none attained statistical significance. Within kea the lack of relationship at the individual level may result in part from noise in individual dietary estimates, the importance of behavioural differences in determining diet, or the importance of unmeasured physiological attributes. Additionally, it is possible that the relationship between diet and morphology only becomes pronounced during times of resource limitation, such as during winter or under high population densities, and may not be significant during the moulting season, when food is abundant.

Overall, these results provide support for the resource competition hypothesis and confirm that kea with more similar bill or head lengths have more similar diets and isotope ratios than dissimilar kea. These correlations were found to be mainly due to morphological and dietary differences among the sexes, and among the high-altitude and rainforest adult kea populations. Surprisingly, given the level of behavioural flexibility common in kea, these correlations explained a higher proportion of the variance in diet and isotopic distance than has been recorded in other species. Future research can investigate if the strength of these relationships increases under conditions of resource limitation.

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CHAPTER SIX

The effect of environmental variables on the isotopic niche of New Zealand's kea

Nestor notabilis

Juvenile kea, Mount Cook (*Photo: Andruis Pašukonis*)

Abstract

Species niche width varies with a number of environmental variables. I investigated how the niche of a highly generalist parrot, New Zealand's kea *Nestor notabilis* varies with differing levels of inter- and intraspecific competition, seasonal resource variability, and dispersal. Using isotopic niche as a proxy for dietary niche, I investigated niche width (using standard ellipse area) and niche shifts (using niche region and overlap metrics) among different populations, sexes and ages of wild kea. My findings contradict the conventional view that interspecific competition has a constraining effect on niche width. Instead, I found that kea had a wider niche under conditions of increased interspecific competition and when providing food for nestlings. I also found the first evidence for sexual segregation in resource use among kea inhabiting high-altitude regions. I propose that in a generalist species, interspecific competition from other generalists may be akin to intraspecific competition and thereby generate resource diversification.

Introduction

The range of environmental conditions within which a species functions, or its 'ecological niche', has far-reaching impacts on its morphology (Svanbäck & Eklöv 2002), its cognitive capabilities (Sol et al. 2005), and its vulnerability to extinction, among others (Kotiaho et al. 2005). The niche concept has become central to the study of ecology since it was defined by Hutchinson (1957) as a region in multidimensional space, where the axes refer to environmental variables or resources (e.g., temperature, possible food sources). A species' niche width is determined relative to those axes, for example, a foraging specialist only exploits a narrow range of possible food sources, whereas a foraging generalist exploits a broad range of foods.

Variables affecting foraging niche width

Five environmental variables are recognised as exerting considerable influence over foraging niche width: interspecific competition, intraspecific competition, temporal variability, environmental heterogeneity, and predation (Futuyma & Moreno 1988). Interspecific competition restricts niche width because some species are 'competitively excluded' from potential resources that are being monopolised by competitors (Hutchinson 1957; Pulliam 2000; Bolnick et al. 2010). An example of this is when native species are marginalised by more competitive invading exotics (Jackson et al. 2012). However, when interspecific competition is reduced, a species may expand its foraging niche to exploit foods previously monopolised by competitors – a phenomenon known as 'ecological release' (Van Valen 1965; Bolnick et al. 2007).

In contrast to interspecific competition, intraspecific competition widens a species' niche (Bolnick 2001; Agashe & Bolnick 2010). A high level of intraspecific competition reduces the overall availability of preferred resources, favouring those individuals able to exploit alternatives (Martin & Pfennig 2010). This may produce intraspecific specialisation on disparate resources, which then increases the niche width of the species as a whole (Araújo et al. 2011). Sexual niche partitioning may evolve to reduce intraspecific competition through the exploitation of different foodstuffs (Radford et al. 2003). For example, whiskered tern *Chlidonias hybrida* males feed by diving into the water,

thereby catching different prey to females, which forage on the surface (Gwiazda & Ledwoń 2014), and male and female purple-throated carib hummingbirds *Eulampis jugularis* have differently shaped bills that are adapted for drinking nectar from different plant species (Temeles et al. 2000).

Overall, foraging niche width "is generally thought to reflect a balance between the diversifying force of intraspecific competition and the constraining effect of interspecific competitors" (Bolnick et al. 2010). However, as both inter- and intraspecific competition affect niche width by limiting food availability, theoretically anything that affects access to food, the need for it, or food availability can also affect niche width. Temporally variable environments tend to favour species that can take advantage of resources as and when they become available (Futuyma & Moreno 1988; Kassan 2002). In this case, generalists or seasonal specialists (e.g., kākā *Nestor meridionalis*; O' Donnell & Rasch 1991) are best able to cope with cyclic differences in food types and abundance. In contrast, environmental heterogeneity, where similar resources are clustered and alternate resources are widely distributed, may promote specialisation, as there is a travel time and energy cost to generalists under such conditions (Ackermann & Doebeli 2004). In addition, the presence of predators can diminish a species' niche width by blocking its access to certain resources (Bednekoff 2006).

In addition to environmental variables, species-specific and within-species attributes also affect niche width. A species may have a feeding apparatus suited to a specific purpose like nectar feeding or nut cracking, which limits exploitation of other resources (Schondube & Martínez del Rio 2003). Animals may also have cognitive limitations that constrain their ability to exploit all available resources, such as only being able to focus attention on gathering one or two food types at a time (Bernays 2001). High levels of neophobia and reluctance to approach a novel resource also limit the likelihood of investigating potentially valuable food sources (Greenberg 1983). An individual's position within a dominance hierarchy can determine its access to highly-prized resources. Finally, those species or individuals with higher rates of dispersal have increased exposure to new food sources and often wider niches (Pulliam 2000).

The unique ecology of New Zealand's kea

The need to reduce both inter- and intraspecific competition has likely played an important role in the kea's (*Nestor notabilis*) evolutionary history and may still have a significant impact on modern kea ecology. Kea are a highly generalist, omnivorous parrot, endemic to New Zealand's South Island (Diamond & Bond 1999). They exploit any potential resource in their high-altitude environment by combining omnivory with a flexible foraging strategy, multi-purpose bill shape, and an extreme degree of neophilia (Diamond & Bond 1999; Chapter 2/Greer et al. 2015). Kea diverged from their sister species, the forest-dwelling kākā, c. 5 MYA when New Zealand's Southern Alps were formed and the alpine niche became available (Wood et al. 2014). Their wide dietary niche likely results from ecological release as kea moved into this new high-altitude region with less interspecific competition, and from the high degree of temporal variability typical of the montane habitat. Evidence for the influence of intraspecific competition on kea ecology comes from morphological differences among the sexes. Male kea have a bill about 14% longer than female kea (Bond & Diamond 1991) and niche partitioning is a likely consequence (or cause) of trophic organ dimorphism (Shine 1989), which reduces intraspecific competition.

Kea have a number of attributes that make them an ideal species in which to study the environmental variables that affect niche width. Due to the wave of extinctions following the arrival of humans in New Zealand there are no extant predators that prey on kea once they have fledged the nest. The New Zealand falcon or kārearea *Falco novaeseelandiae* is theoretically capable of killing a kea, but no successful predation or incidence of kea-eating has been recorded (Diamond & Bond 1999). This is important because predation risk interacts with levels of interspecific competition and resource availability, making the effects on niche width complex and difficult to disentangle (Bolnick & Preisser 2005). Kea are also innovative (Gajdon et al. 2006) and highly intelligent (Auersperg et al. 2009), which suggests few cognitive limitations. Their hierarchy is non-linear, meaning that larger or older individuals cannot necessarily exclude others from accessing highly-prized resources. In addition, there is also likely to be little cost to kea in travel time or effort to obtain alternative resources, as they are a highly mobile, strong-flying species (Kemp 2013).

Crucially for a study of this nature, groups of kea differ in the levels of interspecific and intraspecific competition that they experience. I will investigate two groups of kea from different habitats, high-altitude/montane and temperate rainforest. These groups experience different levels of interspecific, but comparable levels of intraspecific, competition and also incur different degrees of temporal variability. Within the high-altitude habitat, levels of inter- and intraspecific competition vary seasonally, as kea gain access to the mountain-tops and alpine herb-fields that are covered in snow during winter and early spring. Additionally, levels of intraspecific competition and degree of dispersal vary with age in kea, as juvenile birds gather into large, mobile flocks during the moulting season, whereas adults tend to remain alone or in pairs, only joining flocks when they enter their home-range (Wilson 1990, Diamond & Bond 1999).

Studies investigating the influence of inter-and intraspecific competition on niche width in a natural ecosystem are rare (Comas et al. 2014; but see González-Solís et al. 1997; Hsu et al. 2014), as there are many potentially confounding variables in natural systems, as outlined above, and niche width has hitherto been difficult to quantify. However, there are solutions to these problems. The proportions of carbon-13 (δ^{13}C) and nitrogen-15 (δ^{15}N) in animal tissue (e.g., feather, blood) reflect the corresponding stable isotope ratios within the animal's diet. Therefore, the isotopic niche (using δ^{13}C and δ^{15}N as axes) can be used as a proxy for dietary niche, and niche width and position can be easily quantified (as long as the isotopic variability of food sources remains similar across conditions; Cummings et al. 2012). The validity of this approach has been demonstrated using closed systems, such as islands (Rodríguez & Herrera 2013) and lakes (Syväranta & Jones 2007). I will employ recently-developed statistical toolkits that allow meaningful comparisons of niche widths (SIBER: Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011) and niche overlap (NicheROVER: Niche Region and Niche Overlap Metrics for Multidimensional Ecological Niches; Swanson et al. 2015), which are independent of variations in sample size (unlike older metrics e.g., Layman et al. 2007).

Here I take advantage of the unique ecology of kea and newly developed tools for stable isotope analysis in order to investigate how niche width varies in a highly generalist parrot. I will contrast the following six predictions based on the temporal variability, interspecific competition, intraspecific competition, and dispersal hypotheses:

1. Kea living in high-altitude regions will have a wider dietary niche due to greater degree of temporal variability in the high-altitude habitat (*Temporal variability*).
2. Kea in high-altitude habitats will have a narrower niche during the provisioning season (late winter/early spring) than during the moulting season (summer/early autumn) because the level of interspecific competition is higher during winter/spring (*Interspecific competition*).
3. Kea in the rainforest habitat will have a narrower dietary niche during the moulting season than kea in high-altitude habitat, as there is more interspecific competition in the rainforest (*Interspecific competition*).
4. Adult kea in the high-altitude habitat will have a wider niche during the provisioning season as their level of intraspecific competition is higher than during the moulting season (*Intraspecific competition*).
5. Adult kea in rainforest habitat will maintain similar seasonal niche widths as their level of intraspecific competition does not vary greatly across seasons (*Intraspecific competition*).
6. Immature kea will have a wider niche than adults as they experience higher levels of intraspecific competition and disperse more widely (*Intraspecific competition & dispersal*).

Methods

Study Species

Most kea inhabit alpine/sub-alpine regions in New Zealand's South Island (700 to 2000 m a.s.l.), where they feed in the open alpine grasslands, the sub-alpine scrub or the southern beech forests (*Fuscospora* spp. and *Lophozonia menziesii*) that blanket the lower elevations (Robertson et al. 2007). Seasonal variations in weather conditions and food supply are more extreme here than in the lowland temperate rainforest, and semi-permanent snow reaches down to ~1000 m during winter, covering and freezing many food sources (NIWA, 2014a). Kea also inhabit a stretch of temperate rainforest at sea level on the West Coast (c. 50 m a.s.l.). The broadleaf/podocarp mix of vegetation here is dominated by rimu *Dacrydium cupressinum*, kāmahi *Weinmannia racemosa* and southern rātā

Metrosideros umbellata. In contrast with the montane variability, here mean maximum daily temperatures vary by just 8°C across the year and snow is rare (NIWA 2014b).

Kea breed annually, typically laying two or three eggs per clutch in winter (Moorhouse et al. 1999; Diamond & Bond 1999). Eggs take a little over three weeks to hatch and chicks a further 13 weeks to fledge (Jackson 1963). Kea nestlings are fed exclusively by the adult male until they fledge (Jackson 1963). Fledglings retain the feathers they grew in the nest until the next summer when they undergo their first post-fledging moult and kea moult annually from then on. The structure of kea wing moult is highly variable and their moulting season is protracted, lasting from January to May (Davis 2001).

Level of interspecific competition – In the high-altitude habitat, kea forage mainly within the beech forests during winter and spring (provisioning season) but, during summer and autumn, they also exploit the hilltops and alpine herb-fields (Jackson 1960). When foraging within the beech forests, kea have to compete with kākā, kākāriki *Cyanoramphus* spp. and bellbirds *Anthornis melanura*, amongst others, but on the open hilltops, kea have minimal interspecific competition. Young et al. (2012) recorded all foraging observations of bird species at alpine herb-field/hilltop locations during summer and autumn and found that kea accounted for 73% of all foraging observations and almost completely monopolised fruit foraging (95% of observations). In contrast, in a year-round study of the feeding and diet of birds within the rainforest habitat, kea accounted for just 0.4% of foraging observations (O'Donnell & Dilks 1994). This difference cannot be accounted for by differences in population density, so during the moulting season, kea in the rainforest habitat likely experience a far greater degree of interspecific competition than those in the high-altitude habitat.

Level of intraspecific competition – The population density of kea in the high-altitude habitat of Arthur's Pass National Park is estimated at c. one adult female per 250 hectares of beech forest (Kemp 2013). However, during summer and autumn (moulting season), when the alpine herb-fields and hilltops are accessible, this density reduces to c. one adult female per 500 hectares of land. In the rainforest, the kea population density is estimated at one adult female per 500 hectares of forest (Kemp 2013), directly comparable to the level of intraspecific competition experienced by the adult high-altitude population during the moulting season.

Tissue Samples

Feather samples were collected from 136 kea between Dec, 2010 and Oct, 2012. The top 2 cm of a 1st primary (P1) and 10th primary (P10) feather of each bird were clipped and stored in a sealed plastic bag for processing. P1 and P10 feathers were chosen to provide a span of the moult season and their isotopic ratios were later averaged to give a single feather δ^{13}C or δ^{15}N value for each kea. Samples were collected in the high-altitude regions of Arthur's Pass National Park (42°57'S, 171°46'E; 500 – 1,600 m a.s.l.; feather n = 72) and nearby Craigieburn Forest Park (43°6'S, 171°42'E; c. 1,300 m a.s.l.; feather n = 3) and from kea in the rainforest in Okarito and surrounds (43°13'S, 170°10'E; c. 50 m a.s.l.; feather n = 64). Feathers of adult, subadult and juvenile kea represent diet during the moulting season (January to May; Davis 2001). For clarity, I combined results from subadult and juvenile kea into a single 'immature' category, as their niches were extremely similar in both size and position. Feathers from nestling and fledgling kea were grown while being fed by adults in the nest, and so represent diet during the provisioning season.

Sample Preparation

Feathers soaked in 2:1 chloroform:methanol solution for 24 h were rinsed twice in fresh solution and air dried in a fume cupboard for 48 h. The top 1 cm of the inner feather vane was removed and finely clipped. All samples were homogenised and weighed out on an ultra-microbalance (accurate to 0.1 µg; Mettler-Toledo UMX2, Greifensee, Switzerland) to 0.5 - 0.7 mg and inserted into individual 4 x 6 mm tin capsules for mass spectrometer analysis.

Mass Spectrometry

Samples were analysed for δ^{13}C and δ^{15}N using the mass spectrometry protocols laid out in Chapter 3. Isotope values are reported in parts per thousand (‰) as δX, the ratio of heavy to light isotope, relative to the appropriate standard:

$$\delta X = \left(\frac{\mathrm{R_{sample}}}{\mathrm{R_{standard}}} - 1\right) \times 1000$$

where *X* is either ^{13}C or ^{15}N, and R is either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

Statistical Analyses

Measures of Niche Width

To provide a measure of niche width that is insensitive to variations in sample size, I calculated ellipses that contained c. 40% of the data for each compared group of kea (e.g., Jackson et al. 2011). This standard ellipse area (SEA) can be considered to bivariate data what the standard deviation is to univariate data. I employed a small sample size correction (recommended where $n < 30$; Jackson et al. 2011), such that one degree of freedom was removed:

$$SEAc = SEA * (n - 1) / (n - 2)$$

Where SEAc is the standard ellipse area with small sample size correction and *n* is the group sample size. Bayesian estimates of SEAc (Markov chain Monte Carlo simulation with 100,000 replicates) were used to determine if the population niche widths were significantly different. All niche width analyses were carried out in R version 2.15.3 (R Core Team 2013) using the 'SIBER' (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011) routines included in the R package 'SIAR' (Stable Isotope Analysis in R; Parnell et al. 2010).

Measures of Niche Overlap

The degree of SEAc overlap is sometimes used as a quantitative measure of niche similarity among populations (e.g., Jackson et al. 2012). However, geometric calculations of isotopic niche overlap have some associated difficulties. SEAc can be altered from 40% to encompass any percentage of the total niche area, causing the ellipses to overlap to a greater or lesser degree. Moreover, using geometric overlap as a proxy for isotopic niche overlap assumes that individuals are evenly distributed within the ellipse space, which may not be the case. Interpretation also can be difficult; for

example, an overlap of 100% does not mean the populations share exactly the same niche space, as one population may be completely subsumed within the other's much larger space. These issues have recently been addressed within a probabilistic framework. Swanson et al. (2015) describe a method to provide a directional estimate of pairwise niche overlap using stochastic sampling of the group. By repeatedly drawing random A, B pairs from each distribution, the likelihood (%) of finding a randomly selected individual of group A in the isotopic niche region of group B can be determined. The computer codes with which to run these analyses are available in the R package 'nicheROVER' (Swanson et al. 2015). Here, I provide likelihood estimates and 95% Bayesian credibility intervals (CI) of isotopic niche overlap calculated using alpha = 95% and 10,000 sampling repetitions. I categorised probabilities using the following arbitrary breaks: extremely low ≤20%, low ≥21% - 40%, moderate ≥41% - 60%, high ≥61% - 80%, extremely high ≥81%. SEAc overlaps (as calculated using SIBER) are also reported to provide continuity with previous literature.

Niche location

I carried out multivariate analyses of variance (MANOVAs) with $\delta^{13}C$ and $\delta^{15}N$ as dependent variables to establish if kea groups varied significantly from one another in their stable carbon and nitrogen isotope ratios. Increases in $\delta^{15}N$ are generally thought to represent increases in trophic level, whereas differences in $\delta^{13}C$ represent differences in resource pools (DeNiro & Epstein 1978; 1981).

Isotopic variability of food sources

This work is predicated on the assumption that the isotopic variability of kea food sources within the high-altitude and rainforest habitats is the same. To test this assumption I ran Levene's test of equality of variances to compare the variance within stable carbon and nitrogen ratios of plant (n = 122) and invertebrate (n = 29) samples collected from high-altitude and rainforest habitats (Table 1; details of sample collection, preparation and analysis are outlined in Chapter 4). The assumption of equal variances was violated in one instance – plants from the high-altitude habitat were more

variable in their δ^{15}N values than plants in the rainforest habitat (F(1, 120) = 6.74, p = 0.011; high-altitude SD = 3.68, rainforest SD = 2.64).

Table 1. The isotopic variability of stable carbon and nitrogen isotope ratios of food sources in high-altitude and rainforest habitats. * denotes P <0.05.

		δ^{13}C ‰			δ^{15}N ‰		
Food Source	**Habitat**	**Mean**	**SD**	**Levene's Test**	**Mean**	**SD**	**Levene's Test**
Plant	High-altitude	-28.92	2.39	F(1,120) = 0.35,	-4.56	3.68	F(1,120) = 6.74,
	Rainforest	-29.72	2.11	p = 0.554	-2.54	2.64	p = 0.011*
Invertebrates	High-altitude	-25.84	3.02	F(1,27) = 0.49,	2.31	3.00	F(1,27) = 3.42,
	Rainforest	-25.71	4.24	p = 0.492	-0.34	1.47	p = 0.075

Results

Kea, as a whole population, had an SEAc of 11.51. During the moulting season, adult kea sampled in the rainforest had an SEAc >2.5 times the size of that of kea in the high-altitude habitat (Fig. 1A; Table 2). This finding is in contrast to both Predictions 1 (temporal variability hypothesis) and 3 (interspecific competition hypothesis). Kea from these two populations also occupied very disparate isotopic niches, with 0% overlap of the ellipses and a low probability of finding a kea from the rainforest foraging within the high-altitude isotopic niche (Table 3). Adult males and females had similarly sized SEAc's in both habitats, but there was a niche shift between the sexes in that they occupied different niche spaces (Fig. 1B, C; Table 3). This shift was larger in the rainforest population, where the sexes were significantly different in both their δ^{13}C and δ^{15}N values (Table 2) and there was only a low probability of finding males or females feeding in the other sex's niche (Table 3). In the high-altitude habitat, the sexes differed solely on the δ^{13}C axis.

In the high-altitude habitat, the diet provisioned to chicks by adult males had an SEAc 4.5 times the size of that of the diet they ate during the moult (Fig. 1D). This result contradicts the interspecific competition hypothesis (Prediction 2) and supports the intraspecific competition hypothesis (Prediction 4). Adult males did not switch to a completely different niche across seasons, but rather widened their moulting season isotopic niche (Table 3). In the rainforest habitat, the SEAc of the provisioned diet was also marginally larger than the moulting diet (67% greater) and occupied a

similar isotopic niche (Fig 1E; Tables 2 & 3), contradicting Prediction 5 (intraspecific competition hypothesis).

Immature kea from the high-altitude habitat had an SEAc almost three times the size of that of their adult counterparts (Fig. 1F, G). This held true even when restricted to same sex comparisons (adult male: 2.04, immature male: 7.02, p = 0.021; adult female: 1.84, immature female: 5.67, p = 0.084). In high-altitude habitat, the mean δ^{13}C and δ^{15}N values of immature kea were significantly lower (δ^{13}C) and significantly higher (δ^{15}N) than those of adults (Table 2). Although this would seem to indicate a niche shift, in fact, adults were almost certain to be found within the isotopic niche of immature kea (Table 3). Unlike adults, in the high-altitude habitat, immature males and females showed little niche separation (Fig. 1H). There was no difference in niche size between immature and adult kea in the rainforest and there was a moderate to high probability of finding these two age categories in each other's niches (Table 3). When restricted to males only, the size and location of each SEAc remained very similar for kea sampled in the rainforest (adult: 3.34, immature: 3.26, p = 0.583; SEAc overlap: 1.53). The corresponding comparison could not be drawn for females as there was an insufficient sample size for immature females (n = 2). Prediction 6 (intraspecific competition hypothesis) is not supported, as both populations of immature kea experience greater intraspecific competition than their adult counterparts, but only the niche width of juveniles in high-altitude regions is wider.

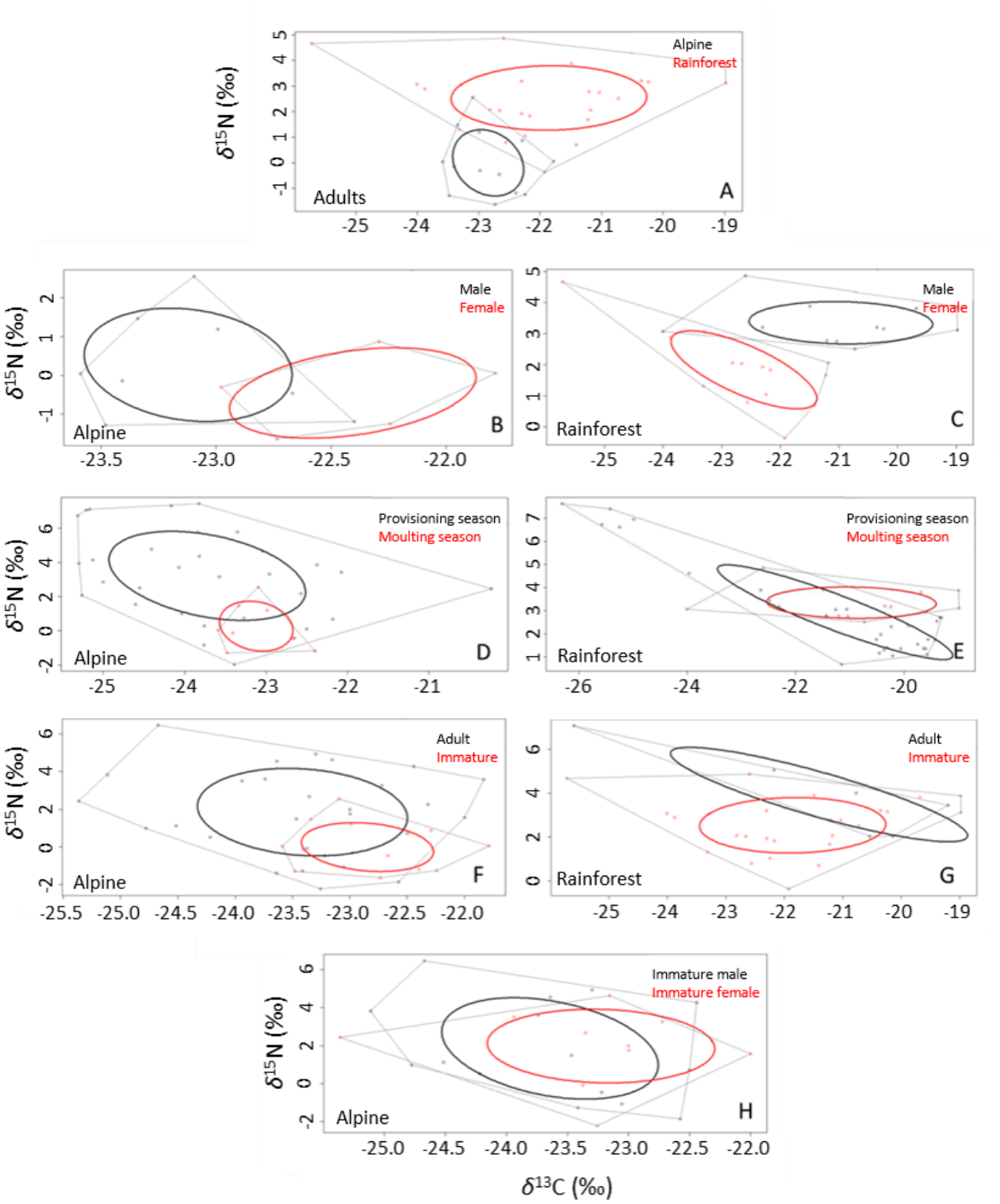

Figure 1. Standard ellipses for each compared group of kea. Black and red lines denote corrected standard ellipses encompassing c. 40% of the data for each group, grey lines denote convex hulls encompassing all data points. A. high-altitude v. rainforest (adults only); B. High-altitude male v. female (adults only); C. Rainforest male v. female (adults only); D. High-altitude provisioning v. moulting season (adult males only); E. Rainforest provisioning v. moulting season (adult males only); F. High-altitude adult v. immature; G. Rainforest adult v. immature; H. High-altitude male v. female (immatures only).

Table 2. Results of multivariate analyses of variance (MANOVAs) for the compared kea groups, along with each group's δ^{13}C and δ^{15}N values and corrected standard ellipse area (SEAc). The SEAc encompasses c. 40% of data points for each group. The probability of each pair of ellipses being the same size (calculated by a Markov chain Monte Carlo simulation with 100,000 replicates) is reported along with the degree of overlap between ellipses. *significant at 0.05, **0.01, ***0.001, †marginally non-significant. Habitat: High-altitude (H); Rainforest (R).

Comparison	**δ^{13}C ‰**				**δ^{15}N ‰**			**SEAc**		**SEAc overlap**
Category & habitat	***n***	***x̄***	***SD***	**MANOVA**	***x̄***	***SD***	**MANOVA**			
Adult (H)	13	-22.85	0.53	$F(1,37) = 4.87$,	-.01	1.19	$F(1,37) = 36.45$,	2.30	$p = 0.008$**	0
Adult (R)	26	-21.86	1.53	$p = 0.034$*	2.53	1.21	$p < 0.001$***	6.31		
Adult male (H)	8	-23.12	0.42	$F(1,11) = 8.31$,	.27	1.35	$F(1,11) = 1.05$,	2.04	$p = 0.441$	0.21
Adult female (H)	5	-22.41	0.46	$p = 0.015$*	-.45	1.01	$p = 0.327$	1.84		
Adult male (R)	12	-20.97	1.49	$F(1,24) = 9.84$,	3.35	0.65	$F(1,24) = 15.14$,	3.34	$p = 0.476$	0
Adult female (R)	14	-22.62	1.19	$p = 0.004$**	1.84	1.21	$p = 0.001$***	3.34		
Moulting season (H)	8	-23.12	0.42	$F(1,37) = 1.96$,	.27	1.35	$F(1,37) = 9.74$,	2.04	p =.0 003**	0.64
Provisioning season (H)	31	-23.72	1.17	$p = 0.170$	3.22	2.52	$p = 0.003$**	9.20		
Moulting season (R)	12	-20.97	1.49	$F(1,42) = 0.20$,	3.35	.65	$F(1,42) = .47$,	3.34	$p = 0.06$†	1.81
Provisioning season (R)	32	-21.27	2.11	$p = 0.657$	2.94	1.98	$p = 0.495$	5.58		
Immature (H)	28	-23.41	0.88	$F(1,39) = 4.38$,	1.85	2.24	$F(1,39) = 7.50$,	6.62	$p = 0.005$**	1.13
Adult (H)	13	-22.85	0.53	$p = 0.043$*	-.01	1.19	$p = 0.009$**	2.30		
Immature (R)	6	-21.41	2.27	$F(1,30) = 0.34$,	3.93	1.92	$F(1,30) = 5.04$,	8.38	$p = 0.366$	1.57
Adult (R)	26	-21.86	1.56	$p = 0.562$	2.53	1.24	$p = 0.032$*	6.31		
Immature male (H)	15	-23.64	0.86	$F(1,24) = 1.48$,	1.85	2.57	$F(1,24) = 0.02$,	7.02	$p = 0.257$	4.07
Immature female (H)	11	-23.22	0.88	$p = 0.236$	1.98	1.84	$p = 0.895$	5.67		

Table 3. The probability of finding a member of each compared pair of kea groups in each other's niche, based on 10,000 sampling repetitions, alpha = 95%.

		Probability of Group A member in Group B niche			**Probability of Group B member in Group A niche**		
Group A	**Group B**	**%**	**Lower CI**	**Upper CI**	**%**	**Lower CI**	**Upper CI**
High-altitude adult	Rainforest adult	57.9	25	90	21.1	7	43
High-altitude adult male	High-altitude adult female	39.6	8	85	56.8	13	97
Rainforest adult male	Rainforest adult female	21.5	4	51	28.7	4	67
High-altitude moulting season	High-altitude provisioning season	96.6	80	100	23.4	10	45
Rainforest moulting season	Rainforest provisioning season	74.2	50	93	47.2	31	69
High-altitude immature	High-altitude adult	43.1	24	67	96.5	83	100
Rainforest immature	Rainforest adult	64.3	33	91	50.2	26	86
High-altitude immature male	High-altitude immature female	77.0	52	96	88.3	64	100

Discussion

Overall, these results indicate that the kea is a highly generalist species with an extremely wide isotopic niche. I found evidence that, contrary to expectations, the kea's isotopic niche (a proxy for dietary niche) widens rather than narrows under increased levels of interspecific competition and only found partial evidence that increased intraspecific competition widens niche width in this species. In spite of the likely role that both interspecific competition (ecological release) and intraspecific competition (sexual dimorphism) have played in the kea's evolutionary history, it seems that other ecological factors have a greater influence on the niche width of modern kea foraging in their native habitats. The results of this study point to the care of nestlings and seasonal changes in food availability as the main environmental variables affecting the kea's dietary niche width; however, it is also possible that an increased level of interspecific competition has an atypical widening effect on the kea's niche.

Adult kea in the rainforest habitat, where interspecific competition is greater, had a far wider isotopic niche than adults in the high-altitude habitat. This is in direct opposition to the predictions of the interspecific competition hypothesis that increased levels of interspecific competition increase niche width. Kea from the rainforest had much higher δ^{13}C and δ^{15}N values, which is indicative of their increased consumption of animal matter, as noted in Chapters 4 & 5. Adult kea from each habitat were unlikely to be found in each other's foraging niche, which indicates a strong degree of fidelity to each habitat type once kea reach adulthood.

The wider isotopic niche of adult kea within the rainforest could be attributed to greater isotopic variability among food sources in the rainforest habitat. However, I tested this degree of variability and found only one difference between habitats, whereby the high-altitude habitat actually had more variable plant sources. An alternative is that the wider isotopic niche of adults in the rainforest habitat could be due to their heavier consumption of invertebrates (Chapters 4 & 5), which may be isotopically more variable than plants. However, again, I tested this assumption and found no difference in the isotopic variability of plant and invertebrate sources. In addition, it should be noted that female kea in the rainforest habitat consume far less animal matter than males (Chapter 4) but

have almost identical niche widths. Therefore, I conclude that this is a real effect and either the increased level of interspecific competition experienced by kea in the rainforest has an atypical widening effect on their niche; or the niche width of adult kea in the high-altitude habitat is particularly narrow during the moulting season, when, due to the super-abundance of food, they may forage exclusively on the most preferred resources. Support for this argument comes from Young et al. (2012), who recorded high-altitude kea feeding from *Podocarpus nivalis* over three times more than any other food source.

Although classical theory predicts that interspecific competition will constrain niche width through competitive exclusion (Van Valen 1965; Pulliam 2000), there are circumstances under which it could have a diversifying effect (Namgail et al. 2009). Remarkably few of the kea's competitors are specialists (O'Donnell & Dilks 1994). This is a typical feature of island ecosystems that may be exacerbated in this case because many of New Zealand's specialist species became extinct after the arrival of humans (e.g., Huia *Heteralocha acutirostris;* O'Donnell & Dilks 1994; Traveset et al. 2015). The actual impact of competition with a large number of other dietary generalists on a generalist species may mimic the effects of intraspecific competition, which typically broadens niche width.

An alternative explanation is that reducing the availability of a valued resource forces the animal to compensate, through increased exploitation of the other resources it currently consumes (as classical theory would dictate), or potentially, if the animal is a generalist and sufficiently explorative or opportunistic, through exploiting new resources, which can mitigate the loss. If more than one alternative resource is incorporated, an animal will have widened its niche as a direct result of interspecific competition. In support of this hypothesis, opportunistic, generalist species of monitor lizards *Varanus* spp. occupy extremely similar niches even when occurring sympatrically, suggesting little competitive exclusion as a result of interspecific competition (Sutherland 2011) and blue sheep *Pseudois nayaur*, a generalist herbivore, maintains its widest niche at moderate levels of interspecific competition (Namgail et al. 2009).

During the provisioning season, kea in high-altitude regions feed predominantly in the beech forest where their levels of both inter and intraspecific competition are higher than during the moult,

when the alpine herb-fields are also accessible (Jackson 1960). In support of the intraspecific competition hypothesis and opposite to the predictions of the interspecific foraging hypothesis, adult male kea in high-altitude habitat maintained a far wider niche during the provisioning season than during the moulting season. However, the niche width of adult males in the rainforest habitat was also wider during the provisioning season, although there is no substantial, seasonal change in the levels of inter- and intraspecific competition in this habitat. Taken together, these results suggest that the additional foraging pressure resulting from the need to provision young causes adult males to widen their dietary niche. Male kea have sole responsibility for feeding the female and young while they are still in the nest (Kemp 2013). Males may be forced to exploit less preferred resources as they seek to provide enough calories to sustain their nesting female and fledglings. Many species differ in their foraging strategy between breeding and non-breeding seasons. However, unlike in kea, the breeding season of many birds coincides with a temporal abundance of valuable resources, which often involves a narrowing of the niche onto preferred resources (e.g., kākā, Powlesland et al. 2009; *Carpodacus* spp., Lu et al. 2011) or sources of nitrogen (e.g., black-headed trogon *Trogon melanocephalus* Riehl & Adelson 2008). The kea's increase in niche width was much more exaggerated in the high-altitude habitat, which may be because, in montane regions, the provisioning season coincides with increased interspecific competition and a major seasonal decrease in food availability.

During the summer and autumn, immature kea gather into large, mobile flocks (Jackson 1960; Kemp 2013) in which their degree of intraspecific competition and dispersal is greatly increased relative to adults. In partial support of the intraspecific competition hypothesis, immature kea in the high-altitude habitat had a significantly wider niche during the moulting season than adults; however, there was no corresponding increase in immature kea sampled in the rainforest habitat. That juveniles in high-altitude regions have a wider niche is somewhat surprising given the findings in Chapter 2 (Greer et al. 2015), which suggests that adults have a broader diet in terms of the number of food types (e.g., roots, fruit, invertebrates) than immature kea. However, adult kea may be carefully selecting only their preferred resources, while juveniles likely select those foods that are readily available, while they learn from experience which are most desirable (Diamond & Bond 1991). The

greater dispersal of immature kea in high-altitude habitats may also account for their increased niche width, as they may ingest a wider variety of foods, or foods with greater isotopic variability, than adults. The degree of dispersal of juvenile kea in the rainforest habitat is unknown, although they also gather into flocks (Jackson 1960; Kemp 2013).

Niche partitioning among male and female kea is suggested by differences in bill morphology and this study provides evidence of sexual niche partitioning in both the high-altitude and rainforest habitats. Adult males from the rainforest habitat occupied a significantly higher niche on both δ^{13}C and δ^{15}N axes than adult females, indicating that they feed at a higher trophic level, as seen in Chapter 4. Here I found the first evidence for sexual niche partitioning within the high-altitude habitat. Adult females in high-altitude regions had higher δ^{13}C, but not δ^{15}N, values than males, indicating that, while the sexes forage at a similar trophic level, they segregate their use of plant resources to some degree. Sexual partitioning only became apparent in adulthood and may reflect the differing physiological needs of the sexes once they have reached breeding age. The non-photosynthetic organs of C3 plants are typically ^{13}C enriched when compared to leaves, so partitioning between plant organs could explain the observed niche shift if males ate more leaves than females (Badeck et al. 2005). However, that male kea would exploit leaves more than females is counter-intuitive, as leaves contain the highest levels of calcium, a valuable resource for females in preparation for egg-laying, and the additional length of the male bill is likely to make it more efficient than the female's for tasks such as digging for roots or extracting larvae rather than plucking leaves. I therefore consider increased leaf foraging by males to be an improbable explanation for the observed difference and consider it more likely that females preferentially forage on different plant species than males, at lower altitudes (Hultine & Marshall 2000), or further abroad of the beech forest canopy (Cerling et al. 2004).

Here I found the first evidence for male and female dietary partitioning in high-altitude kea, providing evidence in support of foraging ecology as a causative or maintaining force for bill sexual size dimorphism in this species (e.g., Moorhouse et al. 1999). In contrast to the predictions of classical theory, I did not find the expected constraining effect of interspecific competition and diversifying effect of intraspecific competition on the kea's resource use. Instead, the kea's niche widened with increased interspecific competition, seasonal decreases in food supply and with the need to provision

young. More research is needed to establish if generalist and opportunistic species can have an atypical reaction to interspecific competition because they may incorporate previously passed-over potential resources; and/or if the effect of interspecific competition within a clade of dietary generalists can mimic intraspecific competition in generalist species.

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CHAPTER SEVEN

Discussion

Kea flying through the Hawdon Valley, Arthur's Pass (*Photo: Andruis Pašukonis*)

The work in this thesis has demonstrated that there is interspecific variation in the foraging ecology of kea *Nestor notabilis* and that this variation has a link with differences in their bill morphology.

Main Findings

By conducting extensive foraging observations and faecal sample analysis, in Chapter 2 (Greer et al. 2015), I demonstrated that there are differences in the foraging ecology of immature and adult kea, of kea in the high-altitude and rainforest habitats, and also seasonal differences. I proposed that the previously estimated proportions of 70% - 95% plant matter eaten by kea (Clarke 1970; Brejaart 1988) do not apply to kea inhabiting Westland temperate rainforest. My results also suggest that the extractive foraging abilities of younger birds do not yet equal those of adults, as they eat less roots and invertebrates. I also found evidence that at least some kea feed in both the rainforest and high-altitude habitats. Finally, I discovered that, in the high-altitude habitat, kea eat more invertebrates in spring, at the time they are raising chicks. This may be how kea maintain their annual nesting cycle, while the other surviving members of the strigops family (kākā and kākāpo) breed only during years of high fruit or seed abundance (masting years; Powlesland et al. 2009).

In Chapter 3 (Greer et al. In Press), I developed a simple and cost-effective methodology to determine stable carbon and nitrogen isotope diet-tissue discrimination factors. I compared the $\delta^{13}C$ and $\delta^{15}N$ values from feathers of a kea population held under their regular conditions at a local zoo, with the $\delta^{13}C$ and $\delta^{15}N$ values from their weekly diet of >30 food items, and presented mass balance calculations that allow researchers to control for the influence of metabolic routing, and zoo animals consuming preferred foods and consuming foods found opportunistically in their enclosure. These discrimination factors are the first to be calculated for a parrot species. Because traditional methods to establish diet-tissue discrimination factors are costly and time-consuming, this work represents a significant advance that will allow the average stable isotope practitioner to determine species-specific discrimination factors rather than relying on generic values established across taxa. As also demonstrated in this Chapter, generic values may be widely different from that of an individual

species so it is of primary importance that researchers should strive to use species-specific values. As the methodology I have outlined can be easily adapted to other species and taxa I hope that researchers will use these simple techniques and discrimination factors for many species will be established.

Also in Chapter 3 (Greer et al. In Press), by sampling simultaneously produced feather and blood tissue from wild kea nestlings, I established regression equations that allow the stable isotope ratios of blood and feather tissues to be compared directly. This will facilitate research that relies on dual-tissue sampling to investigate temporal changes in diet or niche. The carbon stable isotope regression equation for kea and one established for marine birds (Cherel et al. 2014) were remarkably similar, suggesting that $\delta^{13}C$ differences between feathers and blood may be highly consistent across bird taxa in general. However, the nitrogen stable isotope regression equations differed, possibly due to a higher level of cysteine in the diet of marine birds.

In Chapter 4, I used a stable isotope mixing model to investigate differences in the amount of animal matter consumed by kea in the high-altitude and rainforest habitats. Results from both feather and blood samples indicated that in the high-altitude habitat, kea consumed <40% animal matter, whereas in the rainforest their diet was mainly animal-based and kea ate up to 81% animal matter. I also found that within the rainforest habitat, male kea ate significantly more animal matter than females. In addition, I conducted the first comparison of the kea's morphology across populations, revealing that kea in the rainforest had significantly longer bills (male 5.2% longer; female 4.0%) and heads (male 1.9% longer; female 2.5%) than those in nearby high-altitude locations, with no corresponding increase in weight. The increased bill length of male kea and of kea living in the rainforest habitat may improve the efficacy with which they forage on invertebrates, as a longer bill may make ripping through wood or extracting grubs easier. This is the first direct evidence to suggest that ecological factors play a maintaining, or even causative role in the evolution of bill polymorphism within kea.

In Chapter 5, I built on the work done in Chapter 4 by measuring the strength of the relationship that exists between the consumption of animal matter and bill/head length in kea. I found a very strong relationship between diet and morphology across both habitat and sex. My results

provide clear evidence that the relationship between morphology and diet in kea is sufficient that increased reliance on invertebrate foraging may have resulted in the longer bills and heads of kea inhabiting the temperate rainforest habitat; and that resource partitioning among the sexes is a likely causative or maintaining force for the disproportionate sexual dimorphism of the kea's bill (e.g., Moorhouse et al. 1999).

Finally, in Chapter 6, I examined the kea's isotopic niche (a proxy for dietary niche), and compared this across age, sex, habitat and season. I found the first evidence that suggests a difference in the diet of male and female kea in high-altitude regions. Although the niche width and trophic level of male and female kea from high-altitudes was the same, the sexes occupied a different niche space along the δ^{13}C axis, revealing sexual segregation in the resources they consumed. I also found that the kea's isotopic niche is wider during the season in which they are raising chicks, than when they are moulting and that higher levels of interspecific competition may have an atypical widening effect on the kea's niche.

Overall, this thesis represents a significant advance on previous knowledge of the kea's unique foraging ecology, and highlights a number of areas that may prove fruitful for further research.

Best Practice for the use of Stable Isotope Ratios in Dietary and Niche Research

This work has in large part involved using stable isotope analysis to investigate questions concerning diet and niche width in kea. Like all relatively new techniques, researchers are still trying to establish the best and most rigorous protocols for the use of models involving stable carbon and nitrogen isotope ratios. Recently, a paper was published outlining ten best practices for their use (Phillips et al. 2014). Through my own research into the application of such models and a strong drive to conduct the best work possible I managed to address each of these concerns before the publication of these guidelines. I will now outline each best practice guideline in turn, and the steps I took to ensure my work was of the highest possible quality.

Use prior knowledge to identify questions and spatial or temporal scales – While some data were available regarding the kea's diet in the high-altitude habitat and its seasonal variability, until

now there was no information on how the kea's diet may vary across age or sex classes, and very little on the diet of kea within the rainforest habitat. In order to gain the knowledge needed to clearly identify my research questions, I recorded c. 10 h of footage of kea foraging sessions and collected 93 faecal samples. This work resulted in Chapter 2 (Greer et al. 2015), and gave me the data I needed to clarify key areas of interest that would prove fruitful for study with stable isotope analysis.

Consider what is known about the animal's diet – The results of Chapter 2 (Greer et al. 2015) provided sufficient data for me to confidently determine the key food sources on which my specific populations of kea were foraging and to ensure they were included in my sample collection strategy. I collected 84 plant samples from known kea foods in the high-altitude habitat, 38 from the rainforest habitat and 29 invertebrates and deer muscle tissue samples, in order to provide baseline data for kea food sources.

Sample collection: Tissue samples from animals must be carefully selected to reflect the appropriate time period – I analysed feather and/or blood samples from 144 kea of all ages, both sexes and both habitat types. For almost all kea I combined data from two different feathers (1st and 10th Primaries), which together span the season of feather growth. Comparing feathers grown during the moult with those grown by young kea in the nest allowed me to contrast diet during the kea's moulting and provisioning seasons. By collecting blood samples from different periods throughout the year I was able to investigate seasonal variation in diet (Chapter 4). In addition, by collecting both feather and blood tissue from nestling kea I was able to calculate regression equations that allow data from both tissues to be compared directly (Chapter 3/Greer et al. In Press) and represents the first use of this field methodology for a terrestrial species.

Use appropriate diet–tissue discrimination factors – When I began working with stable isotopes I had intended, as is common practice, to use diet-tissue discrimination factors that had been established for birds of a similar size (e.g., American crow *Corvus bruchyrhynchos*; Hobson & Clarke 1992). However, once I began plotting the data points of the first group of kea tissue samples along with their food sources, I realised that there was a serious discrepancy: the kea tissue samples were far lighter in their stable carbon isotope ratios than expected when using a carbon diet-discrimination factor of 1.4‰ (Hobson & Clarke 1992). This (rather uncomfortable) realisation, spurred me to

consider possible methods to establish kea specific diet-tissue discrimination factors. Typically, discrimination factors are determined by holding animals to a catholic diet of one or two food items and comparing their diet and tissue stable isotope ratios; however, ethically this is not possible with kea, which have such a highly varied natural diet. Therefore I developed the methodology described in Chapter 3 (Greer et al. In Press). The results of this additional study showed that kea have an unusually high stable carbon isotope diet-tissue discrimination factor. Incorporating the kea-specific discrimination factors completely reconciled the original discrepancy between the isotope ratios of kea tissues with their diet. Throughout the remainder of this thesis, whereever appropriate, I used these kea-specific diet-tissue discrimination factors rather than relying on literature values that had proved inappropriate for kea. On a personal note, this experience taught me the valuable lesson that sometimes problems should be welcomed, as they can drive progress in unexpected directions.

Plot your data – All data were plotted using stable isotope biplots and further sub-divided in a number of different ways to allow questions of interest to be properly visualised. Plotting my data in this way is what first drew my attention to the problem with using literature diet-tissue discrimination factors.

Include all sources in an informed way – Kea are known to be omnivorous, so comparing the proportion of plant and animal matter in their diet is appropriate for this species. The isotopic variability within different species and organs from plants was too great to allow the sub-division of the plant food source into categories such as root, flower, leaf; or by species.

Consider grouping sources – I grouped the animal sources from both habitats together as they were not significantly different, and kept the plant sources in separate groups as they were different. Statistical significance was determined using a K-nearest neighbour randomisation test, as recommended specifically for use in ecological stable isotope modelling studies (Rosing et al. 1998).

Consider concentration dependence and isotopic routing – I chose to analyse my data with the mixing model MixSIAR (Stock & Semmens 2013), as it allows data on the elemental concentration of food sources to be incorporated into the model. This is particularly important for mixing models involving both plant and animal sources, as animal matter typically contains much higher levels of nitrogen that may distort results if unaccounted for. I accounted for isotopic routing

(in this case the preferential routing of protein from food sources to proteinaceous tissues) when determining kea diet-tissue discrimination factors. However, another factor to be considered is that not all parts of a food are equally digestible. For example, lignin in plants is typically defaecated out rather than assimilated, and there are no data on how digestibility affects kea stable isotope discrimination factors. Therefore, I treated this uncertainty as "an unobservable nuisance" (Phillips et al. 2014) and increased the standard deviation of my discrimination factors to account for the possible influence of differential digestibility of foods.

Consider and incorporate uncertainties – Another advantage of the mixing model MixSIAR (Stock & Semmens 2013) is that it allows uncertainty around the mean to be incorporated into both source data, thereby accounting for the variability of plant and animal stable isotope ratios, and into diet-tissue discrimination factors, as discussed above. Throughout I used standard deviations as a measure of uncertainty.

Report distributions of result – All mixing model results were reported as means and 95% Bayesian credibility intervals throughout this thesis. Probabilities were reported as likelihood estimates and 95% Bayesian credibility intervals. Results of inferential statistics were reported as means ± standard deviations.

Future Research

The population of kea inhabiting New Zealand's temperate rainforest is greatly in need of further study. The degree to which kea disperse between montane and rainforest habitats remains unknown, although in Chapter 2, I found that at least one sub-adult kea must have fed in both habitats (Greer et al. 2015). If some kea disperse into the rainforest from central high-altitude regions, do they then switch to a predominantly animal-based diet? And are they as successful foragers as kea that spent their early years in the rainforest or using both habitats? The greater amount of animal matter in the diet of kea in the rainforest may have knock-on effects on their breeding ecology, as discussed in Chapter 2 (Greer et al. 2015), which also warrants further investigation.

The different foraging ecology and its link with increased bill and head length may point to the seeds of a sympatric speciation within kea. Dussex and colleagues (2014) recently demonstrated, using the neutral genetic marker – microsatellites, that the rainforest population are not a separate sub-species; however, in the early stages of divergence, differences in microsatellites are not expected (Thibert-Plante and Hendry 2010). Darwin's finches represent a classic example of an adaptive radiation where species have different bill morphologies that are suited for exploiting different food types. A population of medium ground finches *Geospiza fortis* has a bimodal distribution of bill size where small morphs feed on smaller softer seeds, and large morphs feed on larger, harder seeds (De León et al. 2012). De León and colleagues consider this species in an intermediate phase of speciation; however, they were unable to identify any differences using microsatellites. As a starting point to determine if an adaptive radiation within kea is a possibility, it would be interesting to investigate to what, if any, degree kea that use both habitat types mate assortatively.

A new technique to analyse the stable isotope ratios of specific amino acids, known as compound specific stable isotope analysis (CSIA) represents a considerable advance in the field of stable isotope ecology. This technique has been used to investigate food web structure (e.g., Chikaraishi et al. 2009), trophic ecology (e.g., Vander Zanden et al. 2014) and which amino acids are responsible for causing the biomagnification of δ^{15}N through the food-chain (e.g., Popp et al. 2007), amongst others. Some amino acids are known as 'source amino acids' as they retain very similar δ^{15}N ratios to those of the animal's diet, which makes them useful to detect the animal's food sources; whereas 'trophic' amino acids increase in δ^{15}N by a consistent amount across each trophic level, meaning that they can be used to identify the trophic level at which the animal is feeding (Evershed et al. 2007). I currently have five kea blood and eleven kea feather samples prepped and awaiting CSIA of carbon and nitrogen, which, due to a lab import permitting issue, I was unable to have processed in time to include as a chapter of my thesis. I will compare the stable isotope ratios obtained for source amino acids to those calculated for the kea's dietary sources, and the trophic levels obtained from individual trophic amino acids with those calculated by the mixing model to verify the accuracy of these approaches. Furthermore, given the very high increase in kea stable carbon isotope ratios relative to their food source, in order to examine why kea have such a high diet-tissue stable carbon

isotope discrimination value I will investigate which amino acids reflect this increase and which are closer to the food source values. To date, there are no published studies that compare the stable isotope ratios of the amino acids in simultaneously produced feathers and blood tissue. I will compare and contrast those amino acids common to both tissue types and those unique to each tissue in order to investigate, firstly, why these tissues vary in their stable isotope ratios and secondly, why there is a difference between marine birds and kea in how much these two tissues differ in their stable nitrogen ratios (Chapter 3/Greer et al. In Press).

It is my hope that the work I have done here will inspire future research into the ecology and evolution of kea, and encourage the protection both of kea overall, and of the unique population in Westland's temperate rainforest.

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