Ecological effects of Tasmanian devil *Sarcophilus harrisii* declines

Submitted by Olivia Charlotte Bell to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences, June 2021.

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

(Signature) Olivia Bell ……

“Knowing trees, I understand the meaning of patience.

Knowing grass, I can appreciate persistence.”

- Hal Borland

Abstract

Species declines, which are both widespread and worsening, affect the ecological dynamics not only of declining populations, but also the species with which they interact. In the case of top carnivores, their marked declines are triggering trophic cascades whereby their structuring influence on communities is lost, resulting in release of prey species from predation pressure and sympatric carnivores from competitive pressure. Changes in competitive pressure both within and between sympatric species are predicted to result in changes in the ecological niches of individuals and populations, according to the niche variation hypothesis. Investigating the niche dynamics of communities experiencing top carnivore loss allows us to test theoretical predictions of how ecological niches respond to competition, as well as furthering our understanding of the role and function of top carnivores.

In this thesis, I have explored the effects of Tasmanian devil *Sarcophilus harrisii* decline, following the emergence of a transmissible cancer, devil facial tumour disease (DFTD). I used stable isotope analysis to characterise the trophic ecology of Tasmanian devils, a top marsupial carnivore, and to investigate the impact of disease and population decline on the trophic niches of devil individuals and populations, and of spotted-tailed quolls *Dasyurus maculatus,* a closely-related sympatric marsupial carnivore.

I first quantified patterns of isotopic variation within a Tasmanian devil population. Using $\delta^{13}C$ and $\delta^{15}N$ values from whisker tissue samples collected from Tasmanian devils at Wilmot, Tasmania, I demonstrated that both $\delta^{13}C$ and $\delta^{15}N$, and group isotopic niche breadth decreased with increasing age in weaned Tasmanian devils. By characterising the isotopic niche breadth of a subset of individuals, I showed that individual niche breadth also decreased with increasing age, and revealed an isotopic signature of weaning in young Tasmanian devils.

Next, I explored the impact of DFTD on the trophic ecology of infected Tasmanian devils. I tested whether DFTD progression, measured as tumour volume, affected $\delta^{13}C$ and $\delta^{15}N$ values of whiskers of Tasmanian devils

collected at six sites across Tasmania. I found isotope values did not change with increasing tumour volume, except at one site, Freycinet, which showed differences in the relative abundance of three common prey species compared to our other sites, based on species distribution models. I also showed that whisker isotope values of individual Tasmanian devils sampled before and after detection of clinical signs of disease do not differ, when compared to healthy control individuals. I conclude that, according to stable isotope analysis, devils do not generally change their diet in response to DFTD but that contextual ecological factors such as prey availability may elicit or allow a change in diet as the disease progresses.

I then used Bayesian stable isotope mixing models to estimate the proportional contribution of prey groups to the diet of Tasmanian devils. I examined variation in $\delta^{13}C$ and $\delta^{15}N$ values from Tasmanian devils and their putative prey species at six sites, and concluded only one site was suitable for mixing model analysis. The results suggested that the devil population at Woodbridge, Tasmania, consumed similar amounts of Tasmanian pademelon *Thylogale billardierii* and small mammals, and fewer Bennett's wallabies *Macropus rufogriseus*. I highlight the importance of further research to quantify trophic discrimination factors in marsupial species, and the difficulty in sampling the prey base of opportunistic carnivores, with large ranges relative to their prey.

Considering the potential impact of Tasmanian devil decline on community niche dynamics, I examined the effect of devil decline upon the population and individual-level isotopic niche breadths of both Tasmanian devils and spotted-tailed quolls. The extent of devil decline, using time since disease arrival as a proxy, had no effect on population level isotopic niche breadths. However, niches of both species were significantly smaller in areas with high coverage of human-modified habitat. I did not find evidence of differences in individual specialisation between sites. I conclude that anthropogenic influences on resource availability have a larger impact on carnivore niches in this system than top carnivore decline.

Finally, I conclude by discussing the key findings of this thesis and placing them within the broader contexts of Tasmanian devil ecology, stable isotope analysis

and ecological niche variation. This work demonstrates the robustness of Tasmanian devil isotopic niches to disease and decline, and shows that ecological context is a key driver of isotopic variation among Tasmanian devils. This research also reveals some of the challenges and opportunities afforded in applying stable isotope analysis to marsupials, particularly in Australia, which is thus far an under-utilised method in this taxon and in this region of the world. Ultimately, the work of this thesis contributes to our understanding of ecological niche dynamics, and highlights the need to consider both community-level and landscape-level perturbations when investigating ecological niches in changing communities.

Acknowledgements

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Contents

Tables and Figures

Author's declaration for co-authored manuscripts

Chapters 2, 3, 4 and 5 have been published or written for publication as authored academic papers. I developed the research design for Chapters 2, 3, 4 and 5 in conjunction with Robbie McDonald, Stuart Bearhop and Menna Jones.

For Chapter 2, Tasmanian devil whiskers were collected by Manuel Ruiz-Avarena (MRA). For Chapter 3, Tasmanian devil whiskers were collected by MRA, Rodrigo Hamede (RH) David Hamilton (DH), Sebastien Comte (SC), Geordie Jennings (GJ) and Samantha James (SJ). For Chapter 4, Tasmanian devil whiskers were collected by MRA, RH, DH, SC, GJ, SJ and Rowena Hamer (RPH). For Chapter 5, Tasmanian devil spotted-tailed quoll and Eastern quoll whiskers were collected by MRA, RH, DH, SC and RPH. I designed all other fieldwork, including prey sample collection (Chapters 2, 4 and 5) and camera trapping (Chapter 5) and conducted it with assistance from volunteers. I conducted all stable isotope sample processing.

For Chapter 2, species distribution models were conducted by Calum Cunningham. I conducted all other statistical analyses in this thesis. I wrote all 4 manuscripts, and amended them in response to suggestions from Robbie McDonald, Menna Jones, Stuart Bearhop and other co-authors.

The cover illustration for this thesis was kindly provided by Cat McNicol. Photographs in this thesis are by myself, RH, DH and SC.

Chapter 1

Introduction

Chapter 1: Introduction

Biodiversity is being lost at an alarming rate. Observed species losses have so outstripped expected background extinction rates that it is clear we are experiencing Earth's sixth mass extinction (Barnosky et al., 2011; Ceballos et al., 2015). Several of the major global drivers of biodiversity loss are related to human activity; including changes in land use, climate change and associated changes in atmospheric CO_2 and nitrogen deposition, and introduction of invasive species to non-native ecosystems (Sala et al., 2000).

Infectious disease represents a real and increasing threat to biodiversity conservation. Pathogens can cause species declines and extinctions, particularly where populations are made vulnerable by anthropogenic environmental change. Famous examples of disease-induced declines include the widespread amphibian declines and extinctions driven by chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis* (Skerratt et al., 2007; Stuart et al., 2004), while white-nose syndrome, caused by the fungus *Geomyces destructans*, has resulted in mass mortality and regional population collapse of bat species in North America (Frick et al., 2010). Pathogens are generally not expected to drive host populations to extinction (Anderson and May, 1992), however some conditions increase extinction likelihood. For example, the transmission rate of some pathogens increases with the fraction of the host population that is infectious, and can therefore continue to cause mortality at low population densities and cause extinction (De Castro and Bolker, 2005; Smith et al., 2009). Extinction also becomes more likely where pathogens have reservoir hosts and are disentangled from single-host pathogen population dynamics. Furthermore, populations driven to low densities by disease may be more vulnerable to extinction through stochastic events or consequences of decreased genetic variability (De Castro and Bolker, 2005; Smith et al., 2009). When populations decline, the drivers of decline can create amplifying feedback loops, interacting cyclically or additively with other drivers of biodiversity loss to intensify pressure upon populations and communities (Brook et al., 2008). Biodiversity loss generally increases disease transmission (Keesing et al., 2010), so when biodiversity has been lost via anthropogenic actions such as land-use change or overexploitation, infectious disease may be

more likely to cause further loss. Then, where disease reduces biodiversity or population sizes, populations will be less resilient to anthropogenic perturbations (Brook et al., 2008). Furthermore, anthropogenic change, including climate change and increased global movement of humans, domestic animals and invasive species, creates conditions for increases in novel pathogen emergence and the spread of existing pathogens into naïve ecosystems (Daszak et al., 2001; Harvell et al., 2002). As humans continue to alter ecosystems, infectious diseases are likely to play an increasing role in wildlife declines.

Ecosystem impacts of species decline

Species declines, whether via infectious disease or other causes, can have wide-ranging effects through ecosystems, altering community structure and function (Eklöf and Ebenman, 2006; Estes et al., 2011; Koh et al., 2004; Paine, 1966). All species interact with others, but consequences of their decline can vary in size and severity depending on their ecological role. Top carnivores, for instance, structure communities by influencing population abundances at lower trophic levels (Beschta and Ripple, 2009; Pace et al., 1999; Ripple et al., 2016). However, top carnivores have undergone widespread and severe declines (Estes et al., 2011; Ripple et al., 2014). The loss of top-down control following carnivore decline can precipitate trophic cascades, defined as “indirect species interactions that originate with predators and spread downward through food webs” (Ripple et al., 2016). This can have severe repercussions for ecosystem conservation, from reductions in biodiversity to changes in disease dynamics (Hollings et al., 2013; Levi et al., 2012; Ostfeld and Holt, 2004), vegetation structure (Beschta and Ripple, 2012, 2009; Letnic and Koch, 2010), human-wildlife conflict (Brashares et al., 2010), and nutrient cycling (Schmitz et al., 2010). Commonly known examples include the sea otters *Enhydra lutris* of the northern Pacific, and the grey wolves *Canis lupus* of Yellowstone National Park. The abundance of sea urchins *Strongylocentrotus* spp*.* and condition of kelp forests in the Aleutian Islands and south Alaska are linked to sea otter abundance (Estes and Duggins, 1995). With increased sea otter abundance, sea urchin populations are suppressed through predation, releasing kelp from urchin grazing pressure, while conversely, in areas of low sea otter abundance,

kelp density is reduced. Similarly, the reintroduction of grey wolves has been linked to a reduction in elk *Cervus canadensis* numbers in Yellowstone National Park, allowing native woody browse species to recover (Beschta and Ripple, 2009; Ripple and Beschta, 2012). Predators are now recognised to influence the abundance of other species in a variety of habitats and ecosystems, and increasingly, through a number of mechanisms.

Top carnivores structure communities by directly altering prey densities through predation, but also by creating a 'landscape of fear' in which prey and smaller predators, also known as mesopredators, alter their behaviour in response to threat from top carnivores (Ritchie and Johnson, 2009; Schmitz et al., 1997; Werner and Peacor, 2003). Ecological cascades resulting from top carnivore loss can therefore be density-mediated, where populations are released from top carnivore predation, and/or behaviourally-mediated, where changes in behaviour as a result of reduced predatory or competitive threat cause changes in fitness in prey or competitor populations (Schmitz et al., 1997; Werner and Peacor, 2003). One behaviourally-mediated and widespread consequence of top carnivore decline is mesopredator release. Top predators can suppress mesopredator populations by outcompeting them for resources, and/or by intraguild aggression and killing (Ritchie and Johnson, 2009). Where top predators are removed, mesopredators may increase in density, distribution or change their behaviour in response, with cascading implications for their prey items or subordinate competitors (Brashares et al., 2010; Prugh et al., 2009). Alongside ecological effects, these trophic cascades can have social and economic consequences, and alter disease transmission. Olive baboon *Papio anubis* abundance in West Africa is correlated with declines in large carnivore species including lions *Panthera leo* and leopards *Panthera pardus* (Brashares et al., 2010). Increased olive baboon abundance in these areas has intensified human-wildlife conflict, as baboons pose a threat to farmers' crops and livestock, resulted in farmers taking children out of school to help protect livestock and crops, and is related to an increase in parasites in local human populations (Brashares et al., 2010). Mesopredator release can also increase the ecological damage caused by invasive species (Prugh et al., 2009), of

which several of the most prolific are mesopredators, including red foxes *Vulpes vulpes*, feral cats *Felis catus* and American mink *Neovison vison*.

The literature describing trophic cascades initially followed traditional food web ecology in focusing on direct consumptive interactions–a predator declines, causing populations of their prey species to increase in size, increasing pressure on their food in turn until the composition of vegetation at the base of the food web is changed (Polis et al., 2000; Polis and Strong, 1996; Strong, 1992). Some of the most striking cascade examples follow this pattern, including the aforementioned wolves and sea otters, likely because of their intuitive simplicity and clarity. However, indirect as well as direct trophic interactions exist between species, and these interactions are sensitive to ecological and environmental context. A growing appreciation of the complexity of top carnivore loss consequences on sympatric species, such as mesopredator release, has widened the scope of trophic cascade research (Ripple et al., 2016). For example, species responding to top carnivore decline may not just increase or decrease their consumption of common prey items, but may alter their trophic behaviour more broadly, including the breadth or evenness of food resources, or the habitats they forage or hunt in, with consequences for species interactions and community dynamics.

Ecological niche partitioning in a biodiversity crisis

The ecological niche was conceptualised by Hutchinson (1957, 1978) as an n-dimensional hypervolume where dimensions include the biological resources exploited by a population (bionomic axes) and the environmental conditions required for population persistence (scenopoetic axes). More recently, Bolnick et al. (2003) highlighted the need for ecologists to consider the total niche width of a population as being a product of both the variation between individuals, and the variation within individuals (Figure 1.1). Generalist populations can be composed of generalist individuals (Type A generalists (Bearhop et al., 2004)), or specialist individuals occupying a small area of the population niche (Type B generalists). Without measuring the extent of individual niche variation, these populations may appear the same, despite ecological differences that have multiple implications, including for intraspecific competition, social interactions

and disease risk. In short, the trophic niche of a population is influenced by the environment in which it lives and forages, the resources it consumes, and the extent of individual specialism or generalism in both of these components.

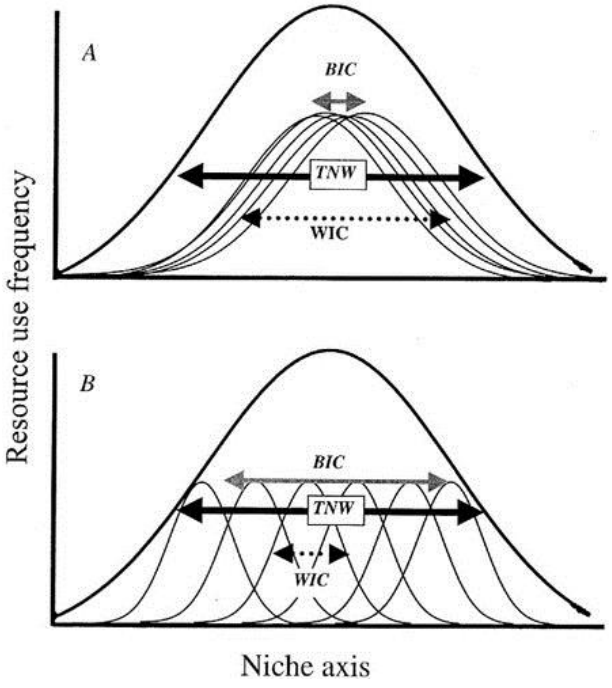

Figure 1.1. A schematic diagram of how individuals can subdivide the population's niche *(thick curve). The total niche width (TNW, black arrow) is the variance of total resource use of all individuals (thin curves). TNW = WIC + BIC, where WIC (dotted arrow) is the average of individual niche widths, and BIC (grey arrow) is the variance in mean resource use among individuals. A, in a population of generalist individuals, WIC is a large proportion of TNW; B, WIC/TNW is small in a population of individual specialists. Although the idealized Gaussian curves used here are a poor description of niche shapes for many real organisms, they usefully convey the concept of between- individual variation. Real populations are likely to contain both generalized and specialized individuals, unlike the schematic diagrams shown here. Bolnick et al. (2002) describe alternative indices that do not rely on assumptions about resource distribution shapes and that can identify variation in individual niche widths. Figure and legend from Bolnick et al. (2003).*

Niche variation

The competitive exclusion principle states that species which compete for exactly the same resources, or occupy identical niches, cannot stably coexist (Gause, 1934; Hardin, 1960). Populations' realised niches generally do not occupy the whole of their fundamental available niche space, therefore populations and individuals can alter their niche based on ecological opportunity or restriction, including the extent of competition experienced. Niche theory predicts intra- and inter-specific competition will affect populations' niches in different ways. Van Valens' niche variation hypothesis posits that intraspecific competition drives niche expansion, which can occur via increased between-individual variation as individuals aim to reduce competition with conspecifics for resources (Van Valen, 1965). Meanwhile, interspecific competition is expected to constrain species niches. Ecological niches can therefore be expected to show responsiveness to shifts in competitive dynamics. Although early empirical work showed mixed support for the niche variation hypothesis (Bernstein, 1979; Soule and Stewart, 1970; Willson, 1969), more recent experimental research has provided supportive evidence. For example, manipulation of three-spine stickleback *Gasterosteus aculeatus* population density demonstrated a positive relationship between intraspecific density and dietary variation among individuals (Svanbäck and Bolnick, 2007), while a reduction in interspecific competitors (cut-throat trout *Oncorhynchus clarki*) increased stickleback population niche width via increased among-individual variation (Bolnick et al., 2010). In wild populations, niche variation will also be sensitive to contextual factors and pressures such as predation or ecological opportunity (Araújo et al., 2011). For example, sea otters show density-dependent individual specialisation in their trophic niches in rocky habitats but not mixed-substrate habitats (Estes et al., 2003; Newsome et al., 2009b; Newsome et al., 2015; Tinker et al., 2008). In mixed-substrate habitats, specialisation occurred at the population level, suggesting differences in prey availability between habitat types constrain the potential for individual specialisation in sea otter populations (Newsome et al., 2015).

Ecological niche variation and the biodiversity crisis

As species decline and community dynamics change, the competitive dynamics within populations and ecological communities will also change. Trophic cascades triggered by top carnivore declines are an example of ecosystem effects driven by changes in predation and competitive dynamics. Trophic cascades therefore provide natural experiments in which to test the predictions of niche theory, as populations and communities will experience changes in the intensity of both intra- and interspecific competition under cascade conditions. Trophic cascades may also take place across areas of ecological heterogeneity, such as different habitat types or levels of anthropogenic disturbance, allowing a comparative understanding of niche dynamics, as well as cascade dynamics, in different contexts. However, understanding ecological niche variation under cascade conditions is also important to develop our understanding of how trophic cascades occur. While predation pressure is a clear mechanism for changes in prey populations following carnivore decline, the mechanisms behind more complex ecological responses, such as mesopredator release, can be harder to clarify. The relaxation of pressure from a competitively dominant species obviously benefits mesopredators, but how exactly? One mechanism may be through an increase in availability of shared prey items following carnivore decline, or another may be increased opportunity to predate new species that were previously dominated by the larger carnivore. Understanding niche dynamics under trophic cascade conditions could increase our understanding of the ecological impacts of species decline on the ecology of declining species in question, and that of the species around them.

Carnivores in Tasmania: devils, decline and disease

Tasmanian carnivore guild

The extant Tasmanian mammalian carnivore guild includes three native marsupial dasyurids: the Tasmanian devil *Sarcophilus harrisii* (males 8.4kg, females 5.4kg; Jones and Barmuta, 1998), the spotted-tailed quoll *Dasyurus maculatus maculatus* (males 3.2kg, females 1.7kg) and the smaller Eastern quoll *Dasyurus viverrinus* (males 1.1kg, females 0.7kg). This guild also included

the Thylacine *Thylacinus cynocephalus* (approximately 25kg), until its extinction in 1936. The feral cat *Felis catus* (males 3.6kg, females 2.8kg; Yamane et al., 1996) was introduced to Tasmania by early European colonisers.

Competition has driven niche partitioning among the native carnivores in Tasmania across evolutionary and recent time scales. Sex and species classes (morphospecies) of native Tasmanian carnivores display character displacement related to bite strength (Jones, 1997), with even spacing in canine and temporalis muscle strength among carnivores from the Thylacine through to Eastern quolls. Accordingly, scat contents analyses suggests size-structured dietary partitioning, with overlap, between the same groups (Jones and Barmuta, 1998). While Tasmanian devils, a top scavenger, predominantly exploit large to medium prey such as Bennett's wallabies *Macropus rufogriseus* and Tasmanian pademelon *Thylogale billardierii,* spotted-tailed quolls exploit a larger proportion of medium-sized prey items including Tasmanian pademelons, birds and small mammals, and Eastern quolls rely largely on smaller mammals, birds, reptiles and invertebrates (Jones and Barmuta, 1998). Niche partitioning also occurs in habitat use (Jones and Barmuta, 2000), as spotted-tailed quolls utilise arboreal habitats to a greater extent than largely ground-based Tasmanian devils, and Eastern quolls appear to use grasslands to a greater extent than either of the larger native dasyurids. Tasmanian devils appear to be competitively dominant in this guild, as spotted-tailed quolls and eastern quolls demonstrate vigilance behaviours in response to devil odour and presence respectively (Andersen et al., 2016; Jones, 1998).

Tasmanian devil decline

Tasmanian devils have experienced severe population declines over the last two decades as a result of a largely fatal transmissible cancer, devil facial tumour disease (DFTD) (Hawkins et al., 2006). First recorded in 1996 in north-east Tasmania, DFTD has since spread to cover at least 80% of the devil's geographic range (Figure 1.2), causing average declines of 80% within five years of emergence in affected populations (Lazenby et al., 2018). DFTD is characterised by locally aggressive ulcerated tumours around the mouth, head and neck (Figure 1.3) (Loh et al., 2006; Pearse and Swift, 2006). Death results

from metastases and organ failure, secondary infection or metabolic starvation, generally within two years of the onset of tumour growth (Pye et al., 2016; Wells et al., 2017). DFTD infection occurs via direct transfer of tumour cells through inflicting and receiving bites, which is common behaviour during feeding and mating interactions (Hamede et al., 2013, 2008; Pearse and Swift, 2006). The transmission pathway of DFTD initially raised fears that the disease might drive devils to extinction, as frequency-dependent diseases such as DFTD lack a threshold density of hosts for disease persistence (Hamede et al., 2008; McCallum et al., 2009). However, rapid evolution of resistance genes, immune responses and occasional tumour regressions in wild devils have been observed (Epstein et al., 2016; Pye et al., 2016). Recent modelling suggests the likelihood of extinction is lower than that of coexistence between disease and host or of DFTD fading out over the next century (Wells et al., 2019). Phylodynamic analyses further suggest that disease spread is slowing (Patton et al., 2020). A second independent transmissible cancer, DFT2, has been recorded in southern Tasmania, but does not appear to have spread beyond a 550km^2 peninsula in south-east Tasmania (James et al., 2019; Pye et al., 2015). Transmissible cancers in wild populations are rare; instances have only been recorded in Tasmanian devils, dogs *Canis familiaris* (Murgia et al. 2006), Syrian hamsters *Mesocricetus auratus* (Cooper et al. 1964), and a handful of bivalves (Metzger et al., 2016, 2015).

Devil facial tumour disease and associated population declines have led to changes in Tasmanian devil life history traits. Disease presence in a population results in a decrease in the proportion of devils 3 years old and over, resulting in a younger population age structure, when compared to healthy populations where devils may be expected to reach 5 or 6 years old (Lazenby et al., 2018; Pemberton, 1990). A 16-fold increase has also been observed in the number of females breeding at 1 year old in populations after DFTD emergence, reflecting a shift from breeding iteroparity towards semelparity due to high mortality rates in adult devils (Jones et al., 2008; Lachish et al., 2009; Lazenby et al., 2018). The occurrence of precocial breeding appears linked to body size rather than age *per se*, suggesting that reduced competition for resources has resulted in an increase in the proportion of female Tasmanian devils reached the critical

weight required for breeding at 1 year old (Lachish et al., 2009). Similarly, reduced competition for resources is thought to have driven a reduction in dispersal rates, especially in females (Lachish et al., 2007).

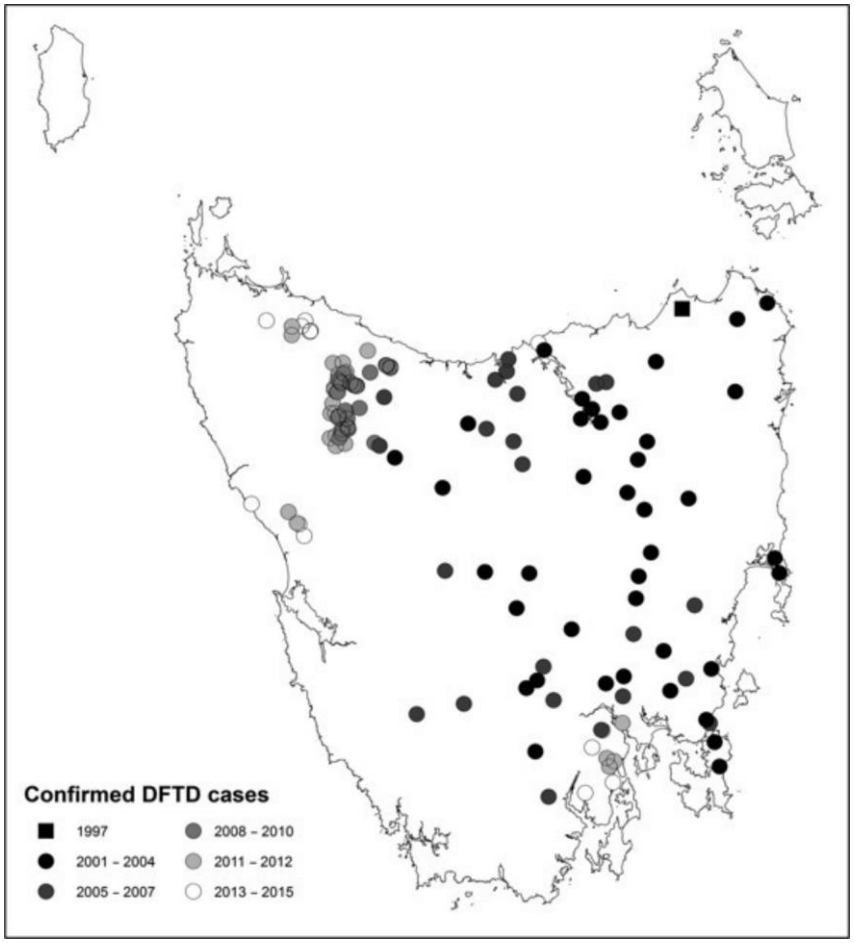

Figure 1.2. Locations with confirmed devil facial tumour disease cases, including year of first diagnosis, by May 2017. *Cases were confirmed via histopathological examination. The black square represents the first confirmed case at Waterhouse. Each circle represents the first confirmed record from an area, with later subsequent records from the same area not shown. Figure and legend from Lazenby et al. (2018).*

Ecosystem impacts of Tasmanian devil decline

Tasmanian devil decline has resulted in mesopredator release. Spotlight surveys from 1985 to 2008 indicate feral cat abundance increases associated

with DFTD arrival, and declines in Eastern quoll populations (Hollings et al., 2014). The response of feral cats to devil decline has been particularly marked in eastern regions, which have experienced the longest and most severe effects of DFTD. While Eastern quoll declines have been largely attributed to unfavourable weather conditions, the proliferation of feral cats following Tasmanian devil declines may have inhibited Eastern quoll recovery (Fancourt et al., 2015). Spotted-tailed quoll abundance appears to be more closely related to herbivore abundance than devil decline (Cunningham et al., 2020). The evidence for density-mediated mesopredator release is geographically inconsistent, with weaker impacts observed in agricultural central Tasmania (Hollings et al., 2014). Besides density-related release, devil decline has caused shifts within the scavenging community; carcasses are discovered sooner, and consumed for longer, by spotted-tailed quolls and forest ravens *Corvus tasmanicus* in areas where devils have declined (Cunningham et al., 2018). Feral cats also demonstrated an increase in foraging behaviour, but scavenging behaviour in general was much lower than for other mesopredators (Cunningham et al., 2018). Temporal partitioning of carnivore activity has also shifted. Where devils are at low densities, devil activity has shifted to later in the evening, presumably due to decreased intraspecific competition driving early foraging in high density populations (Cunningham et al., 2019b). In response, spotted-tailed quoll activity in areas of low devil density shifted forward to early evening from predominantly early morning in areas of high devil density, suggesting competitive release from temporal avoidance behaviours. In contrast, cat temporal activity was not related to devil decline or activity (Cunningham et al., 2019b).

Mesopredator release has had several cascading consequences for native wildlife in Tasmania, including alterations in predation pressure, foraging behaviour and disease-transmission. Where cat density has risen in response to devil decline, this has had a limiting effect on populations of southern brown bandicoots *Isodoon obselus*, although populations of Bennett's wallaby and brushtail possum *Trichosurus vulpecula* increased with time since devil decline (Cunningham et al., 2020). Increased cat density is also positively related to seroprevalence of antibodies for the coccidian parasite *Toxoplasma gondii* in

Tasmanian wildlife (Hollings et al., 2013). Cats are the only known definitive host of the *T. gondii*, however there are many potential intermediate hosts (Hill et al., 2005; Parameswaran et al., 2009), and organisms which have not evolved concurrently with cats and their parasites, such as Australian marsupials, are particularly susceptible to acute infection and mortality following infection (Hartley et al., 1990; Johnson et al., 1989). Tasmanian devil decline appears to have altered the risk-sensitive behaviour of the brushtail possum (Cunningham et al., 2019a; Hollings et al., 2015). On mainland Tasmania, reduced devil density with increasing years since DFTD emergence is associated with possums spending more time foraging on the ground and finding food baits faster in comparison to disease-free areas (Hollings et al., 2015), meanwhile, Tasmanian devil introductions on Maria Island led to reductions in foraging time of possums and higher giving-up densities in experimental food patches (Cunningham et al., 2020).

The spread of DFTD across the island of Tasmania through time has created a gradient in the severity of devil decline, allowing for the research of a carnivore community across a range of ecological contexts. Trophic cascades have been evidenced, including an increase in feral cat abundance in response to devil decline, and implications for disease dynamics and prey behaviour have been recorded, including changing in parasite transmission and temporal activity of predators (Fancourt et al., 2015, 2013; Hollings et al., 2015, 2013). However, the mechanisms behind trophic cascade and mesopredator release in this system are less clearly evidenced. An aspect of Tasmanian mammalian carnivore ecology that may be changing as a result of Tasmanian devil decline is the diet and trophic niche of the relevant carnivore species. In Tasmania, changes in both intra- and inter-specific competition may drive changes in diet and trophic niche width of individuals and populations. The Tasmanian mammalian carnivore guild provides an opportunity to further understand the consequences of carnivore decline for ecological niche partitioning and variation, and further research in this system would benefit from a deeper understanding of the trophic niches of the relevant carnivore species, and how these are responding to Tasmanian devil decline.

Stable isotope analysis and the ecological niche

Stable isotope analysis in ecology

Elements may have multiple naturally occurring stable forms, known as stable isotopes, in which the number of neutrons differ. Natural variation in the ratios of isotopes in abiotic and biotic matter can provide meaningful information used in diverse fields including ecology, earth sciences, archaeology and forensics (Hoefs 1997, Katzenberg & Saunders 2008, Fraser & Meier-Augenstein 2007, Hobson & Clark 1992a). Heavier isotopes, for example ^{15}N compared to ^{14}N, necessarily have a higher energy threshold for participation in physical and chemical processes. This results in isotopic fractionation, in which there is a discrepancy in the ratio of heavy to light isotopes between reactants and products. The resultant variation in ratios of stable isotopes among substrates and tissues can be quantified using isotope-ratio mass spectrometry and expressed in parts per thousand, or 'per mil' (‰) relative to an international standard.

In ecology, particularly in the study of diet, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are the most commonly used stable isotopes (Gannes et al. 1997, Ben-David & Flaherty 2012, Crawford et al. 2008). The isotopic ratios, or signatures, of tissues are the result of the isotopic signatures of the food resources they consume and the physiological processes associated with digesting, assimilating and excreting these resources (Ben-David & Flaherty 2012). Isotopic ratios of carbon in primary producers are heavily influenced by photosynthetic pathway, with C_3 plants exhibiting more depleted $\delta^{13}C$ (-25‰ to -35‰) than those relying on C_4 and CAM pathways (-12‰ to -11‰) (Marshall et al. 2007). Variation in $\delta^{13}C$ can also be affected by environmental variables such as temperature and soil moisture, which affect the water use efficiency of plants (Marshall et al. 2007). As consumers integrate dietary nutrients into their tissues, fractionation results in an enrichment of the heavy isotope compared to the food item, referred to as trophic discrimination factors; this often corresponds to around 1‰ for carbon, but tends to be more noticeable in nitrogen, with around 3‰ enrichment in nitrogen per trophic level (DeNiro & Epstein 1978, DeNiro & Epstein 1981). Causes of variability around these

discrimination factors include (but are not restricted to) body size, diet, metabolic rate, and tissue type (Gannes et al. 1997, Bearhop et al. 2002, Martínez del Rio & Carleton 2012). This predictable increase in $\delta^{15}N$ is widely used by ecologists as a means of investigating the trophic position of consumers relative to one another (Vander Zanden et al. 1997, Layman et al. 2005, Layman et al. 2007). Trophic enrichment in $\delta^{15}N$ can also be used to indicate weaning or starvation, as suckling or starving animals essentially consume tissue of their own species, either by consuming maternal milk or metabolising their own proteins, and thus exhibit elevated $\delta^{15}N$ values (Evacitas et al., 2017; Hobson et al., 1998; Newsome et al., 2009a, Hobson et al., 1993).

Using stable isotopes to study ecological niches

The value of stable isotope analysis lies not as an alternative to traditional dietary analysis methods, but as a tool to address an alternative set of questions. Traditional forms of dietary analysis such as scat and stomach contents analyses enable a qualitative, and semi-quantitative, understanding of a species' food items, but are less appropriate for addressing questions about trophic axes of ecological niches; they do not provide information on the habitats items were foraged in (unless food items are habitat-specific), and it is challenging to build a picture of individual dietary variation through time, as individuals will generally only be sampled once, or scats cannot be attributed to individuals. While stable isotope analysis is not an appropriate tool to identify dietary items where no prior information is known, it can provide information both on proportional consumed diet (bionomic niche axes) and the landscape or habitat in which dietary items were obtained (scenopoetic niche axes) (Bearhop et al. 2004, Newsome et al. 2007). Scenopoetic information can be gathered as $\delta^{13}C$ in consumer tissues is heavily influenced by the primary producers at the base of the food web, alongside environmental factors influencing the water-use efficiency of the primary producers (Marshall et al. 2007), and gradients in $\delta^{15}N$ occur between marine and terrestrial environments, with marine food sources enriched compared to terrestrial sources, and also reflect the degree of environmental eutrophication (Newsome et. al. 2007). How fine-scale this measure is will depend on the scale of the gradient or heterogeneity of the isotope in the sampled environment. Regarding individual dietary variation,

tissues represent diet relative to the turnover of that tissue (e.g. blood), which can be repeatedly sampled, or the length of time taken for an inert tissue, such as keratin, to grow (Bearhop et al., 2004, 2002; Hobson and Clark, 1992a).

The use of stable isotopes to study aspects of ecological niches has been the subject of some criticism, however many of the potential pitfalls of the method can be avoided by applying system-specific ecological knowledge to ensure appropriate sampling protocols and analytical interpretation. Specifically regarding the estimation of isotopic niche breadths, concerns include the potential for physiological processes to disrupt assumed relationships between diet and isotopic ratios in tissues (Ek et al., 2015; Gorokhova, 2018; Karlson et al., 2018) and the ability to quantify aspects of ecological niches using isotopes (Cummings et al., 2012). Physiological process do affect stable isotope ratios in consumer tissues, for example laboratory studies involving invertebrates have demonstrated that isotopic niche metrics can be sensitive to individual variation in growth rate and stress associated with contaminant exposure (Ek et al., 2015; Gorokhova, 2018; Karlson et al., 2018), largely due to variation in discrimination factors. However, in wild systems, the isotopic variation between food sources will often be larger than that introduced by physiological processes, and signals of nutritional stress may be recorded at sampling stage, or be apparent through elevated $\delta^{15}N$. Although isotopic niches are related to ecological niches, they are not exactly equivalent. Some have argued that potential misinterpretations of isotopic niche data mean that using stable isotopes to investigate niches should be avoided. For example, isotopic niches for dietary and habitat specialists can be broader than those of dietary and habitat generalists, due to averaging of isotopic values in generalist tissues and baseline isotopic heterogeneity between habitats (Cummings et al., 2012). Rather than avoid the method, employing appropriate sampling strategies will facilitate appropriate data interpretation. For example, the consumer tissue sampled should be appropriate for the research question being asked. Tissues have different rates of isotopic turnover, and if tissues sampled have a long isotopic turnover, generalists' data will return a narrow, averaged value, perhaps even narrower than specialists, whereas short turnover tissues will demonstrate greater variation among generalists (as demonstrated in Bearhop

et al., 2004). Furthermore, best practise in isotope studies includes the sampling of potential food items across relevant habitats to characterise isotopic baselines, and can be used to avoid erroneous interpretation. Where information on likely food items is lacking, stable isotope analysis may not be an appropriate method until other forms of dietary analyses provide enough information to guide sampling design.

Thesis outline

In this thesis, I apply stable isotope analysis, specifically of $\delta^{13}C$ and $\delta^{15}N$, to investigate niche variation in the Tasmanian mammalian carnivore community. I aim to contribute to the understanding of how carnivore decline impacts species and communities by examining how isotopic niches of populations and individuals respond to the disease-related decline of a top scavenger, the Tasmanian devil. To ground this work in an understanding of isotopic niche partitioning within devil populations, I initially aim to i) quantify variation in the isotopic niches of Tasmanian devil populations, considering the extent and drivers of within- and between-individual variation. As Tasmanian devils are declining due to an infectious disease, particularly one that can disfigure the mouth, jaw and face, there may be physical and physiological drivers of trophic niches in infected devils, so I ii) aim to characterise the impact of DFTD progression on the isotopic niches of infected Tasmanian devils, considering both how tumour volume may affect isotopic signatures and how DFTD infection may alter the course of individual niches. To address these first two aims, I largely use isotopic niche metrics or isotopic signatures in statistical models, which may not reveal the full range of variation in devil populations, therefore I iii) investigate whether variation in the consumption of major mammalian prey species among and within Tasmanian devil populations can be revealed using Bayesian stable isotope mixing models. I then aim to iv) quantify the response of Tasmanian devil and spotted-tailed quoll isotopic niches to the decline of Tasmanian devils across the range of devil decline, considering the response of population and individual niche breadths, and consider the role of other potential ecological drivers of isotopic niches including human disturbance and

prey diversity. This thesis includes four chapters addressing each of these aims, followed by a general discussion.

Following this general introduction, in **Chapter 2**, I analyse variation in the stable isotope composition of Tasmanian devil whisker samples taken from individuals at a single site, Wilmot, Tasmania, from 2014-2017. I analyse the ecological drivers of variation in isotopic signatures among individuals, consider how isotopic niche variation within Tasmanian devil populations affects population niche structure, and analyse the extent of within-individual variation.

In **Chapter 3**, I reveal the impact of devil facial tumour disease infection upon Tasmanian devil whisker $\delta^{13}C$ and $\delta^{15}N$ values and variation. Using whisker samples from infected individuals across 6 study sites, I use a population cross-sectional study to test whether there is a relationship between DFTD tumour volume and isotopic values. I also use an individual-based longitudinal approach to test whether whisker isotope values change after DFTD infection, by comparing a set of individuals sampled before and after DFTD infection with a control set of healthy individuals also sampled on two occasions.

In **Chapter 4**, I test whether Bayesian stable isotope mixing models reveal variation among Tasmanian devils in three main prey species, considering age and DFTD infection status as potential ecological drivers of variation.

In **Chapter 5**, I apply stable isotope analysis to characterise the isotopic niches of Tasmanian devils and spotted-tailed quolls across 5 sites representing a range of stages of devil decline, and the niches of Eastern quolls at 1 of these sites. At the population level, I characterise isotopic niche breadths of each species at each site, and the extent of overlap between them. I then test the potential ecological drivers of between-site and species variation in both isotopic niche breadth and overlap of devils and spotted-tailed quolls, including the time since DFTD infection first occurred at each site. I also quantify individual niche size and individual specialisation in a subset of devil and spotted-tailed quoll individuals at each site.

Chapter 6 is a synthesis and general discussion of the findings of this thesis.

Chapter 2

Age-related variation in the trophic characteristics of a marsupial carnivore, the Tasmanian devil *Sarcophilus harrisii*

Chapter 2: Age-related variation in the trophic characteristics of a carnivorous marsupial, the Tasmanian devil *Sarcophilus harrisii*

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Abstract

Age-related changes in diet have implications for competitive interactions and for predator-prey dynamics, affecting individuals and groups at different life stages. To quantify patterns of variation and ontogenetic change in the diets of Tasmanian devils *Sarcophilus harrisii*, a threatened marsupial carnivore, we analysed variation in the stable isotope composition of whisker tissue samples taken from 91 individual devils from Wilmot, Tasmania from December 2014 to February 2017. Both $\delta^{13}C$ and $\delta^{15}N$ decreased with increasing age in weaned Tasmanian devils, indicating that as they age devils rely less on small mammals and birds, and more on large herbivores. Devils <12 months old had broader group isotopic niches, as estimated by Bayesian standard ellipses (SEA_B mode = 1.042) than devils from 12-23 months old (mode = 0.541) and devils ≥24 months old (mode = 0.532). Devils <24 months old had broader individual isotopic niches (SEA_B mode range 0.492-1.083) than devils ≥24 months old (mode range 0.092-0.240). A decrease in $\delta^{15}N$ from the older whisker sections to the more recently grown sections in devils <24 months old likely reflects the period of weaning in this species, as this pattern was not observed in devils ≥24 months old. Our data reveal changes in the isotopic composition of devil whiskers with increasing age, accompanied by a reduction in isotopic variation both among population age classes and within individuals, reflecting the effect of weaning in early life, and a likely shift from an initially diverse diet of small mammals, birds and invertebrates towards increasing consumption of larger herbivores in adulthood.

Introduction

Intraspecific variation in dietary niches can relate to age, sex or morphological classes, or result from individual specialisation (Polis, 1984; Bolnick et al., 2003), with consequences for individuals, populations and communities. Such dietary niche variation can affect individual fitness, through differences in breeding behaviour and reproductive outcomes (Anderson et al., 2009) or differential exposure to pathogens, parasites and predators (Johnson et al., 2009). Intraspecific dietary variation also has importance for conservation if individuals, sex or age classes are differentially exposed to threats, e.g. use of fisheries discards and entanglement in seabirds (Votier et al., 2010), or in cases where management actions selectively target groups or individuals that have larger impacts upon human interests, e.g. by identifying and managing 'problem individuals' (Swan et al., 2017).

As organisms age, they might experience changes in physiological constraints and competitive pressures, resulting in different realised niches and/or diets at different life stages (Polis, 1984; Werner and Gilliam, 1984). Ontogenetic dietary niche shifts have been well documented in taxa that undergo major morphological, physiological or behavioural shifts during maturation, such as invertebrates, fish and reptiles (Olson, 1996; Reich et al., 2007; Werner and Gilliam, 1984). Mammals experience less extreme ontogenetic changes, yet associated developmental differences in diets will have ramifications for the intensity of intra- and interspecific interactions at different life stages. Clearly, mammals undergo dietary change during infancy, when they are weaned from a diet of maternal milk to solid foods (Geipel et al., 2013; Lee et al., 1991). Even after this point, body size and experience may place additional constraints on diets, particularly for mammalian predators that must find, catch, subdue and consume their prey. Studies of dietary niche development and weaning in mammals have largely focussed on eutherian mammals; both marine (Knoff et al., 2008; Newsome et al., 2009a; Orr et al., 2012; Riccialdelli et al., 2013), and terrestrial (Fahy et al., 2014; Geipel et al., 2013). In contrast, relatively little is known about within-species age-related variation in dietary niches in marsupials (Martins et al. 2008, Albanese et al. 2012).

Tasmanian devils *Sarcophilus harrisii* (Figure 2.1) are the largest extant carnivorous marsupial (Order Daysuromorphia) and are now restricted to the Australian island state of Tasmania. As the largest terrestrial mammalian predator in Tasmanian ecosystems, Tasmanian devils (hereafter, 'devils') exert top-down influences on the behaviour and abundance of sympatric mesopredators and herbivores (Cunningham et al., 2018, 2019a; Hollings et al., 2014, 2015). Since 1996, populations of Tasmanian devils have experienced severe decline associated with the emergence of devil facial tumour disease (DFTD), a transmissible cancer, which is fatal in most cases (Loh et al., 2006; Pearse and Swift, 2006; Pye et al., 2016; Pyecroft et al., 2007). DFTD is characterised by tumours around the face, neck and mouth (Hawkins et al., 2006). Transmission occurs through injurious biting interactions, mostly during the mating season (Hamilton et al., 2019), with mortality of most infected adults by three years of age (Lachish et al. 2007; McCallum et al. 2009). Increased juvenile growth rates in response to reduced density in affected populations means that about 50% of female devils are able to reach sexual maturity in their first year of independent life, instead of at two years old (Jones et al., 2008; Lachish et al., 2007, 2009). The location of DFTD tumours on the mouth and face means that starvation can be a cause of death in DFTD-infected devils, alongside metastasis-related organ failure and secondary infections (Loh et al., 2006). Through disruption to normal feeding ability and metabolic demand as tumours grow in size, DFTD progression may cause changes in the diets of infected devils, with eventual implications for predator-prey and competitive interactions. Furthermore, individual dietary specialisation in healthy individuals might translate into differences in susceptibility to DFTD, if different diets carry different risks of competitive encounters and potential disease transmission. Information on intraspecific dietary variation in devils is therefore desirable to consider potential interactions between DFTD, devil population decline, feeding and community ecology.

***Figure 2.1. Tasmanian devil* Sarcophilus harrisii**, *showing the large vibrissae (whiskers) used in stable isotope analysis of variation in isotopic niche. Photograph Olivia Bell.*

Tasmanian devils are facultative scavengers. Although they are usually solitary, they often feed communally on larger carcasses, making such episodic feeding an important arena of conspecific interaction, competition and, potentially, disease transmission. Scat content analyses suggest devils primarily eat the macropodid herbivores: Bennett's wallabies *Macropus rufogriseus* (average mass of males = 20kg, females = 14kg) and Tasmanian pademelons *Thylogale billardierii* (males = 7kg, females = 4kg) (Strahan, 1983; Andersen *et al*., 2017a; Jones and Barmuta, 1998). Jones and Barmuta (1998) identified dietary differences between juvenile, subadult and adult devils that were associated with age-related body size and climbing ability (Jones and Barmuta, 2000); younger devils ate more birds and small mammals (categorised as antechinus *Antechinus* spp. to sugar glider *Petaurus b. breviceps* ~35-140 g) and fewer large mammals (Bennett's wallaby to bare-nosed wombat *Vombatus ursinus*, ~14-35 kg). Seasonal differences in diet were also found, with devils, particularly males, eating larger prey items in summer compared to winter (Jones and Barmuta, 1998). This is the extent of knowledge of intraspecific dietary variation in devils, and to our knowledge no study has investigated dietary variation at the individual level.

Ontogenetic change in devil diets could be driven by behavioural or morphological development. As devils mature, they encounter multiple food types and gain foraging experience, which could increase foraging efficiency and selectiveness. For example, adult European hedgehogs *Erinaceus europaeus* exhibit narrower dietary niches than juveniles and tend to feed on larger prey, likely due to greater experience and more efficient foraging (Dickman, 1988). A relaxation of anatomical constraints in body size and morphology, could also cause devil dietary niche characteristics to change with age. Tasmanian devils have one of the strongest bite forces (relative to body mass) of any extant predator, with strong canines and musculature enabling the delivery of crushing bites and consumption of bone (Attard et al., 2011; Jones, 1997; Wroe et al., 2005). Another specialised mammalian scavenger, the spotted hyaena *Crocuta crocuta*, does not achieve full adult skull development until after sexual maturity, due to the strong skull morphology and musculature

required for cracking through bone, crucially restricting feeding speed in juvenile and young hyaenas (Tanner et al., 2010). Younger Tasmanian devils may be less capable of killing and/or consuming larger prey species, placing younger devils at a disadvantage, as large carcasses can be a focus for intraspecific competition (Hamede et al., 2008; Jones, 1995; Pemberton and Renouf, 1993). Therefore, both behavioural and anatomical disadvantages may lead young devils to incorporate different, smaller or more diverse prey types into their diets, when compared to adults.

Stable isotope analysis has been widely applied to describe intra-specific variation in resource use, including ontogenetic dietary changes (Inger et al., 2006; Newsome et al., 2009a; Hammerschlag-Peyer et al., 2011; Vales et al., 2015), sex-related differences in resource use (Phillips et al., 2011; Stauss et al., 2012) and individual specialisation (Bodey et al., 2018; Newsome et al., 2009b; Patrick et al., 2015; Robertson et al., 2014). Ratios of stable isotopes, $\delta^{13}C$ and $\delta^{15}N$, in consumer tissues reflect those of their food resources, albeit with alteration following physiological processes associated with digesting, assimilating and routing these resources (Bearhop et al., 2002; Crawford, McDonald and Bearhop, 2008; DeNiro and Epstein, 1978; Hobson and Clark, 1992b). In broad terms, carbon isotopic signatures ($\delta^{13}C$) are influenced by the photosynthetic pathway of resources at the base of the food web, alongside environmental variables, while nitrogen signatures ($\delta^{15}N$) become enriched through the food web, reflecting an organism's trophic level. For example, weaning juvenile mammals are expected to exhibit higher $\delta^{15}N$ than older individuals, as suckled young are feeding on milk derived from maternal tissues and so their apparent trophic level (inferred from stable isotope analyses) should reduce upon weaning (Evacitas et al., 2017; Hobson and Sease, 1998; Newsome et al., 2009a; Orr et al., 2012). Isotopic niches of individuals and groups can be used as a proxy for elements of the ecological niche (Bearhop et al., 2004; Newsome et al., 2007; Layman et al., 2012; Sheppard et al., 2018), as stable isotopes reflect the resources consumed and habitats used to obtain resources (bionomic and scenopoetic niche axes respectively) and variability in these both within and among individuals (Bolnick et al., 2003). Furthermore, a time series of information for individuals can be generated by repeat sampling

of tissues or, less invasively, by subsampling along the length of inert tissues, such as whiskers, that grow continuously and so reflect diet at the time of their synthesis. Importantly, stable isotope analysis also reflects assimilated diet, thereby incorporating easily digestible food sources that might be missed, or differently represented, by stomach and scat contents analysis of undigested or hard parts. In the case of Tasmanian devils, where multiple scat contents analyses have reached similar conclusions regarding the primary diet of devils across multiple years and study sites (Jones and Barmuta, 1998; Andersen et al., 2017a), stable isotope analysis enables further insight into the dietary niche characteristics of devil populations and individuals.

Using whisker samples collected from adult and subadult Tasmanian devils at a site affected by DFTD, we applied stable isotope analysis to quantify variation in the isotopic trophic niches of Tasmanian devils in three ways: (1) among-individual variation, (2) population niche structure and (3) within-individual variation, in each case considering potential drivers of such variation, including age.

Methods

Sample collection

Ninety-one Tasmanian devils were sampled during trapping surveys undertaken every three months between December 2014 and February 2017 at Wilmot (41°23'07.8"S 146°10'02.9"E) in north-west Tasmania. The Wilmot field site is 39.92 km^2 in area and is composed of 28% native eucalypt forest, 42% eucalypt plantation, 28% agricultural land and 2% other vegetation.

One whisker was collected from each devil by cutting close to the skin with scissors. Devils were individually identified by insertion of microchip transponders. Sex and weight were recorded. DFTD status was recorded based on visual diagnosis of clinical signs (tumours on the face, neck or in the oral cavity) (Hawkins et al., 2006). Devils were assigned a year of birth using canine eruption, molar eruption and tooth wear, which is accurate until the animal reaches 3 years of age (M.E. Jones, unpublished data). Tasmanian devils mate in late February/early March (Hesterman et al., 2008), with birth following several weeks later; therefore all devils were given a standardised birth date of 1st April so that age in months could be approximated.

Whisker growth rates

Stable isotope analysis of whiskers involves subsampling whiskers into sections. To estimate the approximate time interval represented by a single whisker and the subsections, we measured whisker growth rates in a feeding trial with five captive Tasmanian devils (of a range of ages from 1.5 to 7 years old), held at Bonorong Wildlife Sanctuary, Tasmania. We fed the devils baits laced with Rhodamine B, which is a biomarker that is integrated into keratinous tissue after ingestion, leaving a distinctive band that can be viewed under a fluorescence microscope (Fisher, 1999). Devils were given a RhB dose of 50 mg/kg body weight, in a gelatine capsule hidden inside a dead day-old chick. After a minimum of two weeks, two whiskers per devil were removed under general anaesthetic during routine veterinary assessments. One devil underwent this procedure three times over a 9 month period, two twice and two only once. Therefore, a total of 18 whiskers from five devils sampled over three

occasions, were examined under a fluorescence microscope and growth rate (in mm per day) was calculated by measuring the distance between the base of the whisker and the basal edge of the fluorescent band, dividing this by the number of days between bait consumption and whisker sampling.

Laboratory procedures

Whiskers collected for isotope analysis were rinsed with distilled water, air dried and placed in a freeze-drier for 24 hours. Chopped whisker pieces with total mass of 0.7 ± 0.1 mg were weighed into tin cups for analysis. To investigate among-individual variation and population niche structure, the basal section of each whisker was used as this likely represents the season in which devils were captured. To investigate within-individual variation, the whiskers of 14 individuals were sampled in their entirety, resulting in between 9 and 15 subsamples per whisker, each representing periods further back in time, moving from the recently grown whisker base to the older whisker tip. Isotope analyses were conducted using a Sercon Integra-2 elemental analyser isotope-ratio mass spectrometer at the University of Exeter, and Thermoquest EA1110 elemental analyser linked to a Europa Scientific 2020 isotope ratio mass spectrometer at Elemtex Ltd, Cornwall UK. In both instances, samples were scale corrected using USGS40 and USGS41 with additional internal standards of bovine liver (University of Exeter and Elemtex Ltd) and alanine (University of Exeter only). Averaging across standards and laboratories, precision was 0.11‰ ± 0.02 (1 standard deviation ± standard error) and 0.13‰ ± 0.02 for $\delta^{13}C$ and $\delta^{15}N$ respectively.

Statistical analysis

All analyses were conducted in R Version 3.5.2 (R Core Team, 2018).

Among-individual variation

To test the correlates of isotopic variation among Tasmanian devils, linear models were fitted with $\delta^{13}C$ and $\delta^{15}N$ as response variables. Models included the fixed effects: age (in months), sex, DFTD infection status (binary), season and year, as well as interactions between season and year, age and sex, age

and DFTD infection, and sex and DFTD infection. Season was categorised as summer (1st October to 31st March) or winter (1st April to 30th September). Year was fitted as a factor with each year (1, 2, 3) beginning on the 1st October, to reflect yearly trapping cycles starting from October 2014 onwards. Linear models were fitted with Gaussian error structure and identity link, and top model sets were generated using the R package MuMIn (Barton, 2018) and selected on the basis of increases in the Akaike information criterion corrected for sample size (AIC_c), where $\Delta AIC < 2$.

Population niche structure

As our linear models revealed the importance of age in influencing variation in both $\delta^{13}C$ and $\delta^{15}N$, the population was divided into three age classes: age class 1 (subadults <12 months, n = 21), class 2 (subadults of 12-23 months, n = 31) and class 3 (adult devils ≥24 months, n = 39). Subadult devils were divided into these two age classes, as subadults under 12 months are certainly immature, whereas occurrences of precocial breeding can occur from 12 months in populations with high prevalence of DFTD (Jones et al., 2008; Lachish et al., 2009). Age class 1 included only two devils (both 8 months old) that would not have been weaned and independent of their mother upon capture. To estimate isotopic trophic niche areas for each age class, Bayesian standard ellipses (SEA_B) were fitted around bivariate $\delta^{13}C$ and $\delta^{15}N$ data from basal whisker sections using the R package SIBER (Jackson et al., 2011). Calculations of SEA_B provide an estimate of uncertainty, with greater uncertainty where sample size is small. We also calculated standard ellipses corrected for sample size (SEA_C); this niche metric captures similar niche properties as SEA_B, but is better suited to graphical depiction of niche variation. Dispersal is male-biased in Tasmanian devils, and genetic evidence indicates female dispersal has reduced in frequency or distance in areas affected by DFTD (Lachish et al., 2011), so Bayesian standard ellipses (SEA_B) were also fitted for the two sexes separately in each age class to assess whether sex and dispersal relates to any differences in niche characteristics between age classes.

Within-individual variation

To examine within-individual variation, we fitted Bayesian standard ellipses (SEA_B) for 14 individual devils, using bivariate $\delta^{13}C$ and $\delta^{15}N$ datapoints generated by sampling all sections of the individuals' whiskers. For this analysis, individuals were divided into two age classes: 'subadult' (<24 months, n = 7) or 'adult' (≥24 months, n = 7). Two, rather than three, age classes were used for this analysis due to small sample size. Broad isotopic niches in this instance could suggest a generalised diet through the period reflected by our data, or could reflect gradual shifts in diet over time. To examine how the isotopic composition of individual whiskers varied through time, we sequentially plotted the mean of the two most basal whisker sections, the two mid whisker sections, and the final two sections at the tip of the whisker.

Results

Whisker growth rates

Of 18 whiskers from 5 individual Tasmanian devils, 13 displayed a fluorescent band, with an average growth rate of 0.48 mm/day (range 0.27-0.69 mm/day). The remaining 5 whiskers showed fluorescence at the very base of the whisker, suggesting whiskers are retained for a period of time after growth is complete. As fewer than one third of the whiskers were retained, this is likely to occur for a relatively short period of time. Our sample size was too small to test any potential relationship between age and whisker growth rate. Applying this growth data to the 14 whiskers fully subsampled to analyse within-individual variation, our data represent between 148 and 264 days of growth (mean 194 days), or approximately 5-9 months of diet, with each subsection representing a median of 10 days growth (95% quantiles = 6-46 days). Applying this growth rate to basal whisker sections sampled to investigate among-individual variation and population niche structure, the median time represented by each section is 6 days (95% quantiles = 4-13 days). Assuming a constant rate of growth, subsections of 14 whiskers fully sampled to investigate within-individual variation represent a greater median number of days comparative to the basal sections sampled to investigate among-individual variation and population niche structure, as whiskers become thinner from the base to the tip, therefore subsections also become longer.

Among-individual variation

For 91 individuals, $\delta^{13}C$ values ranged from -25.7‰ to -23.3‰ (mean -24.5‰), while $\delta^{15}N$ values ranged from 6.3‰ to 9.7‰ (mean 7.8‰). Age and year were important correlates of variation among devils in both $\delta^{13}C$ and $\delta^{15}N$ (n = 90 after removal of missing values) (Table 2.1). Only one model, which included age and year, featured in the top model set for $\delta^{13}C$. Age and year were retained in all four top models of variation in $\delta^{15}N$ (Relative Variable Importance = 1 for both variables), with season retained in three (RVI = 0.79), and sex (RVI = 0.20) and an interaction between the effects of season and year (RVI = 0.17) each featuring in only one model. Both $\delta^{13}C$ and $\delta^{15}N$ decreased with

increasing age (Figure 2.2). As devil reproductive ecology means the youngest devils (<12 months) could only have been trapped in summer, we checked for correlation between age and season, however effects were consistent between single term models and models with both terms included (Table 2.1). $\delta^{15}N$ steadily increased through the three study years, and $\delta^{13}C$ increased in year 2 compared to year 1, with year 3 sitting between the two earlier years.

Table 2.1. Summary of analyses of variation in stable isotope ratios of Tasmanian devil whiskers from Wilmot, Tasmania*. Basal sections of whiskers from 90 Tasmanian devils were analysed for $\delta^{13}C$ and $\delta^{15}N$. The table summarises the top model set for each general linear model of variation in $\delta^{13}C$ and $\delta^{15}N$, where top models sets were restricted to models with ΔAICc < 2. Effect sizes are reported for retained variables in each model. Relative variable importance (RVI) is shown for $\delta^{15}N$ as multiple models had ΔAICc < 2.*

Response	Model Rank	Intercept	Year		Age	Season	Sex	Season:Year		DFTD status	df	logLik	ΔAICc	weight
			Year 2	Year 3				Season: Year 2	Season: Year 3					
$\delta^{13}C$	**1**	-24.63	0.47	0.13	-0.41						5	-39.11	0	1
$\delta^{15}N$	**1**	7.61	0.43	0.25	-0.36	-0.25					6	-69.29	0	0.36
	2	7.62	0.36	0.33	-0.32						5	-71.10	1.33	0.18
	3	7.61	0.43	0.25	-0.34	-0.24	0.11				7	-68.81	1.40	0.18
	4	7.62	0.47	0.26	-0.36	-0.18		-0.21			7	-68.98	1.73	0.15
	RVI		1	1	1	0.79	0.20	0.17						

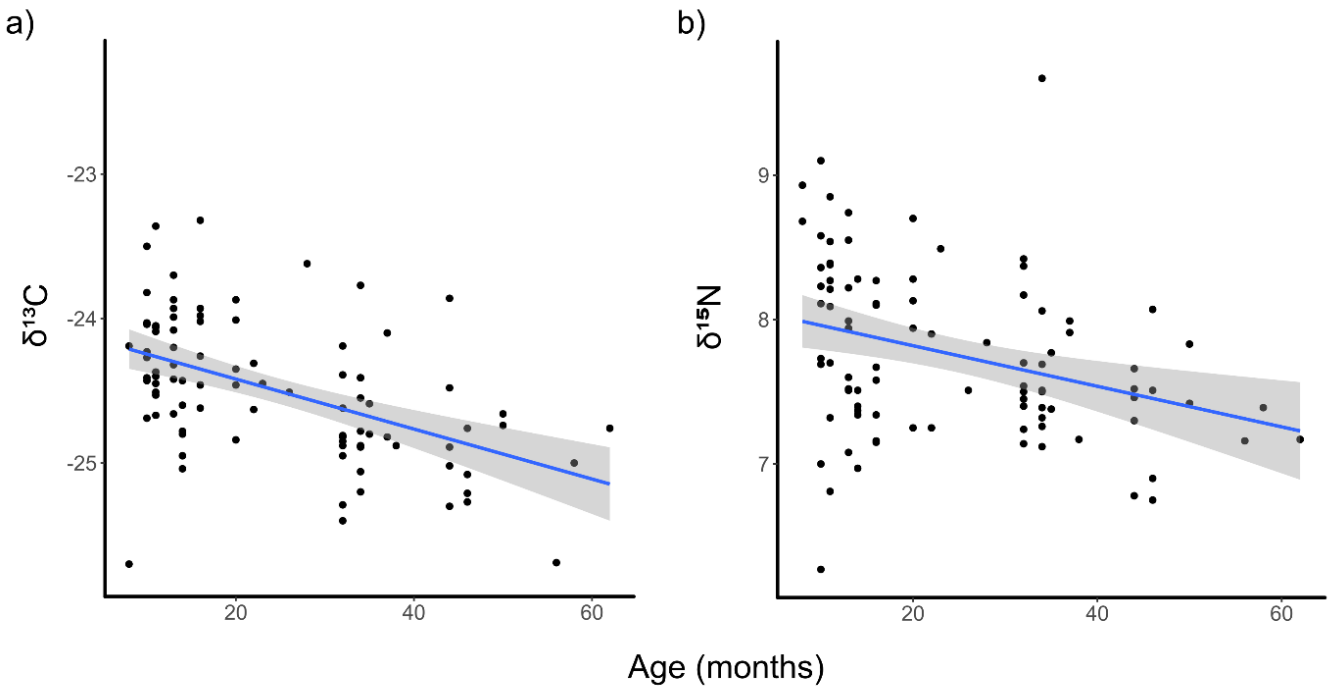

Figure 2.2. Relationships between Tasmanian devil age and a) $\delta^{13}C$ and b) $\delta^{15}N$ values of whisker samples from 91 individuals. *The plotted lines represent linear regressions with standard error.*

Population niche structure

Devils in age class 1 (<12 months) had a greater isotopic niche area, as a group (SEA_B mode = 1.042, 95% CI = 0.664-1.627, SEA_C = 1.130) compared to those in age class 2 (12-23 months; SEA_B mode 0.541, 95% CI = 0.383-0.794, SEA_C = 0.576) and adults in age class 3 (≥24 months; SEA_B mode 0.532, 95% CI = 0.381-0.727, SEA_C = 0.541) (Figure 1.3). Male devils in age class 1 had a greater isotopic niche area as a group than female devils (Male SEA_B mode = 1.288, 95% CI = 0.697-2.385; Female SEA_B mode = 0.325, 95% CI = 0.161-0.716). Similarly, male devils in age class 3 (adults) had a greater isotopic niche area than females (Male SEA_B mode = 0.701, 95% CI = 0.433-1.143; Female SEA_B mode = 0.291, 95% CI = 0.184-0.447). However, in age class 2, females had a greater isotopic niche area than males (Male SEA_B mode = 0.312, 95% CI = 0.189-0.516; Female SEA_B mode = 0.696, 95% CI = 0.415-1.293).

Within-individual variation

Subadult devils (<24 months) exhibited greater within-individual isotopic niche areas (SEA_B mode range 0.492-1.083) than adult devils (≥24 months) (SEA_B mode range 0.092-0.240), with individual isotopic niche areas (SEA_B) decreasing with age over the period 10 to 20 months (Figure 2.4). This greater variability in subadult isotopic niches was also apparent along the axes of both $\delta^{13}C$ (average range of subadults 1.51‰, adults 0.68‰) and $\delta^{15}N$ (average range of subadults 2.23‰, adults 1.01‰). Variation in $\delta^{13}C$ and $\delta^{15}N$ between subadult individuals declined over time, with a reduction in variation between individuals from the distal end (tip) to the base of the whiskers (Figure 2.5); in $\delta^{15}N$, this was accompanied by a reduction in mean $\delta^{15}N$ from 9.13‰ to 7.90‰. By contrast, mean $\delta^{15}N$ among adult devils stayed relatively constant over time, changing from 7.45‰ at the distal end (tip) to 7.35‰ at the base of the whiskers.

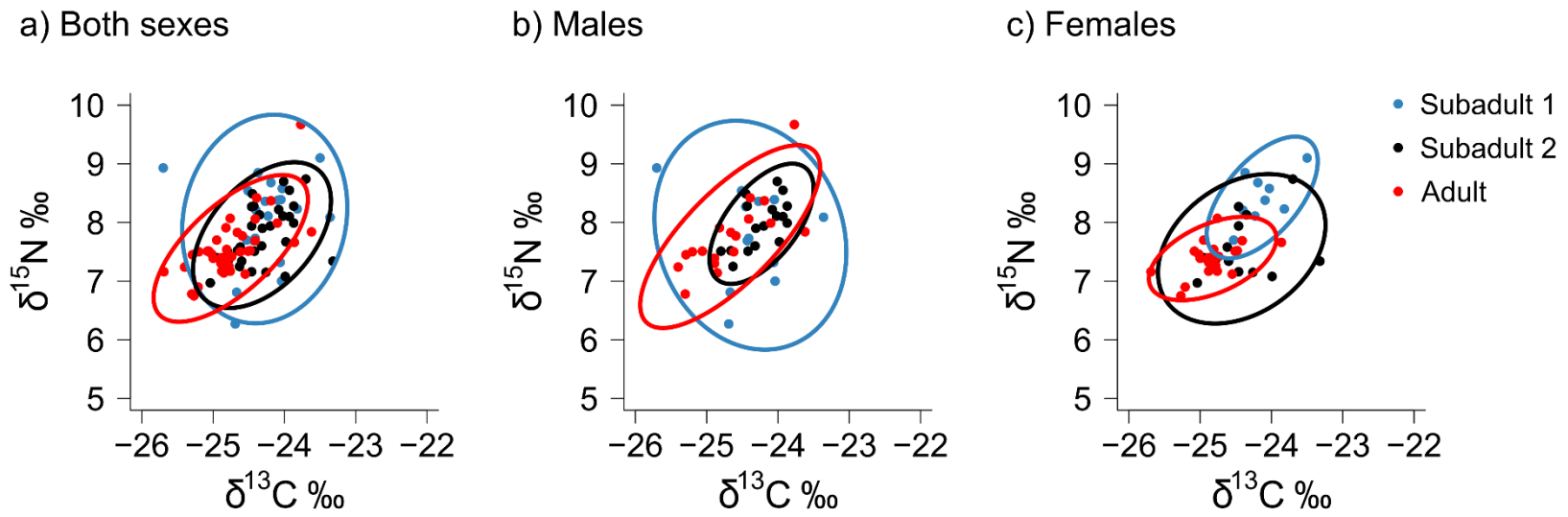

Figure 2.3. Values of $\delta^{13}C$ and $\delta^{15}N$ from analysis of Tasmanian devil whisker samples and isotopic niches by sex and age class*. a) Male and Female devils, b) Male devils and c) Female devils. Three age classes are subadult age class 1 (<12 months; blue), subadult age class 2 (12-23 months; black) and adults (≥24 months; red). Isotopic niches are standard ellipses corrected for sample size (SEA_C).*

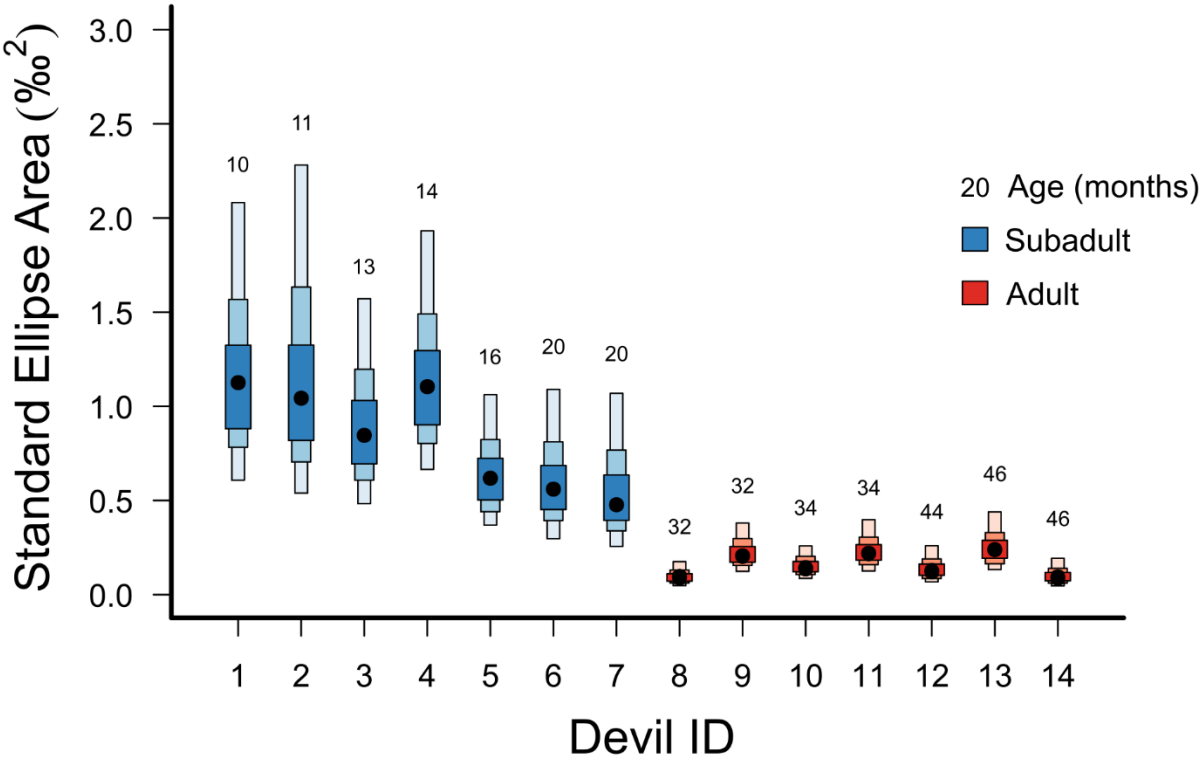

Figure 2.4. Summary of isotopic niche areas for 14 individual subadult and adult Tasmanian devils*. 7 subadults were <24 months old and 7 adults were >24 months. Individuals are shown in order of increasing age from left to right. Age (in months) is indicated above each individual standard ellipse area estimation. Isotopic niche areas are Bayesian standard ellipse area (SEA_B) estimates. SEA_B mode estimates are represented by black dots, while inner, middle and outer boxes represent 50%, 95% and 99% credible intervals, respectively.*

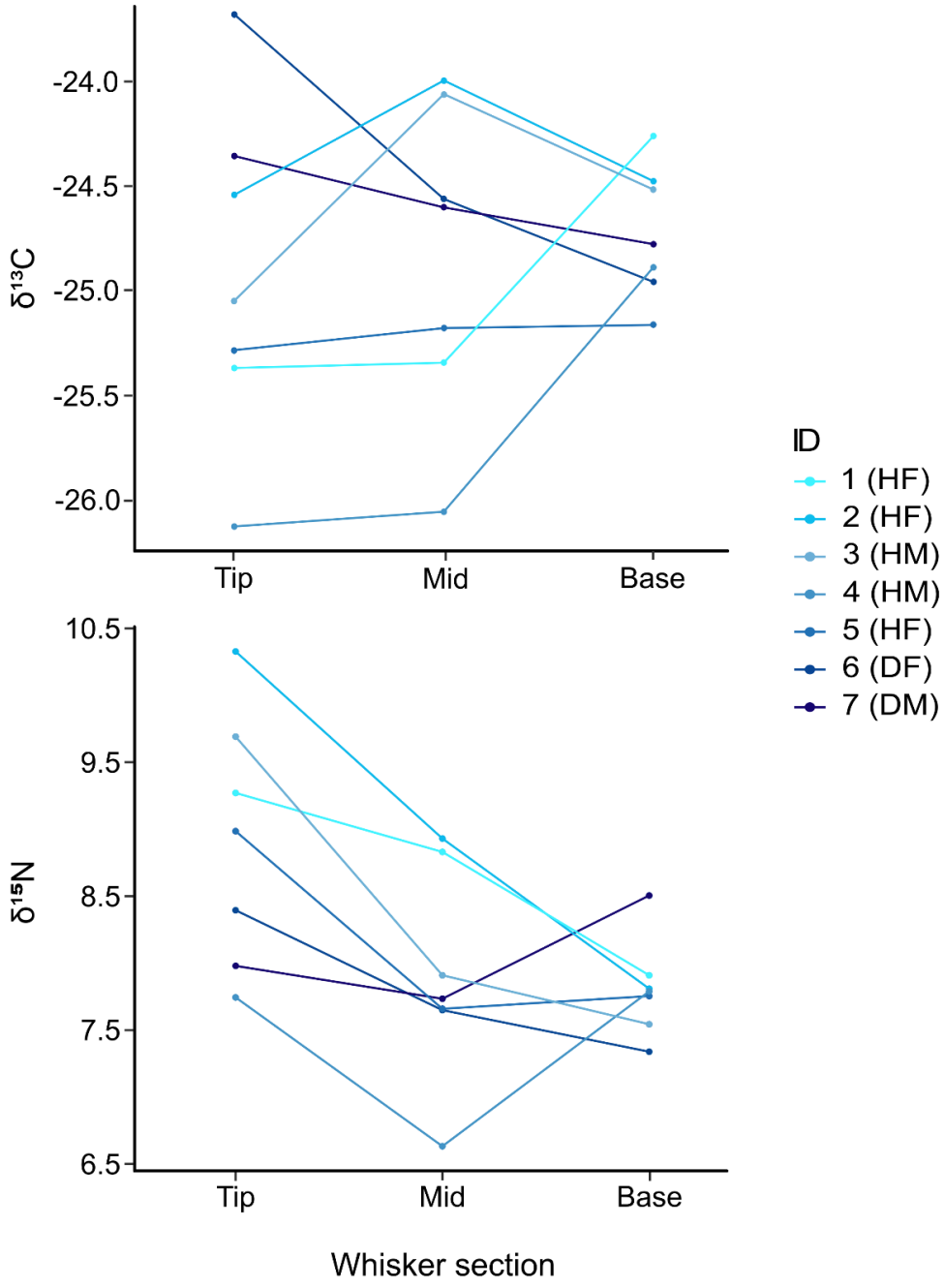

Figure 2.5. Summary of $\delta^{13}C$ and $\delta^{15}N$ values from the tip to the base of whiskers from seven subadult Tasmanian devils. *Subadult devils in this analysis are <24 months old. The tip is the distal and oldest section of the whisker while the base is the most recently grown. Devil ID corresponds with those in Figure 2.4 and incorporates disease (D = DFTD diagnosed, H = DFTD-free) and sex (F = female, M = male).*

Discussion

Our results reveal Tasmanian devils exhibit ontogenetic changes in isotopic niche characteristics. Among-individual variation was largely driven by a decrease in $\delta^{13}C$ and $\delta^{15}N$ values with advancing age. Population niche structure and within-individual variation also changed with age, as isotopic niche areas of population age classes and of individuals both decreased with increasing age. Using isotopic niche as a proxy for dietary niche, we interpret this as indicating broader dietary niches in subadult devils both as an age class, and as individuals.

Decreasing isotopic niche areas of devils from early independence (subadult age class 1) through to adulthood was driven by a contraction towards the lower end of $\delta^{15}N$ values, rather than a shift of a consistently broad niche towards lower trophic levels. Maternal milk, with elevated $\delta^{15}N$ values, will likely have contributed to the higher $\delta^{15}N$ values of the youngest devils. Alongside any effects of weaning, higher $\delta^{15}N$ in younger Tasmanian devils and the age-related decline could also reflect diets in younger animals that are higher in trophic level, incorporating medium to small mammals and birds, with larger, herbivorous mammals increasing in their importance in diets as the devils age. An ontogenetic shift in diet as devils grow from subadult to adult body size would also contribute to the observed contraction in isotopic trophic niches. Of the prey base available to Tasmanian devils, the larger marsupials are herbivorous, including the bare-nosed wombat, and the macropodid Bennett's wallaby and Tasmanian pademelon, and the smaller mammals are omnivores to varying degrees, including the potoroid macropods (long-nosed potoroo *Potorous tridactylus apicalis* and eastern bettong *Bettongia gaimardi*) and peramelids or bandicoots (eastern barred bandicoot *Perameles gunnii* and southern brown bandicoot *Isoodon obesulus*) (Bennett and Baxter, 1989; Quin, 1988; Taylor, 2006). A high proportion of bird species in Tasmania are also insectivorous (Ridpath and Moreau, 1965). Jones and Barmuta (1998) found the diet of subadult devils, based on scat contents, contained a lower biomass of large mammal prey species and a higher biomass of birds, relative to adult devils which fed primarily on larger mammals. Unweaned juvenile devils (defined in Jones and Barmuta (1998) as first year young trapped between

October and January that were still suckling) were also found to have a higher percentage of small mammals and birds in their diet than adult devils. The observed differences in isotopic niche size and position through ontogeny in Tasmanian devils could therefore be the result of changes in the type of food resources consumed, as well as the effect of weaning.

Isotopic signatures in consumer tissues can reflect spatial or temporal variation in the environmental isotopic baseline, rather than actual variation in resources consumed. If ignored, this can present a problem for the interpretation of isotopic data (Cummings et al., 2012). However, with application of prior ecological knowledge, variation in the isotopic baseline can be an advantage, for example in investigating the consistency of foraging habitat use in the face of ecological change (Bodey et al., 2010). Upon reaching independence, Tasmanian devils disperse from the natal den. Dispersal in devils is male-biased, similar to other mammalian species, although both sexes disperse (Lachish et al., 2011). As such, greater isotopic heterogeneity in subadults could be driven by mobile, newly independent devils passing through our study site, having foraged in multiple locations outside of the immediate study area. A reduction in female dispersal has been found in sites where DFTD is present, although this has not affected male dispersal (Lachish et al., 2011). Subadult males (age class 1) did exhibit broader isotopic niches than females. On the other hand, genetic and observational data suggest the majority of devil dispersal events occur over distances of 14-30km (Lachish et al., 2011). Our study site, Wilmot (almost 40km^2), is situated within a relatively uniform landscape of native and plantation forest and agriculture, reducing the likelihood of large changes in isotopic baseline within the trapping area, although some localised environmental heterogeneity may still occur.

Subadult devils had a broader individual isotopic niche than adults, along both the $\delta^{15}N$ and the $\delta^{13}C$ axes. The depletion in $\delta^{15}N$ from the older whisker sections to the base of the whisker in young Tasmanian devils (Figure 2.5) is evidence of the weaning process in Tasmanian devils. Tasmanian devils are born after approximately 3 weeks gestation (Keeley et al., 2017), when the young crawl into the mother's pouch and attach to one of four available teats. Juveniles remain in the pouch until they are 5-6 months old, at which point they

are left in the den (Guiler, 1970). Records of lactating adult females suggest weaning is completed approximately 10 months after birth, coinciding with independence and dispersal from the natal den (Pemberton, 1990). The process of weaning has been predicted to result in an enrichment of $\delta^{13}C$, on account of the high lipid content in milk; lipids are depleted in $\delta^{13}C$ relative to proteins and carbohydrates (Tieszen et al., 1983). However, the evidence for this in eutherian mammals is mixed (Knoff et al., 2008; Newsome et al., 2009a; Orr et al., 2012; Vales et al., 2015). Furthermore, this prediction may not hold for marsupial species. Eutherian mammals have a long gestation, and neonates are precocial in comparison to marsupial neonates, which are born before the completion of organogenesis. To match the evolving needs of a suckling marsupial juvenile, milk composition in marsupials changes through lactation; for example, lipid and protein content increases while carbohydrate content decreases in late stage lactation in the herbivorous tammar wallaby *Macropus eugenii* (Pharo, 2019). That said, as devils in our analysis of within-individual variation were at least 10 months old, whiskers are most likely to represent the second stage of lactation, where devil joeys are capable of life outside of the pouch, at which point milk composition may be more stable and similar to eutherian milk. A more likely explanation for $\delta^{13}C$ variation in individual diets of young devils would be that an increasing variety of solid food resources was being consumed as weaning progressed. Prior to independence, young individuals roam increasing distances from the natal den, gradually increasing their dietary independence and mothers also bring some food back to the den (M.E. Jones, unpublished trapping records at and near dens).

Devil facial tumour disease infection status was not included in any of our top models (Table 2.1). It may be that our binary measure of infection status is too coarse to reveal an effect, as behavioural or physiological effects of DFTD may not manifest during early infection but increase with tumour burden (Ruiz-Aravena et al., 2018). Furthermore, only 24 devils in our sample of 90 were infected with DFTD, therefore our sample size may be too small to reveal any subtle effects or significant interaction terms relating to the effect of DFTD upon isotopic variability. Our dataset includes a number of devils unlikely to display clinical signs of DFTD due to their age; most of the transmission-relevant

injurious biting occurs during the mating season between adults (Hamede et al., 2008; Hamilton et al., 2019). If there is an effect of DFTD upon isotopic signatures, this could influence our findings related to age and isotopic variability due to the age skew of the diseased population. To account for this, we included an interaction between DFTD infection status and age in our GLMs, though this was not retained in any top model.

In conclusion, Tasmanian devil isotopic values and isotopic niche area change with age, likely following a pattern of increased prey size and reduced dietary niche breadth with increasing devil maturity, alongside a reduction in the influence of weaning from a diet of maternal milk. The abundance of devils has declined dramatically due to outbreaks of DFTD, resulting in changing age structures, behaviours and ecological interactions. Moving forward, this research provides a basis to consider in more detailed terms the potential impacts of DFTD-related decline upon the trophic ecology of Tasmanian devils and the ecological communities they occupy and influence.

Chapter 3

Isotopic niche variation in Tasmanian devils *Sarcophilus harrisii* with progression of devil facial tumour disease

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Abstract

Devil facial tumour disease (DFTD) is a transmissible cancer affecting Tasmanian devils *Sarcophilus harrisii*. The disease has caused severe population declines and is associated with demographic and behavioural changes, including earlier breeding, younger age structures, and reduced dispersal and social interactions. Devils are generally solitary, but social encounters are commonplace when feeding upon large carcasses. DFTD tumours can disfigure the jaw and mouth and so diseased individuals might alter their diets to enable ingestion of alternative foods, to avoid conspecific interactions, or to reduce competition. Using stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of whiskers, we tested whether DFTD progression, measured as tumour volume, affected the isotope ratios and isotopic niches of 94 infected Tasmanian devils from six sites in Tasmania, comprising four eucalypt plantations, an area of smallholdings and a national park. Then, using tissue from 10 devils sampled before and after detection of tumours and 8 devils where no tumours were detected, we examined whether mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ of the same individuals changed between healthy and diseased states. $\delta^{13}C$ and $\delta^{15}N$ values were generally not related to tumour volume in infected devils, though at one site, Freycinet National Park, $\delta^{15}N$ values increased significantly as tumour volume increased. Infection with DFTD was not associated with significant changes in the mean or standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values in individual devils sampled before and after detection

of tumours. Our analysis suggests that devils tend to maintain their isotopic niche in the face of DFTD infection and progression, except where ecological conditions facilitate a shift in diets and feeding behaviours, demonstrating that ecological context, alongside disease severity, can modulate the behavioural responses of Tasmanian devils to DFTD.

Introduction

Animals can alter their behaviour in response to infection or disease, whether caused by viral, bacterial or macroparasitic pathogens, or cancers (Aubert, 1999; Hart, 1988; Vittecoq et al., 2015). Infectious diseases often result in a suite of responses termed 'sickness behaviours' that may be associated with variation in host survival, including reductions in movement, food and water intake, aggression and altered rates of social contacts (Adelman and Martin, 2009; Anderson and Behringer, 2013; Aubert, 1999; Bohn et al., 2016; Hart, 1988; Lopes et al., 2016). Alongside host-mediated behaviours, some changes in behaviour are driven by conspecifics that recognise, and aim to avoid, infected individuals (Behringer et al., 2006; Curtis, 2014); although both sickness behaviours and avoidance can depend on social context (Fairbanks et al., 2015; Lopes et al., 2012). These behavioural changes have implications for population connectivity, predation risk and transmission of infection through populations and communities (Behringer and Butler, 2010; Bouwman and Hawley, 2010; Lopes et al., 2016).

Compared to the better understood sickness behaviours listed above, relatively little is known about the impact of disease on diet and feeding behaviours (Hite et al., 2020). Sickness behaviours related to diet include reduced food intake, or illness-induced anorexia, and dietary alterations as a form of self-medication, or to compensate for the nutritional demands of immune responses (Adamo et al., 2010; Bos et al., 2015; Ghai et al., 2015; Hite et al., 2020; Lee et al., 2006). From an ecological perspective, infection, disease and associated changes in the social environment create new sets of physiological and ecological constraints for hosts. Food items that were previously considered suboptimal may become preferred. Available resources may also be restricted if the potential for agonistic interactions with healthy conspecifics excludes diseased individuals from preferred food types. Dietary or foraging changes as an ecological response to disease may be particularly likely to occur when a species' diet is closely linked to social or competitive interactions.

Tasmanian devils *Sarcophilus harrisii* are known for intense agonistic interactions, which have indirectly effected severe population declines, following

the emergence of a transmissible cancer, devil facial tumour disease (DFTD) (Pearse and Swift, 2006; Lazenby et al., 2018; Cunningham et al., 2021). Transmission of DFTD occurs via direct inoculation of clonal tumour cells from one individual to another during injurious biting behaviour, which occurs during agonistic feeding and mating interactions (Hamede et al., 2013, 2008; Hamilton et al., 2019; Pearse and Swift, 2006). DFTD is almost always fatal and the clinical signs present as destructive tumours around the head, neck and mouth (Figure 3.1), which lead to death from a combination of metabolic starvation, metastasis and subsequent organ failure, and secondary infections (Loh et al., 2006). Two evolutionarily distinct transmissible cancers have emerged in Tasmanian devils over a 20 year period. DFTD was first recognised in 1996 in the north east of Tasmania, and has spread south and west, resulting in a gradient of disease prevalence and population decline, providing a natural experiment in which to study the impacts of DFTD (Hawkins et al., 2006; Lazenby et al., 2018). A second transmissible cancer, DFT2, emerged in southern Tasmania in 2014, although this is clinically indistinguishable from DFTD and does not yet appear to have spread beyond an area of approximately 550km^2 in the d'Entrecasteaux peninsula in south-east Tasmania (James et al., 2019; Pye et al., 2015).

Tasmanian devils have responded to DFTD in their life history and their behaviour. Lower conspecific densities in affected populations have resulted in increased growth rates in subadults, probably as a consequence of lower competition for resources, leading to almost 50% of females reaching sexual maturity after their first year, rather than their second year, as in healthy populations (Jones et al., 2008; Lachish et al., 2009). Reduced food competition has also led to reduced dispersal rates in females, probably because of reduced competition (Lachish et al., 2011). Individuals with DFTD display a sustained reduction in conspecific contact rates with disease progression, altering their connectivity in affected populations (Hamilton et al., 2020). Individuals with DFTD also appear to reduce their daily activity; this effect is stronger in males than females, possibly due to the necessity for female devils to travel between den sites and feeding grounds while caring for young (Comte et al. 2019). Such findings are in line with evidence that diseased females maintain their body

condition for longer than males, suggesting they have higher tolerance to DFTD infection, potentially ensuring their survival until any dependent young are weaned (Ruiz-Aravena et al., 2018).

In response to the progression of DFTD, Tasmanian devils may change their diets to maintain condition and enhance their survival. Devils are facultative scavengers and ordinarily largely eat medium to large herbivores such as Bennett's wallaby *Macropus rufogriseus* and Tasmanian pademelon *Thylogale billardierii*, though scat contents analyses show sub-adult diets to contain lower proportions of these larger species, and higher proportions of birds compared to adults (Andersen et al., 2017a; Jones and Barmuta, 1998). Devils are generally solitary, though multiple devils may feed simultaneously at a single carcass (Hamede et al., 2008; Jones, 1995; Pemberton and Renouf, 1993), and feeding is therefore regarded as a source of costly intra-specific interactions and competition in this species. DFTD has high metabolic and physiological costs, evidenced by a reduction in body condition as tumour volume increases (Ruiz-Aravena et al., 2018). As DFTD tumours grow, they can grossly disrupt the structure of the mouth and jaw, causing necrosis, ulcerations and secondary infections (Figure 3.1) (Loh et al., 2006; Pye et al., 2016), potentially resulting in a competitive disadvantage for some infected individuals during feeding interactions. Given that devils reduce their social contacts in response to DFTD infection both inside and outside of the mating season (Hamilton et al., 2020), an alteration in diet may facilitate reduced competition and avoidance of conspecific aggression, or be the result of ostracisation of diseased individuals by healthy conspecifics. Devils may switch to a diet that can be consumed relatively quickly, easily, and solitarily, carrying a lower risk of costly competition. This could occur via a proportional increase in the consumption of smaller, generally omnivorous, prey, compared to the medium to large, primarily herbivorous, marsupials that usually comprise the bulk of adult devil diets (Andersen et al., 2017a; Jones and Barmuta, 1998).

Figure 3.1. Four individual Tasmanian devils, pictured at four different stages of DFTD progression*, with (a) being the earliest disease stage, through to (d) being most severe. DFTD can occur inside or close to the oral cavity (a), which can disrupt the mouth parts and maculatory feeding organs (d). As demonstrated in all images, DFTD tumours often ulcerate which can lead to secondary infection.*

We have applied stable isotope analysis to investigate the effects of DFTD upon Tasmanian devil trophic ecology. Stable isotopes in consumer proteins broadly reflect those of the dietary proteins they have assimilated, subject to alterations as a result of digestion and routing of food sources (Bearhop et al., 2002; DeNiro and Epstein, 1978; Hobson and Clark, 1992b). In ecological studies, $\delta^{13}C$ and $\delta^{15}N$ are commonly used isotopes. Variation in $\delta^{13}C$ across primary producers provides information on consumer's dietary carbon sources, which may vary among organisms according to movement, foraging grounds or dietary selectivity (Araújo et al., 2007; Bearhop et al., 2004; Cherel and Hobson, 2007; Crawford et al., 2008; DeNiro and Epstein, 1978). $\delta^{15}N$ can provide information on trophic position and food web structure, since ^{15}N becomes enriched with each trophic level (DeNiro and Epstein, 1981). Trophic enrichment in $\delta^{15}N$ can also be used to indicate weaning or starvation, as young mammals consuming maternal milk, essentially tissues of their own species, will have high $\delta^{15}N$ which reduces through the weaning process (Evacitas et al., 2017; Hobson et al., 1998; Newsome et al., 2009a). Starving animals metabolise their own protein tissues, so are also expected to exhibit elevated $\delta^{15}N$ values (Hobson et al., 1993). Traditional dietary analysis methods, generally of scat and stomach contents, provide qualitative information on dietary composition, but tend to underrepresent easily digested food items and over-represent less easily digested items. Stable isotope analysis reflects assimilated diet and can be conducted on inert tissues, such as whiskers and feathers. These tissues may then be subsampled, or sampled on multiple occasions, to build a time series of dietary data representing the period of time the tissue was actively growing. Stable isotope approaches can therefore be particularly useful in building an integrated picture of individual diets and investigating how wild animals respond to change, such as ontogenetic changes in body size and foraging capabilities (Jeglinski et al., 2012; Newsome et al., 2009a), seasonal food availability (Inger et al., 2006), ecosystem fragmentation (Layman et al., 2007), or anthropogenic management activities (Bodey et al., 2010). Our stable isotope analysis of Tasmanian devil whiskers showed a significant decrease in $\delta^{13}C$ and $\delta^{15}N$ with increasing age, accompanied by a narrowing isotopic niche, of devils as a group and individually, from subadults to adults (Bell et al., 2020). This reduction in trophic

level and niche breadth reflects the isotopic effect of weaning, alongside a probable shift in diet from smaller, omnivorous to larger, herbivorous prey species, and a reduction in dietary diversity (Bell et al., 2020). In the context of disease, devil behavioural changes may occur gradually, or might change more swiftly upon individuals reaching an infection load 'tipping point' (Szyszka and Kyriazakis, 2013). Stable isotope analysis provides a means of analysing whether disease progression, not just infection, is associated with changes in foraging ecology.

We used stable isotope analysis of Tasmanian devil whiskers to investigate the impact of devil facial tumour disease on devil foraging ecology in two ways. First, we used a population cross-sectional study to test whether there is a relationship between tumour volume and whisker isotope values in Tasmanian devils sampled at a range of stages of DFTD progression. We hypothesised that $\delta^{13}C$ and $\delta^{15}N$ would change with increasing tumour volume, perhaps via a shift in $\delta^{13}C$ and an increase in values of $\delta^{15}N$ associated with a diet comprising fewer large herbivorous marsupials and greater proportions of smaller, more omnivorous species. We considered how this effect could vary based on other ecological variables, including sex, on account of differential tolerance to DFTD (Ruiz-Aravena et al., 2018), or site. To consider ecological differences among sites that could influence devils' trophic responses to DFTD, we also estimated the relative availability of three of the devils' main prey species: Bennett's wallabies, Tasmanian pademelons and brushtail possums *Trichosurus vulpecula*, at all sites. Second, we used an individual-based longitudinal approach to test whether whisker isotope values change in individual devils after DFTD infection, by comparing a set of individuals sampled before and after clinical signs of DFTD were observed, and a comparison set of individuals with no clinical signs of infection, for which we had whisker samples from two time points separated by a comparable interval. We hypothesised that devils that developed DFTD between capture events would exhibit a change in mean and standard deviation $\delta^{13}C$ and $\delta^{15}N$, possibly characterised by an increase in mean $\delta^{15}N$ associated with feeding at a higher trophic level, and an increase in standard deviation to reflect greater dietary diversity, if diseased devils incorporate previously suboptimal food items.

Methods

Field sites

To test the effect of DFTD tumour growth we selected six field sites with varying environments and histories of DFTD infection (Figure 3.2); Freycinet National Park (-42.107 E, 148.277 S), Woodbridge (-43.131 E, 147.224 S), West Pencil Pine (-41.541 E, 145.823 S), Wilmot (-41.377 E, 146.152 S), Takone (-41.156 E, 145.580 S) and Black River (-40.980 E, 145.263 S). DFTD (DFT1) presence has been recorded at Freycinet since 2001, at West Pencil Pine since 2006, Wilmot since 2008, Takone since 2010 and at Black River since 2016. At Woodbridge, DFT1 was first recorded in 2012, followed by the emergence of DFT2 in 2014. Freycinet National Park is a coastal site predominantly composed of native dry eucalypt forest. Woodbridge is an area of smallholdings interspersed by native eucalypt woodlands, while Black River, Takone and Wilmot are commercial eucalypt plantations situated within agricultural landscapes. West Pencil Pine is also a commercial eucalypt plantation, situated close to a large protected area.

Sample collection

Tasmanian devils were caught for sample collection during monitoring surveys carried out at each site at 3 month intervals. Individuals were identified via subcutaneously implanted microchips (AllFlex© ISO FDX-B), and age, sex, weight and head width were recorded. Age was determined using wear on canine and molar teeth, and canine tooth over-eruption; this method is accurate because devils sustain predictable high tooth wear and over-eruption, and senesce and usually die by five years of age in wild populations (M.E. Jones, unpublished data). Devils were given a standardised birth date of 1st April, as Tasmanian devils give birth three weeks after mating in late February/early March (Hesterman et al., 2008).

DFTD status was based on visual diagnosis of clinical signs of the disease (Hawkins et al., 2006). The number and location of tumours present was recorded and the length, width and depth of each tumour was measured.

Individual tumour volume (mm^3) was calculated using the formula:

$$ellipsoid\ volume = \frac{4}{3}\pi abc$$

Where a, b and c are linear tumour measurements (in this case half the length, width and depth). Total volume was then estimated as the sum of the individual volumes of all tumours present on an individual devil. This measure has previously been used to successfully describe DFTD severity in Tasmanian devils (Ruiz-Aravena et al., 2018).

One whisker was collected at each capture for isotope analysis by cutting close to the skin with scissors. In total, whiskers were collected from 112 Tasmanian devils between February 2015 and February 2019 at the six sites (Table 3.1). Whiskers were stored individually in plastic zip-lock bags in a -20°C freezer or fridge prior to laboratory preparation.

Table 3.1. Summary of the numbers of individual Tasmanian devils sampled at each site for our populational cross-sectional study and longitudinal study*. Our population cross-sectional study included no individual repeats, while each devil included in our longitudinal study was sampled on two occasions.*

Site	**Sample size**	
	Population cross-sectional study	**Longitudinal study**
Freycinet National Park	18	7
Woodbridge	8	7
West Pencil Pine	17	NA
Wilmot	24	NA
Takone	10	NA
Black River	17	4
Total	**94**	**18**

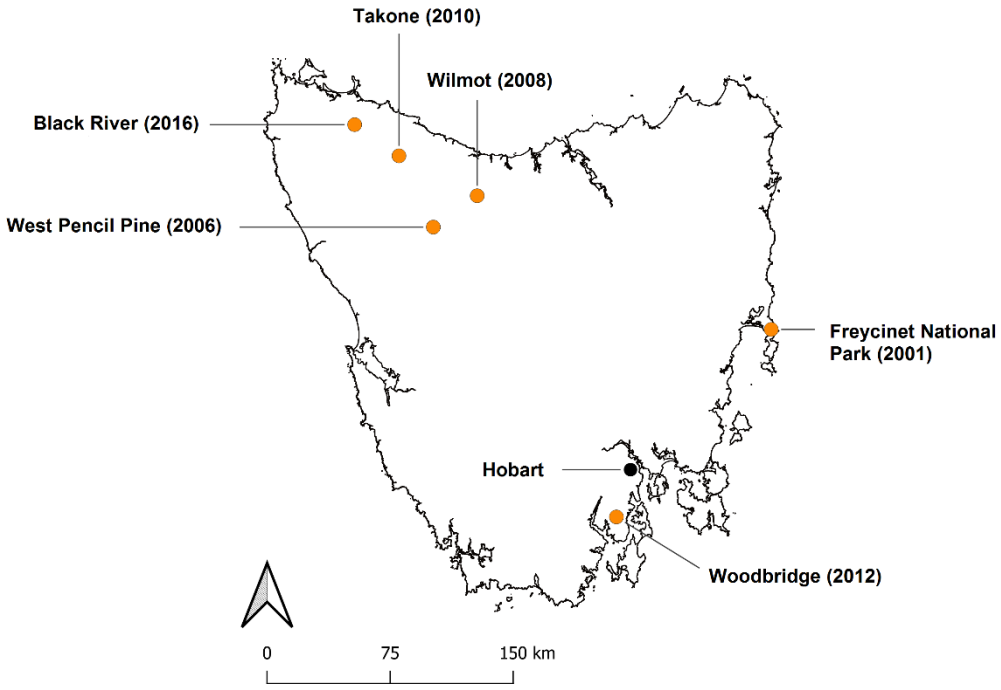

Figure 3.2. Locations of sites in Tasmania at which Tasmanian devils were sampled*. Hobart, the capital city of Tasmania is shown for reference (black circle). Study sites (orange circles) are labelled with site name and the year DFTD was first recorded at the site in brackets.*

Sample preparation

In the laboratory, whiskers were rinsed with distilled water to remove surface contaminants and left to air-dry, then placed in a freeze-dryer for 24 hours. Samples were prepared by chopping small whisker sections into a tin cup until the required analytical sample weight of 0.7 mg ± 0.1 mg was reached.

For the population cross-sectional study, 94 devils with DFTD were sampled. One sample per individual was taken from the base of each whisker, as this had grown most recently and is therefore most likely to correspond to the tumour volume recorded at the time of capture.

For the individual longitudinal study, we included individuals based on three constraining factors, where individuals must have been: free from clinical signs of DFTD when the first whisker was sampled, at least 18 months old at the first sampling occasion to reduce likelihood of age effects masking variation (Bell et al., 2020), and the two whiskers must have been sampled at least 6 months apart, to maximise the likelihood of independence of the two time points. In total 18 individuals were selected; 10 had DFTD when sampled on the second occasion, while 8 'control' individuals were free of tumours on both sampling occasions. Each whisker was divided into 4 sections (base, 2 middle sections and tip), and each section was subsectioned. One sample per section was taken, by randomly selecting small subsections until the required sample weight was reached, resulting in four samples per whisker.

Stable isotope analysis

All stable isotope analyses were conducted using a Sercon Integra-2 elemental analyser isotope-ratio mass spectrometer at the University of Exeter. Stable isotope ratios are expressed as delta (δ) values expressed in parts per thousand, or 'per mil' (‰) relative to international standards, according to:

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

Where X = ^{13}C or ^{15}N and R = measured ratio of ^{13}C to ^{12}C, or ^{15}N to ^{14}N. If the heavy to light ratio is higher in the sample than the standard this results in a greater, or 'enriched', δX. Conversely, heavy to light ratios that are lower than

the standard, lead to lower, or 'depleted', δX values. The standards for $\delta^{13}C$ and $\delta^{15}N$ are the Vienna Pee Dee Belemnite and atmospheric nitrogen respectively, however other materials calibrated against these standards are routinely used. Our samples were scale corrected using the international standards USGS40 and USGS41, with additional internal standards of bovine liver and alanine. Average precision was 0.05‰ ± 0.01 (1 standard deviation ± standard error) for both $\delta^{13}C$ and $\delta^{15}N$.

Statistical analysis

All statistical analyses were conducted in R Version 3.5.2 (R Core Team, 2018).

Population cross sectional study

For the cross-sectional study, we tested the effect of tumour volume on isotope values from basal whisker sections of 94 devils by fitting two linear models using the R package lme4 (Bates et al., 2015), with $\delta^{13}C$ and $\delta^{15}N$ as response variables. Our main variable of interest, tumour volume (mm^3) was included as a fixed effect after we applied a $\log_{10}$ transformation to account for positive skew. Age (in months), sex, and a sex by $\log_{10}$-transformed tumour volume interaction term were also included. Body condition was added as a fixed effect, as a change in isotope values with changing body condition may be reflective of a change in diet or changing metabolic processes, as animals under nutritional stress exhibit enriched $\delta^{15}N$ (Hobson et al., 1993). Body condition was estimated using the scaled mass index (SMI) (Peig and Green, 2009):

$$scaled\ mass\ index: \widehat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

Where M_i and L_i are the body mass and a linear body measurement of individual i (in this case head width), and L_0 is an arbitrary value of L (in this case mean head width). To calculate the SMI for each individual, Tasmanian devil mass was first corrected by subtracting tumour mass from the total devil mass (assuming a tumour density of 1.1 g/ml, based on the average density of soft tissues), then the SMI was calculated using the corrected devil mass and

head width. Site was added to our model as a fixed effect, alongside a site*tumour volume interaction term, as any patterns of isotopic variation may differ based on site-specific ecological conditions. Year was fitted as a fixed effect, and was an integer, based on yearly trapping cycles from October-October.

Model selection was conducted using the package MuMIn (Bartoń, 2018). To place all variables on the same scale, our predictor variables were rescaled to have a mean of 0 and a standard deviation of 0.5. We then used our global models, built using biologically realistic predictor variables, to create sets of top models where ΔAIC was lower than 2. These top model sets were then averaged, resulting in one final model for $\delta^{13}C$ and one for $\delta^{15}N$. We used the conditional average for model interpretation; as these models avoid shrinkage of model estimates, and are preferable when there is a variable of particular *a priori* interest (Grueber et al., 2011; Nakagawa and Freckleton, 2011), in this case disease progression.

Species Distribution Models

Any site-based differences in the relationship between $\delta^{13}C$ and $\delta^{15}N$ values of infected devil whiskers and DFTD tumour volume could be driven by ecological differences among sites, including variation in prey availability. To explore differences in prey availability among the sites, we modelled the relative abundance of three of the main prey species of Tasmanian devils: Bennett's wallabies, Tasmanian pademelon and brushtail possums (Andersen et al. 2017a) across the whole of Tasmania. To do this, we used data from standardised spotlight surveys (see Appendix), conducted annually from 1985-2019, at up to 172 10km transects across Tasmania (Figure 3.5a) (Hocking and Driessen, 1992).

We modelled the spotlight counts for each species using integrated nested Laplace approximation (INLA), fitted using the inlabru R package (Bachl et al., 2019; Illian et al., 2013; Lindgren et al., 2011). For each species, we modelled the count of animals detected per transect in response to explanatory variables for the proportional cover of the four main vegetation classes in Tasmania: wet

Eucalypt/rainforest (*%wetEuc*; 28% of Tasmania), dry Eucalypt forests and woodlands (*%dryEuc*; 24%), agricultural land (*%agric*; 23%) and button grass moorlands (*%butGrass;* 9%). We omitted *%dryEuc* from the analysis because it was negatively correlated with *%wetEuc* (Pearson's r = -0.65). In addition to simple linear effects, we also modelled non-linear effects of these covariates (see Appendix). We accounted for spatial dependency in the spotlight counts, as well as correlations between repeated surveys of transects, with the use of a spatial random field. Following the advice of Illian et al. (2013) for models that include spatial covariates and spatial random fields, we first fitted all combinations of explanatory variables. Then, using the best-performing covariate model, we tested whether adding a random field improved model fit. Models were compared using the Widely Applicable Information Criterion (Watanabe, 2010).

Using the best-performing models, we produced predictive maps of each species' relative abundance across Tasmania. From the predicted maps, we estimated the relative abundance (± standard deviation) of each prey species, within a buffer around devil trap locations of 3.22 km, which is the radius of the mean 95% kernel density estimate recorded for female devils at Freycinet National Park prior to the first recorded DFTD infection in the area (S. Comte and M. Jones, unpublished data). We used female ranges as female devils have larger home ranges than males. For further details of the spatial modelling, see the Appendix.

Individual longitudinal study

For the longitudinal study of 18 individuals sampled on two occasions, we tested whether DFTD infection results in a shift in individual isotope values, by fitting two linear models, with the response variables as the mean $\delta^{13}C$ and mean $\delta^{15}N$ of the whisker sampled on the second capture occasion. We regressed this against the mean $\delta^{13}C$ and mean $\delta^{15}N$ of the whisker sampled on the first capture occasion and $\log_{10}$ transformed tumour volume (mm^3, calculated as above). To test whether DFTD infection results in changes in individual isotopic variation, we then fitted two linear models, with the standard deviation of $\delta^{13}C$ or $\delta^{15}N$ of the whisker sampled on the second capture as the

response variables. Our models of standard deviation followed the same structure as those of mean $\delta^{13}C$ and $\delta^{15}N$; fixed terms were the standard deviation of $\delta^{13}C$ or $\delta^{15}N$ of the whisker sampled on the first capture occasion and $\log_{10}$ transformed tumour volume. Parameter standardisation was conducted for all four models using the methods described above. Although standard deviation data tend to be skewed, our data were not strongly skewed and our checks for residual normality, homogeneity of variance and unduly influential data points did not raise any concerns.

Differences in foraging behaviour could lead to differential likelihood of agonistic interactions with conspecifics and subsequent DFTD infection. To test whether isotopic ratios at the first sampling occasion predict subsequent appearance of tumours, we fitted a binomial model with a logit link function, with DFTD infection status at the second sampling occasion as the response variable, and the mean $\delta^{13}C$ and $\delta^{15}N$ at the first sampling occasion, and the time in months between sampling events (as likelihood of contracting DFTD may increase with increased time between observations) as predictor variables.

Results

Population cross sectional study

Tumour volume in our sample of 94 devils with DFTD was strongly skewed towards small volumes. The median volume of tumours was 10,676mm^3 (95% quantiles = 204 – 147,061mm^3). Mean $\delta^{13}C$ of devil whiskers was -23.66‰ (standard deviation = 1.22‰), while mean $\delta^{15}N$ was 7.89‰ (SD = 1.26‰). Two models featured in the top model set for variation in $\delta^{15}N$ and four models were in the top set for $\delta^{13}C$ (Table 3.2). Prior to model selection, our global models for $\delta^{13}C$ and $\delta^{15}N$ had adjusted R^2 values of 0.71 and 0.63, suggesting a high proportion of the variation in our data is explained by the variables included in our global model. For $\delta^{15}N$, there was a strong effect of the site*tumour volume interaction; at Freycinet National Park, but not at the other sites, $\delta^{15}N$ increased positively with tumour volume (estimate = 1.44, 95% confidence interval = 0.26–2.61) (Figure 3.3). Tumour volume was not included in our averaged model as a main effect (Figure 3.4). $\delta^{15}N$ varied significantly among sites, reflecting variation in the isotopic baseline of different sites. Body condition was also retained in the averaged model, showing a positive trend with increasing tumour volume. For $\delta^{13}C$, neither tumour volume nor the site*tumour volume interaction influenced variation among devils, though tumour volume was retained in the average model with a slight negative effect. Site was included in all the top models used to create the averaged model, again reflecting differences in the isotopic baseline of our sites. Age was retained in our averaged model, but with only a slight negative effect of increasing age on $\delta^{13}C$. We tested the role of variation in prey availability among sites on isotopic values by replacing site as a stand-alone variable, and in an interaction term with tumour volume, with our estimates of mean relative abundance for Bennett's wallabies, Tasmanian pademelon and brushtail possums. However these models had an AIC estimate at least 2 units higher than models with site, therefore we retained site as a variable in our model.

Table 3.2. Summary of analyses of variation in the stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of whiskers from 94 Tasmanian devils infected by devil facial tumour disease*. The conditional averaged results for two linear models, with each isotope as a response variable are presented. Tumour volume was log10 transformed before adding to the model, and all predictors were standardised.*

Response Variable	Model Variables	Estimate	Standard Error	Lower Confidence Interval	Upper Confidence Interval	Z value	P value	Relative Variable Importance
$\delta^{13}C$	Intercept	-23.77	0.15	-24.08	-23.45	147.81	<0.001	NA
	Site (Woodbridge)	-0.39	0.29	-0.96	0.18	1.34	0.18	1.0
	Site (Freycinet)	2.11	0.22	1.67	2.55	9.45	<0.001	
	Site (Takone)	-0.12	0.28	-0.67	0.43	0.43	0.67	
	Site (West Pencil Pine)	-0.03	0.23	-0.49	0.43	0.12	0.90	
	Site (Wilmot)	-0.95	0.21	-1.36	-0.53	4.49	<0.001	
	Tumour volume	-0.19	0.14	-0.46	0.08	1.30	0.19	0.45
	Age	-0.21	0.16	-0.52	0.12	1.30	0.19	0.43
$\delta^{15}N$	Intercept	8.32	0.19	7.94	8.70	43.17	<0.001	NA
	Site (Woodbridge)	0.36	0.33	-0.30	1.01	1.07	0.29	1.00
	Site (Freycinet)	0.83	0.26	0.31	1.34	3.15	0.002	
	Site (Takone)	-1.27	0.33	-1.93	-0.62	3.82	<0.001	
	Site (West Pencil Pine)	-1.80	0.27	-2.33	-1.27	6.61	<0.001	
	Site (Wilmot)	-0.76	0.25	-1.27	-0.26	3.0	0.003	
	Tumour Volume	0.20	0.44	-0.66	1.07	0.46	0.65	1.00
	Tumour Volume: Woodbridge	-0.41	0.74	-1.87	1.05	0.55	0.58	1.00
	Tumour Volume: Freycinet	1.44	0.59	0.26	2.61	2.40	0.02	
	Tumour Volume: Takone	-0.18	0.58	-1.33	0.97	0.31	0.76	
	Tumour Volume: West Pencil Pine	0.35	0.63	-0.91	1.61	0.54	0.59	
	Tumour Volume: Wilmot	-0.47	0.53	-1.53	0.58	0.88	0.38	
	Body Condition	0.36	0.18	-0.01	0.74	1.93	0.05	0.69

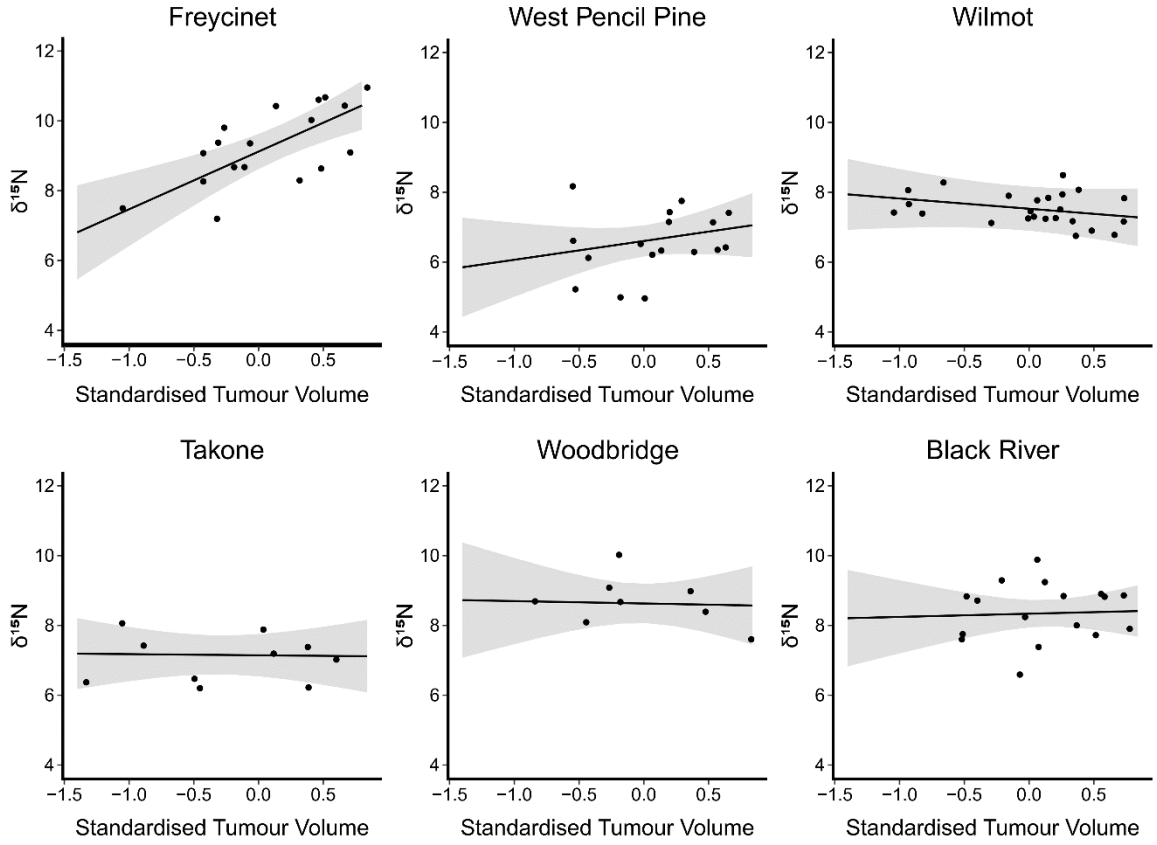

Figure 3.3. The effect of increasing DFTD tumour volume (log10 transformed and standardised) on $\delta^{15}N$ values of Tasmanian devil whiskers at six study sites across Tasmania*. $\delta^{15}N$ data is presented with slopes predicted from our standardised linear model. Devils at Freycinet, but not the other sites, show a sharp increase in $\delta^{15}N$ with increasing tumour volume (estimate = 2.11, 95% CI = 1.67-2.55).*

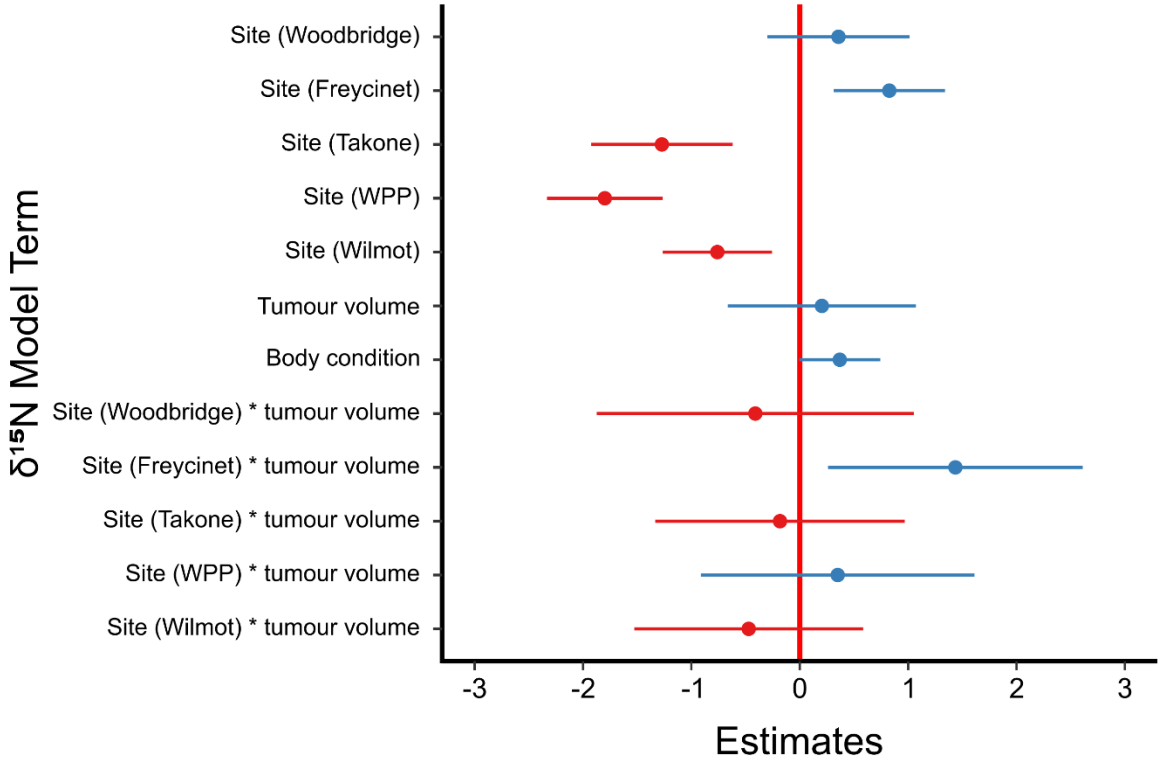

Figure 3.4. Results from a linear averaged model of variation in stable isotope ratios ($\delta^{15}N$) from whisker samples of 94 Tasmanian devils infected with devil facial tumour disease*. The site West Pencil Pine is denoted by (WPP). Effect sizes and confidence intervals are presented from our model, produced by averaging two models with a ΔAIC >2.*

Species Distribution Models

Our top-performing species distribution models suggest that the prey community differs substantially between Freycinet and our other sites. The model-estimated relative abundance of the three prey species showed that Freycinet had the highest mean predicted relative abundance of both Bennett's wallabies (17.01 ± 2.36 SD predicted animals per transect) and the omnivorous brushtail possums (15.83 ± 7.36 SD predicted animals per transect) (Figure 3.5e). Tasmanian pademelons appear to be relatively consistently abundant across all sites (Figure 3.5e). The distributions of three major prey species of devils, Bennett's wallabies, Tasmanian pademelons and brushtail possums, were all influenced by wet eucalypt, agricultural habitat and button grass moorlands, and the top-performing model for each species contained a spatial random field (Appendix, Table A.1).

Individual longitudinal study

Of the 18 devils sampled on two occasions to study the longitudinal effects of DFTD on devil isotopic signatures, the 10 individuals that developed DFTD between capture occasions had a median tumour volume of 19,305mm^3 (95% quantiles = 602 – 72,514mm^3).

Tumour volume did not explain variation in mean or standard deviation of $\delta^{13}C$ or $\delta^{15}N$ at capture occasion 2 (Table 3.3). Across both $\delta^{13}C$ and $\delta^{15}N$ models, the mean ratios at capture occasion 1 were closely correlated with mean ratios at capture occasion 2 ($\delta^{13}C$ estimate = 3.47, 95% CI = 2.87–4.08; $\delta^{15}N$ estimate = 0.69, 95% CI = 0.04–1.34; Figure 4.6). The adjusted R^2 for our mean $\delta^{13}C$ model was 0.89 and the adjusted R^2 for our mean $\delta^{15}N$ model was 0.19. The standard deviation in $\delta^{13}C$ at capture occasion 2 was closely correlated with the same metric at capture occasion 1 (estimate = 0.40, 95% CI = 0.05–0.74). Neither standard deviation in $\delta^{15}N$ at capture occasion 1 nor tumour volume predicted standard deviation in $\delta^{15}N$ at capture occasion 2. The adjusted R^2 for our standard deviation $\delta^{13}C$ and $\delta^{15}N$ models were 0.23 and 0.17.

Our binomial model demonstrated that mean $\delta^{13}C$ and $\delta^{15}N$ of individuals at the first capture event (while healthy) and the time between capture events did not

predict subsequent appearance of DFTD tumours (mean $\delta^{13}C$ estimate = -0.08, 95% CI = -0.72–0.58 ; mean $\delta^{15}N$ estimate = -0.45, 95% CI = -1.99–0.88; time (in months) estimate = -0.14, 95% CI = -0.40–0.03).

Table 3.3. Summary of analyses of variation in the mean and standard deviation of stable isotope rations ($\delta^{13}C$ and $\delta^{15}N$) of whiskers from Tasmanian devils sampled before and after detection of clinical signs of devil facial tumour disease*. Devils were each sampled at two separate time points. 10 individuals developed DFTD between capture occasion 1 (t_1) and 2 (t_2), while 8 remained disease free. The results for four linear models are presented, with the mean and standard deviation of each isotope at the individuals' second capture occasions as response variables. Across both $\delta^{13}C$ and $\delta^{15}N$, tumour volume did not predict mean or standard deviation isotope values at capture occasion 2.*

Response Variable	Model Variables	Estimate	Standard Error	Lower Confidence Interval	Upper Confidence Interval	t value	P value
Mean $\delta^{13}C$ (t_2)	Intercept	-22.70	0.14	-22.99	-22.41	-165.97	<0.001
	Mean $\delta^{13}C$ (t_1)	3.47	0.28	2.87	4.08	12.30	**<0.001**
	Tumour volume	0.18	0.28	-0.42	0.78	0.63	0.54
Standard deviation of $\delta^{13}C$ (t_2)	Intercept	0.46	0.08	0.30	0.63	5.98	<0.001
	Standard deviation of $\delta^{13}C$ (t_1)	0.40	0.16	0.05	0.74	2.46	**0.02**
	Tumour volume	0.22	0.16	-0.12	0.57	1.39	0.18
Mean $\delta^{15}N$ (t_2)	Intercept	8.84	0.15	8.53	9.15	60.76	<0.001
	Mean $\delta^{15}N$ (t_1)	0.69	0.31	0.04	1.34	2.25	**0.04**
	Tumour volume	-0.17	0.31	-0.82	0.48	2.25	0.59
Standard deviation of $\delta^{15}N$ (t_2)	Intercept	0.39	0.03	0.32	0.46	11.66	<0.001
	Standard deviation of $\delta^{15}N$ (t_1)	-0.10	0.07	-0.25	0.04	-1.51	0.15
	Tumour volume	0.10	0.07	-0.05	0.25	1.48	0.16

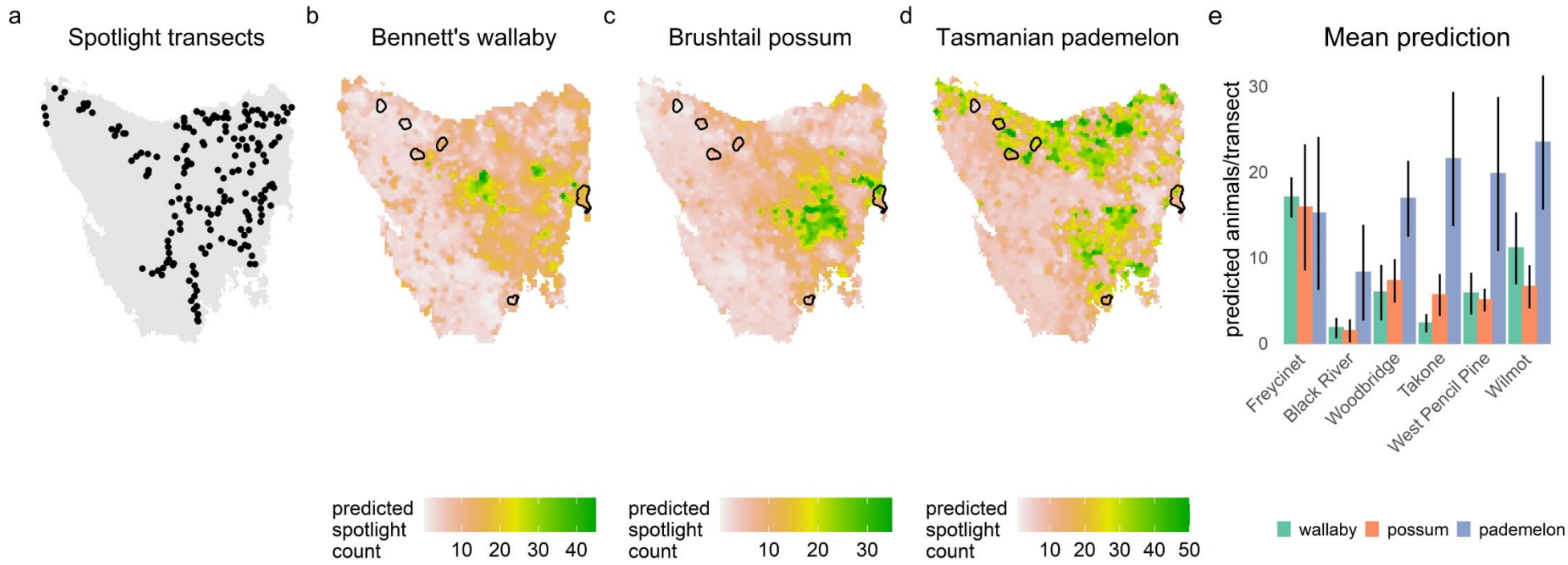

Figure 3.5. Distribution of a) spotlight transect surveys, b-d) species distribution models and e) predicted abundances for Bennett's wallabies, brushtail possums and Tasmanian pademelons*. For our study sites (e), predictions are of mean counts of animals per transect (± standard deviation). Species distribution models were created using data from standardised spotlight surveys, conducted annually from 1985-2019, at up to 172 10km transects across Tasmania.*

Discussion

Stable isotope ratios in Tasmanian devil whiskers do not, for the most part, vary in response to the progression of DFTD infection. Variation in $\delta^{13}C$ and in $\delta^{15}N$ values among infected devils was not associated with tumour volume as a main effect, and neither did mean or standard deviation in $\delta^{13}C$ or $\delta^{15}N$ alter after clinical signs of DFTD infection were detected. Tumour volume was, however, related to a pronounced increase in $\delta^{15}N$ values at Freycinet National Park. The composition of the prey community differed significantly at Freycinet National Park, when compared to all other sites, suggesting that any dietary response by devils to infection may depend on local environmental or ecological conditions.

We predicted that stable isotope ratios of Tasmanian devil whiskers would alter in response to DFTD progression, on the basis that diseased devils might experience reduced energy for foraging, increased difficulty eating, and changes in diet to avoid social contacts (Loh et al., 2006; Pye et al., 2016; Hamilton et al., 2020). Specifically, we suggested that $\delta^{13}C$ and $\delta^{15}N$ may increase with increasing tumour volume, to reflect a shift from a diet relying heavily upon herbivorous macropods, such as Bennett's wallabies and Tasmanian pademelons, towards smaller prey, which tend to be omnivorous and are less likely to attract competition. At Freycinet, although we observed no change in $\delta^{13}C$, we found $\delta^{15}N$ values increased significantly with increasing tumour volume, which may reflect an increase in the trophic level of devil food items specifically reflecting a shift from a diet largely comprising herbivorous macropods towards more omnivorous prey species. Our relative prey availability estimates suggest that prey composition at Freycinet differed substantially from our other sites, with higher estimated relative abundance of Bennett's wallabies and brushtail possums, whereas Tasmanian pademelons dominated our other sites. The higher availability of omnivorous prey species at Freycinet, like brushtail possums, could allow devils to prey-shift when tumours become larger, which would result in an increase in $\delta^{15}N$ values. Our results could indicate more broad differences in prey assemblage and availability of other species not modelled here, such as bandicoots, birds or skink species, which allow devils to transition to a diet richer in omnivores and lower in

herbivorous macropods as DFTD infection progresses. Although we do not have data on relative carcass availability between sites, it is reasonable to presume differences in relative abundance of prey species among sites will also lead to differences in the proportion of these carcass types availabile to scavengers, which may influence the ability of scavengers to shift their diet. Of all our sites, Freycinet is the only site largely comprised of protected areas, and has the most pristine habitat, alongside a drier and warmer climate. This likely leads to differences in relative prey abundance, but other environmental differences, such as predominant foraging habitat, could influence the likelihood that devils will shift their diet with increasing DFTD tumour volume. $\delta^{15}N$ can increase due to nutritional stress (Hobson et al., 1993), however if starvation was driving variation in $\delta^{15}N$, we would have expected to see a negative, rather than a positive relationship between body condition and $\delta^{15}N$ (Table 3.2). We therefore suggest the most likely explanation is that differences in the ecological and environmental context of Freycinet compared to other sites, including prey availability, facilitate changes in devil diets and their isotopic niches as tumour volumes increase.

Given the extent of facial deformation associated with advancing DFTD (Figure 3.1), it is remarkable that, in sites other than Freycinet, Tasmanian devils retain such consistency in their isotopic signatures with tumour progression. Our longitudinal study showed that mean isotopic signatures, and standard deviation in $\delta^{13}C$ as a measure of isotopic niche variation, were most strongly predicted by an individuals' prior measures of mean and standard deviation, rather than other ecological predictors, or disease severity, demonstrating the extent to which consistency of inferred diet and dietary variation is maintained among adult devils, regardless of tumour volume. This new information suggests that, while Tasmanian devils exhibit some sickness behaviours in response to DFTD, this generally does not encompass, and is not facilitated by change in the types of food they eat.

Where devils appear not to shift their diets in response to DFTD, they may alter their spatial and social behaviour with DFTD progression while maintaining their existing diet and foraging behaviour. Outside of the mating season, feeding has been assumed to be a major focus of social and competitive interactions among

Tasmanian devils, with direct observational studies of social behaviour usually focusing on behaviour at carcasses of large prey species (Hamede et al., 2008; Jones, 1995; Pemberton and Renouf, 1993). However, recent video collar evidence found a lower proportion of intraspecific interactions occurred at carcasses than expected, with the majority of interactions occurring while devils were moving (Andersen et al., 2020). The relative importance of social interactions outside of large carcass feeding and mating behaviours may have been underestimated, and as such, foraging may carry a lower relative risk of competitive interactions allowing devils to maintain their usual feeding behaviour. Additionally, the preferred food items of devils (including pademelons, wallabies and possums) may be in high enough abundance that the prospect of intraspecific competition at carcasses is fairly low and there is generally no need for a disease-related dietary shift. The main prey species of Tasmanian devils are abundant; furthermore, Tasmania has high roadkill density compared to other areas in Australia (Hobday and Minstrell, 2008), providing increased opportunities for facultative scavengers such as Tasmanian devils. The abundance of preferred prey items relative to devil density may vary geographically, resulting in a dietary shift in some areas, but allowing devils to maintain their diet in others.

Devils could maintain the proportional prey composition of their diets, but change aspects of their feeding ecology to accommodate the physical and competitive disadvantages of DFTD infection. For example, infected devils could reduce the overall amount of food they consume. Illness-induced anorexia can result from a trade-off between acquiring necessary calories and nutrients, and minimising energy expended on finding and/or hunting, consuming and digesting food items (Adamo et al., 2010; McMillan et al., 2018). For Tasmanian devils, reduced food consumption may incidentally result in a reduction in social contact. However, we did not see a sharp increase in $\delta^{15}N$ in response to DFTD progression or with reduced body condition, which would be expected if food intake reduction and nutritional stress were occurring (Hobson et al., 1993). A further possibility is that devils with DFTD reduce the likelihood of agonistic contacts with other devils by shifting their activity to times of the diel cycle when devil activity is relatively low. Tasmanian devils have been shown to exhibit

flexibility in their temporal activity (Cunningham et al., 2019b), likely driven by intraspecific competition for carcasses and times of peak herbivore activity. In low-density sites, peak devil activity is later (around 22:00) than in high density sites (peak activity around 18:00) (Cunningham et al., 2019b), therefore infected devils in disease-affected low-density sites could avoid competition by foraging in the early evening. Devils could also modify their feeding behaviour by abandoning food items more readily on the approach of conspecifics. While direct observations of intraspecific interactions at carcasses have not found strong evidence of dominance hierarchies among devils (Pemberton and Renouf, 1993), larger devils are more likely to displace smaller devils of the same age class (Jones, 1995). This suggests that costly agonistic interactions over carcasses are more likely if an individual is at a competitive disadvantage relative to other devils at a carcass. Diseased devils may therefore have a lower threshold for retreat from carcasses compared to healthier devils, reducing the chance for close agonistic encounters with conspecifics.

We considered whether individuals that contract DFTD, and those that do not, exhibit differences in their foraging that influence their susceptibility to DFTD infection. Tasmanian devils have variable likelihoods of developing DFTD based on their behaviour (Hamede et al., 2013); devils with fewer bites are more likely to develop DFTD, predominantly inside the oral cavity, suggesting dominant, aggressive individuals are more at risk (Hamede et al., 2013). We found that isotopic signatures of healthy devils did not predict whether the same individuals subsequently developed DFTD. However, as not all devils were recaptured after the second sampling occasion, we cannot confirm that that our healthy control individuals did not also go on to show symptoms or contract DFTD at a later date or rule out the possibility that individual differences in foraging behaviour may influence the likelihood of DFTD infection.

Stable isotope analysis is a robust method of inferring animal diets and ecological niches (Bearhop et al., 2004; Newsome et al., 2007), and its application has enabled us to provide insight into the ecology of individual devils both before and during DFTD progression. However, isotopic signatures and niches are related to, but are not an exact reflection of, diet and ecological niche. Therefore, we cannot exclude the possibility that Tasmanian devils do

change their diet with increasing tumour volume, but that stable isotope ratios of whisker samples are not sensitive enough to reveal this change. If different prey species are isotopically similar, a shift in devil diet with increasing tumour volume may not result in a noticeable change in devil isotope signatures. Equally, if prey species are isotopically distinct but vary in their position in isotopic space geographically, stable isotope analysis of devil tissues from multiple sites may not reveal a consistent directional change in isotopic signatures if devils alter their diet with DFTD progression. Furthermore, our ability to capture changes in the standard deviation of $\delta^{13}C$ and $\delta^{15}N$ in whiskers before and after DFTD infection depends on whether dietary variation integrates into the whisker at a rate that matches our sampling protocol. If animals consistently eat a broad range of prey items, this could average out within each whisker section, potentially resulting in a low amount of variation between the four whisker sections we analysed in our longitudinal study. If dietary variation occurs over a longer period of time, variation between whisker sections may be evident.

Sickness behaviours, and individual and population behavioural responses to disease, have implications for immune responses, disease transmission, and disease management (Bouwman and Hawley, 2010; Johnson, 2002; Lopes et al., 2016, 2014; Silk et al., 2019). It is striking that devils with DFTD generally maintain their isotopic niches, given the pathological severity of, and metabolic demands imposed by, cancer. While our data demonstrate that Tasmanian devils largely maintain their isotopic niches as the disease progresses, where ecological conditions permit devils exhibit greater trophic flexibility. This suggests that any sickness behaviours they manifest are dependent as much upon their ecological context as their pathology.

Appendix

Relative abundance of prey species

We modelled the Tasmania-wide relative abundance of three of the main prey species of devils (Andersen *et al.* 2017a). This included the omnivorous brushtail possum, as well as two macropod herbivores, the Bennett's wallaby and the Tasmanian pademelon. We made use of a dataset of standardised annual spotlight surveys that have been conducted annually from 1985-2019 at up to 172 transects across Tasmania (Figure 5), totalling 5,761 surveys (Hocking & Driessen 1992). Each transect follows a 10-km segment of road, with one person operating a handheld spotlight and another recording sightings of all wild animals (Hocking & Driessen 1992). We considered the count of each species per transect as a measure of relative abundance.

We modelled the distributions of these prey species using integrated nested Laplace approximation (INLA). INLA is a computationally efficient method for Bayesian inference with spatial data. INLA facilitates the use of Gaussian random fields to model spatial dependence between observations (Bachl *et al.* 2019). Using INLA, a Gaussian random field is approximated using the solution to a stochastic partial differential equation (SPDE) (Lindgren *et al.* 2011), which requires discretising space into a tiling of adjacent triangles, known as a mesh (Bachl *et al.* 2019). We constructed the mesh to have maximum interior edge lengths of 10 km (matching the scale of the spotlight transects). To avoid problematic boundary effects, we extended the mesh beyond the boundaries of Tasmania, using larger edge lengths (30 km) to reduce fitting time (Bakka *et al.* 2018). We used a Matérn correlation structure for the SPDE (priors = 0.1 probability that the range is less than 10 km and a 0.5 probability that the standard deviation was greater than 10 km). See Lindgren *et al.* (2011) for a fuller explanation of SPDEs and INLA. We fitted the models using the 'inlabru' R package (Bachl *et al.* 2019), which builds upon the R-INLA package (Illian *et al.* 2013).

We constructed four spatial covariates reflecting the percentage cover of the four main vegetation classed in Tasmania: wet Eucalypt/rainforest (*%wetEuc*;

28% of Tasmania), dry Eucalypt forests and woodlands (*%dryEuc*; 24%), agricultural land (*%agric*; 23%) and button grass moorlands (*%butGrass;* 9%). Using a raster (cell size = 1 km^2) of the TasVeg 3.0 GIS layer (Department of Primary Industries Parks Water and Environment 2014), we extracted the mean proportional cover of each vegetation class within a 5 km^2 area around each spotlight transect (for further details, see Cunningham *et al.* 2021). We omitted *%dryEuc* from the analysis because it was negatively correlated with *%wetEuc* (Pearson's r = -0.65). In addition to simple linear effects, we modelled non-linear effects of these covariates using a one-dimensional Matérn SPDE with five evenly spaced B-spline knots.

We followed the advice of Illian *et al.* (2013) for selecting models that include spatial covariates and spatial random fields, which can compete for explanatory power. We first aimed to select the most informative environmental covariates. For each species, we tested whether linear or non-linear effects of vegetation covariate produced the best model fit. Next, we tested all simpler combinations of those environmental covariates, comparing models using the Widely Applicable Information Criterion (Watanabe 2010). Then, we added a spatial random field to the best covariate model. This has two purposes: first, to model spatial dependency not captured by the vegetation covariates, and second, to account for correlations between repeated surveys at each transect. Finally, we compared all models using WAIC, and from the best model, produced predictive maps of relative abundance across Tasmania ('predict' function of 'inlabru', which takes many draws from the model posteriors).

Table A.1. Model selection table for the species distribution models of prey species. "Order model fitted" shows the order in which the models were fitted, which followed a model selection approach that first aimed to select the important environmental covariates, and then test whether adding a spatial random field improved model fit. Models were ranked according to the Widely Applicable Information Criterion (WAIC), with Delta.WAIC showing the difference between the best model. We tested linear (e.g., "wetEuc") and non-linear (e.g., "f(wetEuc)") effects of each vegetation covariate.

Order model fitted	WAIC	Delta.WAIC	Model
Bennett's wallaby			
12	30569.7	0.0	f(wetEuc) + f(agric) + butGrass + random field
3	32756.4	2186.7	f(wetEuc) + f(agric) + butGrass
4	32759.0	2189.3	f(wetEuc) + f(agric) + f(butGrass)
2	32861.9	2292.2	wetEuc + f(agric) + butGrass
1	32879.1	2309.3	wetEuc + agric + butGrass
6	32883.4	2313.7	f(wetEuc) + f(agric)
5	33011.8	2442.1	f(wetEuc) + butGrass
8	33107.1	2537.4	f(wetEuc)
7	33941.7	3372.0	f(agric) + butGrass
10	34008.2	3438.5	butGrass
9	34135.5	3565.8	f(agric)
11	34199.3	3629.6	null
Tasmanian pademelon			
12	39345.9	0.0	f(wetEuc) + f(agric) + f(butGrass) + random field
4	41898.3	2552.4	f(wetEuc) + f(agric) + f(butGrass)
3	41904.8	2558.9	f(wetEuc) + f(agric) + butGrass
2	41908.8	2562.9	wetEuc + f(agric) + butGrass
6	41914.6	2568.7	f(wetEuc) + f(agric)
7	41922.3	2576.4	f(agric) + f(butGrass)
9	41938.1	2592.2	f(agric)
1	42110.0	2764.1	wetEuc + agric + butGrass
5	42573.0	3227.1	f(wetEuc) + f(butGrass)
10	42651.9	3306.0	f(butGrass)
8	42657.7	3311.8	f(wetEuc)
11	42746.9	3401.0	null

Brushtail possum

12	30388.6	0.0	f(wetEuc) + f(agric) + f(butGrass) + random field
4	33289.6	2901.1	f(wetEuc) + f(agric) + f(butGrass)
6	33404.1	3015.5	f(wetEuc) + f(agric)
5	33404.9	3016.3	f(wetEuc) + f(butGrass)
3	33406.0	3017.4	f(wetEuc) + agric + f(butGrass)
2	33417.8	3029.2	f(wetEuc) + agric + butGrass
8	33568.3	3179.7	f(wetEuc)
1	33691.8	3303.2	wetEuc + agric + butGrass
7	33960.4	3571.8	f(agric) + f(butGrass)
9	34177.3	3788.7	f(agric)
10	34214.8	3826.2	f(butGrass)
11	34557.5	4168.9	null

Chapter 4

Using Bayesian stable isotope mixing models to investigate dietary variation among Tasmanian devils *Sarcophilus harrisii*

Chapter 4: Using Bayesian stable isotope mixing models to investigate dietary variation among Tasmanian devils *Sarcophilus harrisii*

Abstract

Tasmanian devils *Sarcophilus harrisii* are the largest extant carnivorous marsupial, and the top scavenger of the Tasmanian ecosystem. They are experiencing population declines as a result of a transmissible cancer, devil facial tumour disease (DTFD), with population declines of around 80% across 80% of their range. Despite a number of dietary studies, our understanding of the trophic ecology of Tasmanian devils remains limited, particularly with respect to how it might be linked to DTFD. To address this, we investigated Tasmanian devil diet using stable isotope analysis. We aimed to i) characterise the proportional contributions of potential foods to Tasmanian devil diet, ii) consider dietary variation in Tasmanian devil diet by age and DFTD infection status, and iii) to consider dietary variation among sites. We collected Tasmanian devil whiskers (n = 426) at six locations across Tasmania, alongside 249 muscle tissue samples of potential prey species from the same locations. After plotting our isotopic data, only one site, Woodbridge, was suitable for dietary proportion analysis. We applied two Bayesian stable isotope mixing models (BSIMMs) to investigate the diet of Tasmanian devils at Woodbridge. Our first BSIMM was a null model, estimating the diet of the devil population as a whole, while our second BSIMM included the fixed effect of population class (subadult, <24 months, adult ≥24 months with DFTD, adult free from DFTD). Our null model carried the most weight after model comparison, and estimated that devils at Woodbridge consumed similar proportions of Tasmanian pademelons and small mammals (pademelon mean ± SD = 38% ±8%, small mammal = 43% ±10%), while Bennett's wallabies contributed less (19 ± 9%). This is the first known use of Bayesian stable isotope mixing models on a marsupial species, and we highlight both opportunities and challenges for applying this method in these taxa, while underlining the importance of Tasmanian pademelons to the diet of Tasmanian devils.

Introduction

Tasmanian devils *Sarcophilus harrisii*, of the Order Dasyuromorphia, are the largest extant carnivorous marsupial. Tasmanian devil populations were once spread across continental Australia, but wild populations are currently restricted to the island state of Tasmania, possibly due to a combination of competitive and/or predation pressure from dingos, increases in human population density, and climatic changes around 3000 years ago (Johnson and Wroe, 2003; Letnic et al., 2014). Although Tasmanian devils can hunt, they are primarily a scavenger in the Tasmanian ecosystem (Cunningham et al., 2018; Jones and Stoddart, 1998), but exert competitive control over smaller sympatric mesopredators such as the spotted-tailed quoll *Dasyurus maculatus maculatus*, Eastern quoll *Dasyurus viverrinus* and feral cat *Felis catus* (Andersen et al., 2016; Cunningham et al., 2020, 2018; Hollings et al., 2014; Jones, 1998). Tasmanian devils are generally solitary, with interactions between individuals occurring mostly during movement, feeding at carcasses and during mating season (Andersen et al., 2020; Pemberton, 1990). These interactions can be intensely aggressive and injurious, resulting in bite wounds (Hamede et al., 2013, 2008; Pemberton, 1990). As Tasmanian devils are cryptic and nocturnal, they are difficult to observe undisturbed in the wild. Therefore, the detail of our understanding of natural devil behaviour is increasing with advances in ecological methods and telemetry technology, including VHF, GPS or proximity-sensing radio collars and video collars (Andersen et al., 2020, 2017b; Comte et al., 2020; Hamede et al., 2009; Hamilton et al., 2019).

Tasmanian devils have experienced extensive population declines since 1996, due to the emergence of devil facial tumour disease (DFTD) (Lazenby et al., 2018; Pearse and Swift, 2006; Cunningham et al., 2021). DFTD is a transmissible cancer spread via direct inoculation of tumour cells when individuals bite one another (Hamede et al., 2013; Pearse and Swift, 2006). Presenting clinically as tumours around the head, face and mouth, DFTD is almost always fatal, death results from a combination of metastasis and organ failure, metabolic starvation, and secondary infection (Loh et al., 2006). DFTD has now spread across approximately 80% of the devil's range, causing population declines of an average of 80% in affected areas (Lazenby et al.,

2018). A second transmissible cancer, DFT2, emerged in 2014, but is clinically indistinguishable from DFTD and has not yet spread beyond an area of approximately 550km^2 in the d'Entrecasteux peninsula in southern Tasmania (James et al., 2019; Pye et al., 2015). Initial research raised serious concerns that DFTD may cause Tasmanian devil extinction (McCallum et al., 2009), however local extinctions of long-affected populations have not occurred, and recent modelling suggests either eventual coexistence between devils and DFTD, or DFTD gradually fading out of devil populations, are more likely scenarios than host extinction (Wells et al., 2019). Nevertheless, severe declines have resulted in a suite of conservation strategies aimed at reducing the incidence of DFTD in wild populations, including selective culling programs and vaccination research (Flies et al., 2020; Lachish et al., 2010; Owen and Siddle, 2019; Rout et al., 2018), and aimed at creating healthy 'insurance' populations on Tasmanian islands and continental Australia (Hogg et al., 2015; Hunter et al., 2015).

A solid understanding of a species' ecology is important in order to predict the needs and consequences of reintroduction or translocations at the level of populations, meta-populations, and ecosystems (Armstrong and Seddon, 2008), especially in areas where a species has been absent for a considerable length of time, and may encounter novel habitats, competitive environments or available niche space. The diet of Tasmanian devils has been investigated using scat contents analysis (Andersen et al., 2017a; Jones and Barmuta, 1998), and stable isotope analysis has been used to estimate isotopic niche metrics of devil population classes (Bell et al., 2021, 2020). Scat contents studies have demonstrated the importance of large and medium mammals such as Bennett's wallabies *Macropus rufogriseus* and Tasmanian pademelons *Thylogale billardierii* in the diet of Tasmanian devils, although there are some differences in findings between studies (Andersen et al., 2017a; Jones and Barmuta, 1998). A multi-site scat contents study found devils consume more pademelons than wallabies (Andersen et al., 2017a), while a single-site study at West Pencil Pine, a large eucalypt plantation, found large mammals, including wallabies, were consumed more than medium mammals such as pademelons (Jones and Barmuta, 1998). Andersen et al. (2017a) found that birds and small

mammals often contributed to devil diets according to scat contents analysis (birds frequency of occurrence 22.1%, relative volume 7.0%; small mammals frequency of occurrence 10.4%, relative volume 5.1%), although these food groups were rarely found in scats by Jones and Barmuta (1998). Stable isotope analyses have found a reduction in Tasmanian devil trophic level and isotopic niche breadth with increasing age, possibly reflecting a shift from a broad subadult diet including omnivorous, small prey items, to a narrower adult diet mostly consisting of large herbivores (Bell et al., 2020). This age effect was also noted by Jones and Barmuta (1998). Stable isotope analysis has also indicated that, while devils generally do not alter their diets in response to DFTD infection, this may be dependent on ecological context, as infected devils in one study location showed an increase in trophic level with increasing tumour volume, perhaps indicating a dietary shift to smaller, omnivorous prey types from large herbivores (Bell et al., 2021). However, stable isotope approaches have not thus far been used to estimate the proportional contributions of dietary items consumed by devils, and scat contents analyses have not considered potential within-population dietary variation across multiple ecological contexts. Furthermore, as scat contents analyses provide a snapshot of dietary intake, this method may not capture the full extent or limit of dietary variation between individuals. For example in a generalist population, scats may indicate a high degree of variation between individuals, however over a longer period of a week or month, all individuals may consume a similar, if varied, diet.

Bayesian stable isotope mixing models (BSIMMs) are powerful tools that enable researchers to convert measurements of stable isotopes in consumer tissues, in combination with those of putative food sources, into estimates of the dietary proportions of different isotopic sources, or food types (Moore and Semmens, 2008). BSIMMs are constructed with consumer and source (prey items) isotopic data such as such as $\delta^{13}C$ and $\delta^{15}N$ values, and an estimation of the systematic isotopic change that occurs between source and consumer tissues after source consumption, known as the trophic discrimination factor (TDF) (Phillips, 2012). BSIMMs then characterise the proportional contributions of each dietary source to consumer diet, returning probability distributions for each source. Recent BSIMMs can account for uncertainty around TDF estimates and concentration

dependence where elemental concentrations differ substantially between sources, and be fitted with hierarchical population structures, or fixed or random covariates (Semmens et al., 2009; Stock et al., 2018). Estimates of dietary proportions can then help answer broader ecological questions. For example, BSIMMs have been used investigate the effect of diet on risk of Guinea-worm infection in domestic dogs *Canis familiaris* in Chad and Ethiopia (McDonald et al., 2020; Wilson-Aggarwal et al., 2021), to demonstrate the dietary flexibility of hazel dormice *Muscardinus avellanarius* in response to local food availability (Goodwin et al., 2020), and also to confirm individual specialisation in the diet and foraging locations of northern gannets *Morus bassanus*, which holds consequences for the degree of competition experienced by individuals (Bodey et al., 2018). As with all modelling techniques, not all data will be suitable for a BSIMM approach and care should be taken to avoid erroneous interpretations, for example, appropriate TDFs should be applied, and baseline isotopic variation may vary spatially and temporally and could influence BSIMM results if this is not accounted for (Phillips et al., 2014). Applying best practise methodology from sampling design through to analysis and reporting of results can reduce the likelihood of inaccurate or misrepresented dietary proportional estimations. These practises include, but are not limited to, careful study design guided by clear questions and prior ecological understanding, using appropriate TDFs, plotting data before analysis, grouping ecologically and isotopically similar dietary sources, and reporting the full distributions of results (Phillips et al., 2014).

In this study, we use isotopic data from Tasmanian devil whiskers, and muscle tissue of their putative prey, to characterise the dietary composition of Tasmanian devils, using BSIMMs. We aimed to characterise diets at six study sites across Tasmania, to consider whether these vary based on age or DFTD infection status, and to consider whether Tasmanian devil diet varied between locations.

Methods

Study sites

We selected six field sites, located across Tasmania (Figure 4.1); Freycinet, (-42.107E, 148.277S), Woodbridge (-43.131 E, 147.224 S), the Midlands (the Forest, -41.860E, 147.505S; Oatlands -42.257E, 147.349S), West Pencil Pine (-41.541E, 145.823S), Wilmot (-41.377E, 146.152S), and Arthur River (-41.055E, 144.679S). Freycinet is a coastal site predominantly composed of native dry eucalypt forest. Woodbridge is an area of smallholdings interspersed by native eucalypt woodlands. The Midlands site is a composite of two sites, which we have combined due to low sample sizes related to predator density, as well as geographic proximity and habitat similarity. Both sites are farmland with fragmented patches of dry eucalypt woodland. Wilmot largely comprises of a commercial eucalypt plantation situated within an agricultural landscape. West Pencil Pine is also a commercial eucalypt plantation, situated close to a large protected area. Arthur River is a coastal site predominantly composed of coastal scrub. Freycinet and Arthur River are largely protected national park or conservation areas. DFTD has been recorded at Freycinet since 2001, at West Pencil Pine since 2006 and at Wilmot since 2008. DFTD arrived in the Midlands between 2000 and 2003. In Woodbridge, DFTD was first recorded in 2012, followed by the emergence of DFT2 in 2014. Arthur River was DFTD-free at the time of fieldwork for this study.

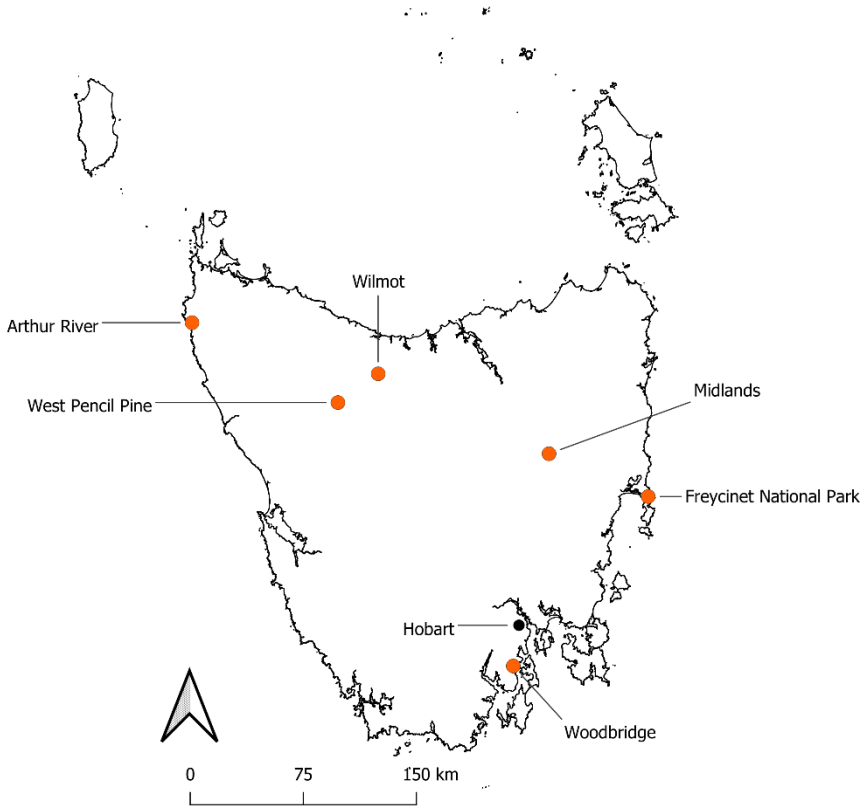

Figure 4.1. Locations of sites in Tasmania where Tasmanian devil whiskers were sampled*. Hobart, the capital city of Tasmania, is shown for reference (black circle). Study sites (orange circles) are labelled by site name.*

Sample collection

A total of 426 Tasmanian devil whiskers were collected during three-monthly live trapping periods from 2013-2019 (Table 4.1). One whisker was collected from each Tasmanian devil by cutting close to the skin with scissors. Tasmanian devils were identified using unique microchip transponders, and individual data including age, sex and weight were recorded. Individuals were aged using canine eruption, molar eruption and wear, which is accurate up to three years of age in Tasmanian devils (M.E. Jones, unpublished data). Whiskers were stored in plastic bags at -20°C prior to laboratory preparation.

Samples of muscle tissue from prey species were collected opportunistically at each study site (n = 249, Table 4.1). Muscle samples were taken from the hind leg of road-killed wildlife at each site, and their immediate surrounding areas. Prey sample collection occurred in every season at all sites throughout 2018. In addition, in the Midlands only, muscle samples for brushtail possums *Trichosurus vulpecula*, Bennett's wallabies, Tasmanian pademelon, rabbit *Oryctolagus cuniculus* and hare *Lepus europaeus* were also obtained from landowners managing wildlife numbers under Crop Protection Permits. Muscle samples were stored at -20°C, then oven dried at 60°C for 48 hours and stored in sealed Eppendorf tubes.

Sample preparation

Whiskers were rinsed in distilled water to remove surface contaminants and left to air-dry, before being placed in a freeze dryer for 24 hours. For isotope analysis, the base section of each whisker was chopped and weighed into a tin cup until a weight of 0.7 ± 0.1 mg. The base section was sampled as this whisker section is the most recently grown, and therefore isotopic data from this section is the most likely to reflect the trophic ecology of animals as the time at which they were sampled. Oven-dried lean muscle samples of prey species were placed in a freeze-dryer for 24 hours to remove any moisture built-up during storage, and then homogenised using a pestle and mortar. Homogenised tissue was then weighed into tin cups for isotope analysis, to a weight of 0.7 ± 0.1 mg.

Table 4.1. A summary of the number of Tasmanian devil whiskers collected at each site*. Each whisker was collected from a different individual. Prey muscle samples were collected at all sites in 2018.*

Site	Devil samples	Devil sample collection period	Prey samples
Freycinet	106	2015-2018	46
Woodbridge	42	2017-2018	32
The Midlands	17	2015-2017	60
West Pencil Pine	88	2014-2019	34
Wilmot	94	2014-2017	59
Arthur River	79	2015-2018	18
Total	426	NA	249

Stable isotope analysis

Stable isotope analyses were conducted using a Sercon INTEGRA2 elemental analyser-isotope ratio mass spectrometer at the University of Exeter, a Thermoquest EA1110 elemental analyser linked to a Europa Scientific 2020 isotope ratio mass spectrometer at Elemtex Ltd, Cornwall UK, and an Elementar Pyrocube Elemental Analyser linked to a Thermo-Fisher-Scientific Delta XP Plus isotope ratio mass spectrometer at the Stable Isotope Ecology Laboratory at the Scottish Universities Environmental Research Centre. Stable isotope ratios are expressed as delta (δ) values in parts per thousand, or 'per mil' (‰) relative to international standards, according to:

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

Where X is ^{13}C or ^{15}N, R_{sample} is the heavy to light isotope ratio of the sample and $R_{standard}$ is the heavy to light isotope ratio of an international standard (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). In laboratory settings, materials calibrated against the international standards are routinely used. Our samples were scale corrected using the standards USGS40 and USGS41, and internal standards of bovine liver, alanine, alagel, gel and glygel. Across laboratories and standards, average precision was 0.08 ± 0.007‰ (1 standard deviation ± standard error) for $\delta^{13}C$ and 0.12 ± 0.009‰ for $\delta^{15}N$.

Statistical analysis

No studies have yet taken place to estimate trophic discrimination factors (TDFs) in Tasmanian devils, or any marsupial species. Therefore, we estimated TDFs using an R package, SIDER (Healy et al., 2017). SIDER uses Bayesian inference to impute the TDF of a consumer based on its phylogenetic relationships to species with known TDF data, tissue type, and feeding ecology. The package then returns posterior probability distributions for consumer TDFs, providing means and estimates of uncertainty. We ran SIDER models to

estimate TDFs of Tasmanian devils for $\delta^{13}C$ and $\delta^{15}N$, using default formulae for fixed and random terms, and non-informative priors. We checked that our chains in each model converged and had effective sample sizes of over 1000, and plotted our posterior distributions to check for multi-modality (Healy et al., 2017). Our SIDER estimated TDFs (mean ± SD) were 2.1 ± 2.1‰ for $\delta^{13}C$ and 3.3 ± 1.8‰ for $\delta^{15}N$.

Before running any BSIMMs, we plotted our data to check that consumer isotope values at each site fell within the convex hull polygon created by the outermost mean signatures of prey sources (by species). This is a required feature of the geometric arrangement of data, or 'mixing space' if there are to be solutions for diet composition of consumer values (Phillips et al., 2014). This was the case at only two of our sites, Wilmot and Woodbridge (Figure 4.2). At Wilmot, prey, or 'source' signatures fell largely into two groups, approximately equidistant from the mean devil signatures, therefore the mixing model may struggle to resolve between species and grouping would result in 50/50 generalist solution. We therefore chose to proceed by fitting Bayesian stable isotope mixing models to data from Woodbridge. Our consumer sample size was therefore 42 Tasmanian devil samples, sampled at Woodbridge from 2017-2018.

We chose to include three prey sources for our mixing models, including Tasmanian pademelons, Bennett's wallabies, and small mammals (Figure 4.3). Small mammals represent a grouped source comprised of data from native Eastern barred bandicoots *Perameles gunnii* and Southern brown bandicoots *Isoodon obesulus*, and introduced brown rats *Rattus norvegicus* and European rabbits *Oryctolagus cuniculus*. Initial analyses treating bandicoot species and introduced mammals as separate sources revealed strong negative correlation between the two groups, therefore we combined these isotopically similar sources to increase model precision (Phillips et al., 2014; Figure 4.3). In previous scat contents analyses no distinction was made between native and non-native mammals of similar size (Andersen et al., 2017a; Jones and Barmuta, 1998). Although Eastern bettong *Bettongia gaimardi* data were collected at Woodbridge, we excluded these due to low sample size (n = 2) and a lack of both ecologically and isotopically similar species for potential grouping.

To investigate the diets of Tasmanian devils at Woodbridge, we fitted two Bayesian stable isotope mixing models using the R package MixSIAR (Stock et al., 2018). First, we fitted a null model with no fixed or random effects. Second, we fitted a model with the fixed effect of devil population class, including three groups: subadult (<24 months old, n = 18), adults without DFTD (≥24 months old, n = 15), and adults with DFTD at the time of sampling (n = 7). We checked that our models had converged using Gelman-Rubin and Geweke diagnostics, and then calculated relative support for each of our models using leave-one-out cross-validation (LOO) (Vehtari et al., 2017).

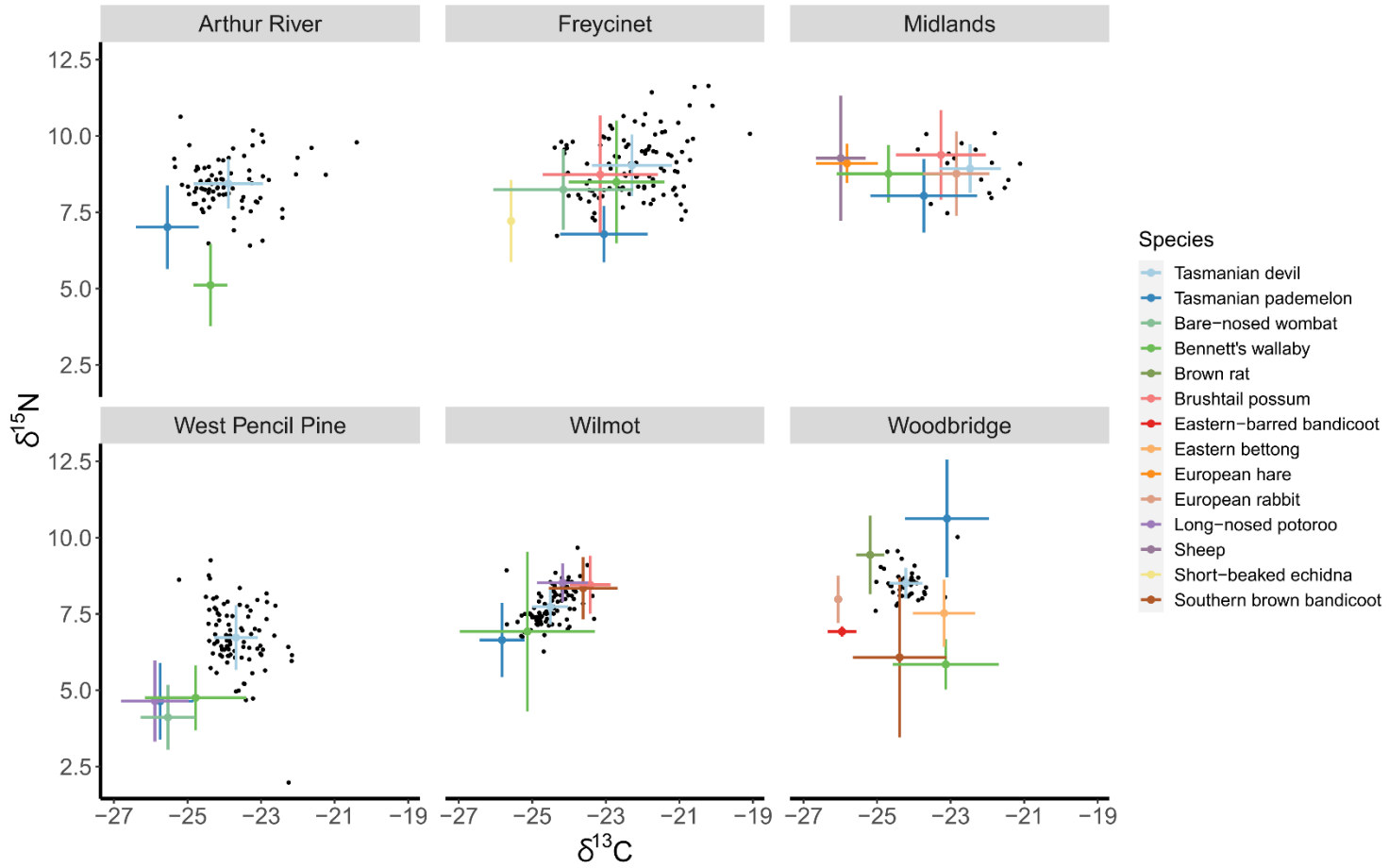

Figure 4.2. The mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ of Tasmanian devils (pale blue) and mammalian prey species *for which we had over 1 tissue sample per site. Individual Tasmanian devil $\delta^{13}C$ and $\delta^{15}N$ are represented as black dots. Isotope ratios of prey species have been adjusted based on trophic discrimination factors estimated using the R package SIDER (Healy et al., 2017).*

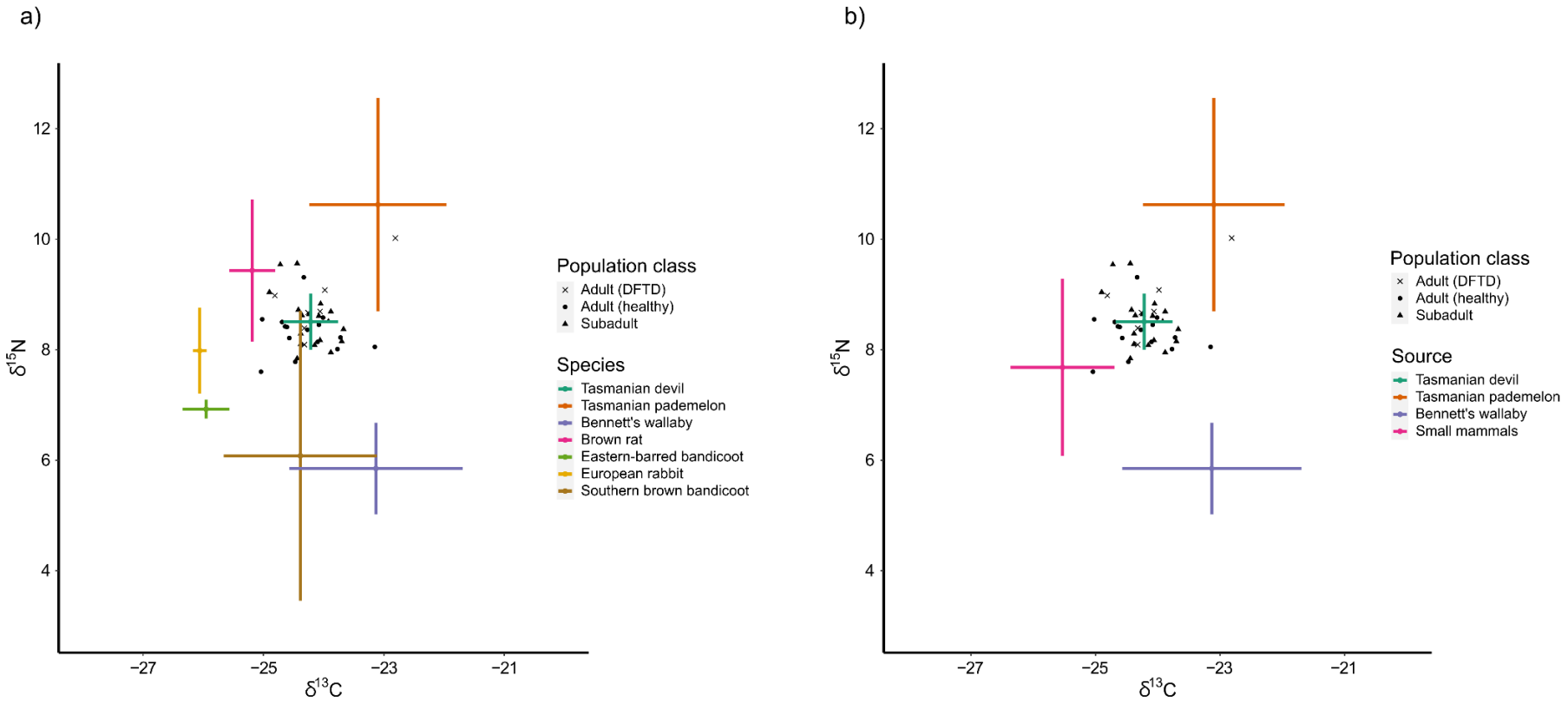

Figure 4.3. Mean stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of Tasmanian devils and a) dietary species at Woodbridge, and b) dietary sources grouped for Bayesian stable isotope mixing model analysis*. Bars represent standard deviation of stable isotope values. Stable isotope values of individual Tasmanian devils are represented as either black triangles (subadults; <24 months old), black circles (healthy adults), or black crosses (adults with clinically diagnosed DFTD). Dietary species & group isotope values have been adjusted based on trophic discrimination factors estimated using the R package SIDER (Healy et al., 2017).*

Results

The degree of isotopic variation observed within Tasmanian devil populations differed between sites (Figure 4.2). The highest relative levels of variation were seen in the two protected conservation area sites, Freycinet ($\delta^{13}C$ SD = 1.1‰, $\delta^{15}N$ SD = 1.0‰) and Arthur River ($\delta^{13}C$ SD = 0.8‰, $\delta^{15}N$ SD = 0.9‰), while the lowest amount of variation was observed at Wilmot ($\delta^{13}C$ SD = 0.5‰, $\delta^{15}N$ SD = 0.6‰) and Woodbridge ($\delta^{13}C$ and $\delta^{15}N$ SD = 0.5‰). Generally, the degree of variation in $\delta^{13}C$ and $\delta^{15}N$ was positively correlated for each site, however at West Pencil Pine, devils were much more varied in $\delta^{15}N$ (SD = 1.1‰) than $\delta^{13}C$ (SD = 0.6‰). For prey species collected at multiple sites, including Tasmanian pademelons and Bennett's wallabies, we observed very little consistency in the relative isotopic position of prey species to devil isotope values between sites (Figure 4.2).

Our null BSIMM, with no fixed effects, estimated that devils in the Woodbridge population as a whole consumed similar proportions of small mammals and pademelons (small mammal mean ± standard deviation = 43 ± 10%; pademelon = 38 ± 8%). Bennett's wallabies were consumed the least (19 ± 9%) (Figure 4.4). Negative correlation was found between each source dyad (Figure 4.5), suggesting devils consuming a higher proportion of one food type necessarily consume fewer of both of the other sources. However, this did not result in multi-modal probability distributions for our estimated mixing proportions (Figure 4.4). Our BSIMM with population class as a fixed effect revealed some differences between groups (Figure 4.6). Subadult devils consumed small mammals (43 ± 11%) and pademelons (42 ± 9%) in very similar proportions, with Bennett's wallabies consumed least (15 ± 9%). Adults without DFTD consumed small mammals in the highest proportions (48 ± 12%), followed by pademelons (35 ± 9%) and Bennett's wallabies (17 ± 10%). Adult devils with DFTD consumed pademelons the most (51 ± 11%), followed by small mammals (37 ± 12%) and finally Bennett's wallabies (13 ± 9%).

Our null model, with no fixed effects, had a lower LOO information criterion (LOOic) than our model with a fixed effect of devil population class (Table 4.2), and received 61% of the Akaike weight, indicating a 61% probability that it is the

better model. Therefore, we will discuss the results of our BSIMM estimating Tasmanian devil population diet as a whole, as this model is the most likely to better reflect the ecological reality at Woodbridge.

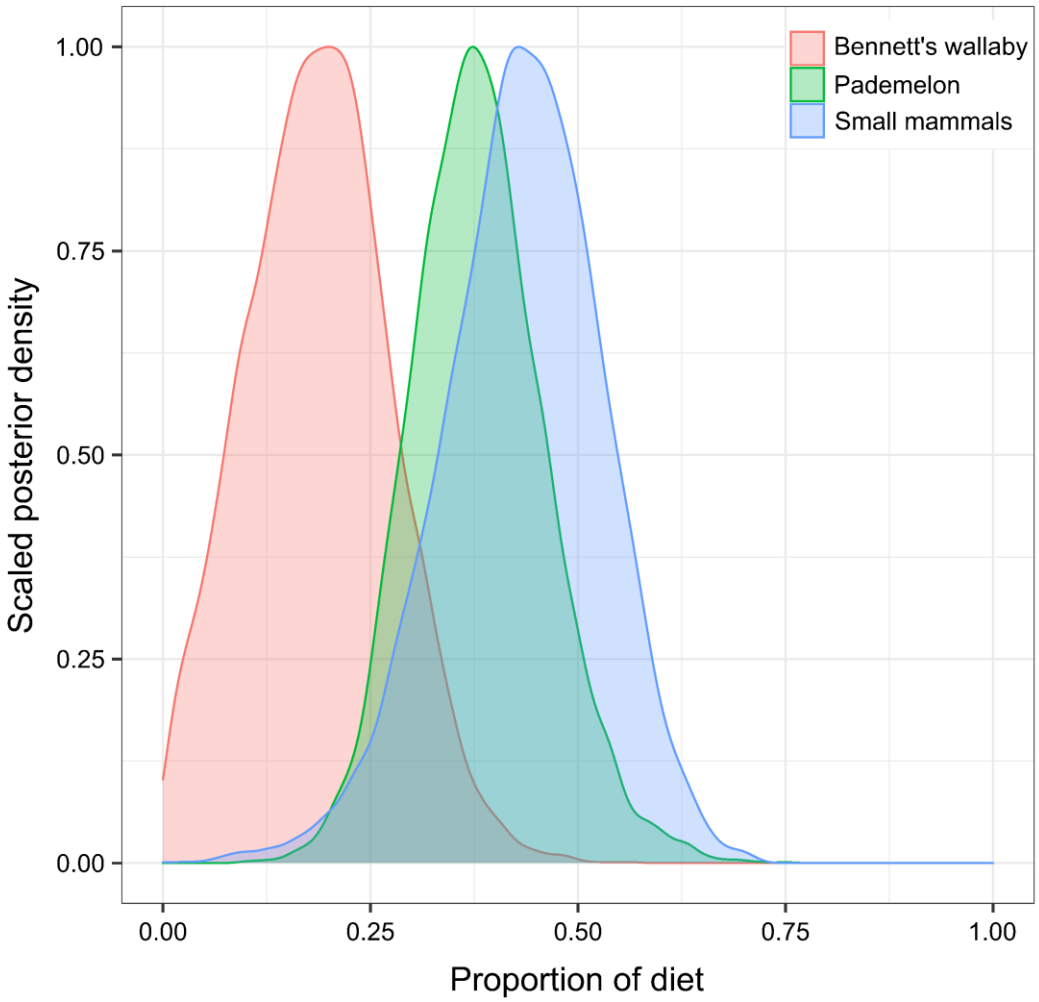

Figure 4.4. Posterior density distributions of the contributions of three dietary food sources (Bennett's wallabies, Tasmanian pademelons and small mammals) to the diet of a population of 42 Tasmanian devils at Woodbridge*. Proportional dietary contributions were estimated using a null Bayesian stable isotope mixing model in MixSIAR with no fixed effects (Stock et al., 2018).*

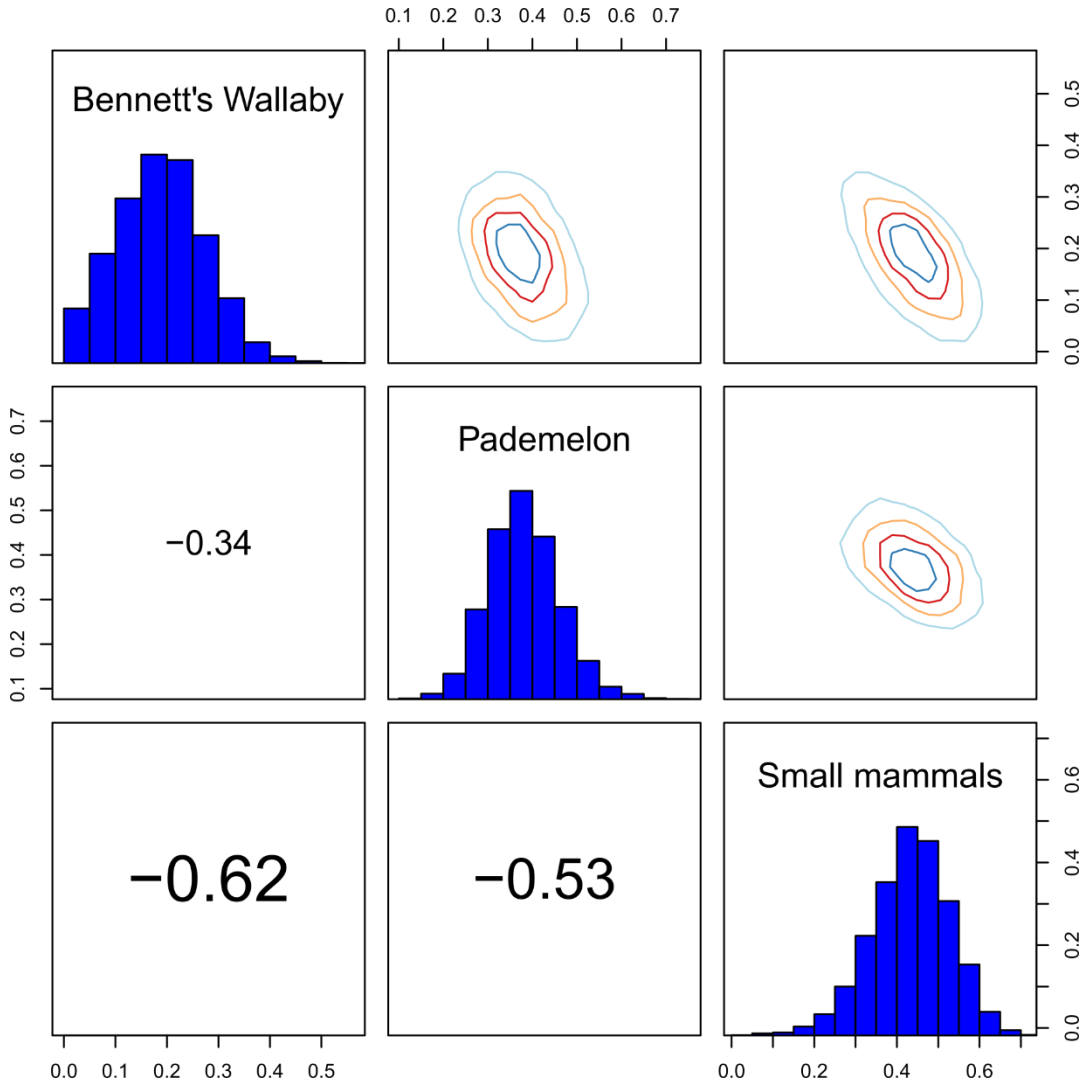

Figure 4.5. Matrix plot of proportional contributions of three food sources of Tasmanian devils; Bennett's wallabies, Tasmanian pademelons and small mammals*. The diagonal cells show the posterior probability distributions of proportional dietary contribution for each source. The cells below the posterior probability distributions show the correlations between contributions of each pair of sources. The cells above the posterior probability distributions show the joint posterior distribution for dietary contributions of each pair of sources.*

Table 4.2. Model comparison table for two Bayesian stable isotope mixing models (BSIMMs), using leave-one-out cross validation*. Our null model estimated the proportional contributions of Bennett's wallabies, Tasmanian pademelons and small mammals to the diet of Tasmanian devils at Woodbridge. Our population class model included a fixed effect of devil population class (subadult, <24 months; adult (≥24 months) with DFTD; adult without DFTD). The leave-one-out cross validation criterion (LOOic), standard error of LOOic, the standard errors for the difference in LOOic between models (dLOOic) and the relative support for each model based on Akaike weights (Weight) are shown below. The best model was the null, as it had the lowest LOOic and received the highest relative model support.*

Model	LOOic	Standard error LOOic	dLOOic	Standard error dLOOic	Weight
Null model	18.3	21.2	0.0	NA	0.61
Population class	19.2	18.4	0.9	5.7	0.39

Discussion

Tasmanian devils at Woodbridge, South Tasmania appear to consume similar proportions of Tasmanian pademelons and small mammals, and consume fewer Bennett's wallabies, based on a 3-source Bayesian stable isotope mixing model using $\delta^{13}C$ and $\delta^{15}N$ values of devil whiskers and prey tissue (Figure 4.4). Due to poor mixing geometry, we were unable to estimate proportional dietary intake of Tasmanian devils at the 5 other sites where data were available, so were unable to compare devil diet across multiple ecological contexts (Figure 4.2).

Our results indicate that, of the prey species included in our analysis, Tasmanian pademelons are the most important for Tasmanian devils at Woodbridge (Figure 4.4). Although small mammals were predicted to contribute a similar proportion of devil diet, this is a composite group, likely reducing the importance of each of the four species included. The importance of pademelons in devil diet in our study compliment the scat-contents based findings of Andersen et al. (2017a), although Jones & Barmuta (1998) found that large mammals, including Bennett's wallabies, were consumed by devils at a higher frequency and volume than medium mammals, including pademelons. Both scat contents studies estimated small mammals form a lower proportion of devil diet than our study suggests (Andersen et al., 2017a; Jones and Barmuta, 1998).

Differences between our study and prior scat contents analyses could be driven by ecological variation between the sampled locations. Our study site, Woodbridge, is an area of smallholdings interspersed by native eucalypt woodlands, while West Pencil Pine, sampled for scats in Jones and Barmuta (1998), is a eucalypt plantation close to a protected area. These differences in habitat will likely influence the prey assemblage available to Tasmanian devils and could result in dietary differences between devils in the two areas. Andersen et al. (2017a) analysed whether devil diet between sites varied based on rainfall, but not other ecological factors such as habitat or prey assemblage. Human-disturbed landscapes and habitat fragmentation can benefit invasive, generalist species (Marvier et al., 2004), and as Woodbridge has higher

availability of agricultural fields, gardens and buildings than sites in large plantations or protected areas, this could increase the availability of introduced species including brown rats and European rabbits. Although scat content analyses provide powerful qualitative estimates of diet, the contribution of dietary sources can be under or overestimated depending on their digestibility. As small mammals will have a higher hard-part to digestible protein ratio than large herbivores, frequency of occurrence measures may actually overestimate the contribution of small mammals to Tasmanian devils, and Andersen et al. (2017a) estimated larger contributions of small mammals based on frequency of occurrence compared to percentage volume estimations. Therefore it is particularly striking that at Woodbridge, small mammals appear to comprise a significant portion of devil diet. We suggest that ecological differences in prey abundance or availability, perhaps due to habitat type, drive higher consumption of small mammal species at Woodbridge compared to sites previously studied using scat contents analyses.

Site-specific ecological conditions may also help explain the isotopic distribution of individuals and species at our sites, and our ability to distinguish between certain groups. Across all our sites, we observed greater variation in devil isotopic signatures at our two protected sites, suggesting differences in ecological context such as habitat and prey availability could drive differences in diet and isotopic variation, care needs to be taken to ensure this effect is not driven by differences in isotopic baseline variation before this can be concluded, which is beyond the scope of this study. Tasmanian pademelons are herbivores and would not be expected to show high $\delta^{15}N$ values relative to other herbivores or omnivores, as they do at Woodbridge (DeNiro and Epstein, 1981) (Figure 4.3). Improved pasture and agricultural habitats tend to be enriched in $\delta^{15}N$ (Gibb and Cunningham, 2011; Harrington et al., 1998). Although Tasmanian pademelons and Bennett's wallabies both feed on grasses and herbs, pademelons tend to select for herbaceous species while Bennett's wallabies select for grasses (Sprent and McArthur, 2002). Potentially, niche separation between pademelons and wallabies at Woodbridge lead pademelons to feed on plants in improved areas to a greater extent than wallabies, resulting in higher $\delta^{15}N$ values. This apparent niche separation could occur without clear

differences in $\delta^{13}C$ values between wallabies and pademelons, as $\delta^{13}C$ in plants will be influenced by their photosynthetic pathway rather than fertilisation regimes, and grasses in Tasmania are predominantly C_3 species, relying on the same photosynthetic pathway as herbaceous species (Hattersley, 1983). The isotopic differentiation between Tasmanian pademelons and Bennett's wallabies has enabled us to assess differences in consumption of these two macropod species, which almost always form the majority of devil diet (Andersen et al. 2017a; Jones and Barmuta 1998), revealing pademelons to be the predominant dietary source at Woodbridge.

When applying Bayesian stable isotope mixing models, it is important to collect tissue samples from all potential prey items. This is to ensure reasonably accurate representation of consumed proportions of all prey types, and also to prevent the model becoming skewed inaccurately towards a particular source (Phillips et al., 2014). For example, if a consumed prey group is isotopically similar to Source A, but is not collected, the BSIMM will inaccurately inflate the importance of Source A. Due to the practicalities and biases associated with opportunistically sampling roadkill, our BSIMMs have only included mammalian species, and are unlikely to represent every prey item consumed by Tasmanian devils. For example, Andersen et al. (2017a) found evidence of bird consumption in 22% of scats, with 7% relative volume, although birds were not an important dietary source in Jones and Barmuta (1998). We cannot rule out that our mixing space is missing consumed prey groups, or that these groups, such as birds, could be influencing our results. However, Tasmanian pademelon and Bennett's wallabies constituted the bulk of devil diet in both scat contents studies (Andersen et al., 2017a; Jones and Barmuta, 1998), therefore we can be confident that we have included the most important food sources in our mixing space. In the case of birds, these will be opportunistically hunted (Andersen et al., 2020, 2017a), be drawn from a number of functional groups and be isotopically varied, therefore unlikely to skew the model in a single direction. Furthermore, MixSIAR and similar BSIMMs provide residual error terms which partly account for uncertainty in source proportions (Parnell et al., 2013, 2010; Stock et al., 2018). Even so, to account for the potential that

consumed prey groups are missing, we have discussed our results in terms of relative consumption, rather than absolute dietary proportions.

Of our two BSIMMs, our null model with no fixed effects had a lower LOOic and received a higher Akaike weight that our model with the fixed effect of population class. Two reasons account for this; first, the null model will have performed better than our fixed effect model due to low sample sizes once our devil population is broken down into three constituent groups. Second, our fixed effect model will have had fewer degrees of freedom, and will have been penalised for this during model comparison via leave-one-out cross validation. Some differences were apparent between groups in our fixed effects model. In particular, our models estimated that healthy adults ate a larger proportion of small mammals than pademelons, while adults with DFTD ate a larger proportion of pademelons than small mammals. Some isotopic differences between healthy adult devils and adults with DFTD have previously been observed, although not at this study site (Bell et al., 2021). However, due to low sample size, our estimate of the dietary proportions of adults with DFTD has likely been unduly influenced by a single data point lying close to pademelon data in our mixing space (Figure 4.3). Therefore we do not feel that the results of our fixed effects model provide an indication of ecologically realistic differences within groups, and instead place higher confidence in the null model, which received higher model support.

Of six sites where we collected data, only data for one site returned a feasible mixing polygon (Figure 4.2). Where consumer data do not fall within the polygon created by the outermost food source values, this can be due to incorrect TDFs causing a mismatch between consumer and source data, or can occur if important food sources are missing from the sampled putative food items. The most accurate way to obtain species-specific TDFs is to feed captive animals an isotopically consistent, or known, diet for an adequate length of time for the tissue being sampled, and measure the difference between food source and consumer isotopic ratios (Gannes et al., 1997; Hobson and Clark, 1992a; Martínez Del Rio et al., 2009). However, we did not undertake a controlled TDF study due to logistical impracticalities associated with ensuring captive devils are fed an isotopically consistent diet. Therefore we imputed TDFs for devils

using SIDER, as SIDER takes phylogeny, ecology and tissue types into account and provides uncertainty around mean estimates (Healy et al., 2017). Results from Bayesian mixing models using TDFs estimated by SIDER have been shown to accord well with direct observations of prey provisioning in common buzzard *Buteo buteo* chicks (Swan et al., 2020), demonstrating that in the absence of species-specific TDFs, estimations generated using SIDER are a robust method. Stable isotope analysis has not been widely used in marsupial species, and SIDER does not contain any TDF data for any marsupial species, therefore our SIDER estimates of TDFs are necessarily generic and uncertain. Generic TDFs could then result in a mismatch between consumer and source data, resulting in unfeasible mixing models. However, SIDER does not estimate TDFs from phylogeny alone, but also tissue and diet type. SIDER is informed by TDF data from multiple carnivores and for keratinous tissues (Healy et al., 2017). Furthermore, SIDER provides estimates of uncertainty, and this uncertainty is passed into our BSIMMs. While this may result in greater uncertainty in our proportional diet estimates for our site with a feasible mixing space, this is preferable to running nonsensical mixing models using generic point estimates.

Our data demonstrate the difficulty of comprehensively sampling the prey base of consumers for isotope analyses, particularly for opportunistic species with relatively large home ranges far bigger than that of their prey. Tasmanian devils have average home ranges of 30km^2 (males) and 26km^2 (females) (Comte et al., 2020), while home ranges of Bennett's wallabies and Tasmanian pademelons are under 1km^2 (Le Mar et al., 2003). Both the volume and diversity of prey samples we collected varied between sites. While this variance could be due to genuine ecological variation in species abundances and distribution between sites, they could easily have been influenced by other factors such as variation in the number of roadkill incidents between areas and habitat or road topography affecting observation of road-killed species. Our prey isotope values, and the isotopic relationship between different species, varied by area and often did not result in a feasible mixing space. Although sampling was carefully considered during study design, it is possible that our method of sampling muscle tissue is not fully representative of consumed devil diet. While

Tasmanian devils will certainly consume large amounts of muscle, as scavengers they may also consume tissues such as skin and bone (Attard et al., 2011; Jones and Stoddart, 1998; Wroe et al., 2005). Due to different rates of isotopic turnover (Bearhop et al., 2002; Hobson and Clark, 1992a; Tieszen et al., 1983), and metabolic routing of macronutrients to different tissues (Tieszen and Fagre, 1993), these tissues will have different isotopic values to muscle and may influence devil isotopic values.

Our results underline the importance of Tasmanian pademelons in the diet of Tasmanian devils at Woodbridge, and, when considered alongside previous scat contents analyses, suggest the relative importance of key macropodid species such as pademelons and Bennett's wallabies may vary by area, likely due to ecological factors such as relative availability and habitat. We have raised important issues regarding the difficulty in sampling the prey base of opportunistic consumers with relatively large ranges, but have also demonstrated, for the first time, that Bayesian stable isotope analysis may be used to investigate proportional dietary consumption in marsupial species. In doing so, we have helped to further understanding of the trophic ecology of a charismatic but cryptic species, the Tasmanian devil.

Chapter 5

Habitat, not Tasmanian devil *Sarcophilus harrisii* decline, drives isotopic niche variation in Tasmanian mammalian carnivores

Chapter 5: Habitat, not Tasmanian devil *Sarcophilus harrisii* decline, drives isotopic niche variation in Tasmanian mammalian carnivores

Abstract

Top carnivores can structure communities via competition with and predation of sympatric species. As a consequence, changes in top carnivore abundance and distribution can lead to changes in mesopredator population sizes and behaviours, with cascading impacts on prey species and wider ecological communities and processes. The niche variation hypothesis generally predicts that increasing intraspecific competition drives niche expansion and/or increased individual differentiation and specialisation, while interspecific competition constrains species niches. Using stable isotope analysis, we studied the effects of the disease-related decline of Tasmanian devils *Sarcophilus harrisii*, upon the population- and individual-level isotopic niches of devils, sympatric spotted-tailed quolls *Dasyurus maculatus* subsp. *maculatus* and Eastern quolls *Dasyurus viverrinus.* We investigated whether time since onset of devil decline, as a proxy for the severity of decline, and habitat characteristics, affected the isotopic niche breadth and overlap of devils and quolls. At the individual level, we took a subset of individuals of Tasmanian devils and spotted-tailed quolls from five sites, fully subsampled their whiskers to quantify individual isotopic niche variation, and assessed whether between-site population niche variation was driven by individual-level specialisation. Tasmanian devils and spotted-tailed quolls had similar isotopic niche breadths across sites, with neither species having a consistently larger isotopic niche than the other. At West Pencil Pine, Eastern quolls had a smaller isotopic niche (SEA_B mode = 1.41, 95% Credible Intervals = 0.88-2.15) than devils or spotted-tailed quolls (devil SEA_B mode = 1.86, 95% CI = 1.50-2.28; spotted-tailed quoll SEA_B mode = 1.75, 95% CI = 1.02-3.05). Time since disease-related declines did not have any effect on population-level isotopic niche estimates or isotopic niche overlap between Tasmanian devils and spotted-tailed quolls, however populations of both species had smaller isotopic niches in sites with a higher percentage cover of human-modified habitat ($p = 0.007$, critical p value =

0.008). Individual isotopic niche estimates were positively associated with total group isotopic niche areas ($p = 0.01$), but relative individual niche estimates (as a proportion of the total group niche) did not change significantly with total group niche area, suggesting that variation in individual isotopic niche breadths drives niche expansion, but not via variation in individual specialisation. Our results suggest that across varied landscapes, Tasmanian mammalian carnivore niches are more sensitive to the bottom-up forces of anthropogenic habitat disturbance than the top-down effects of devil facial tumour disease and associated top carnivore declines.

Introduction

Top carnivores play a significant role in structuring communities and ecosystems, but many species have undergone local extirpation and widespread decline, and are globally threatened (Estes et al., 2011; Ripple et al., 2014). By exerting predation pressure on prey, and competitive pressure on mesopredators, top carnivores can influence the abundance and behaviours of multiple sympatric species. This occurs directly, through the effects of predation and competition, and indirectly, for example by creating a 'landscape of fear', in which animals modify their behaviour to reduce the chance of predation or competition (Laundré et al., 2010; Ritchie and Johnson, 2009; Schmitz et al., 1997). Carnivore losses therefore often lead to ecosystem-scale consequences in the form of trophic cascades. For example, extirpation of top carnivores in Scandinavia is related to red fox *Vulpes vulpes* range expansion and population growth (Elmhagen and Rushton, 2007). Subsequent recovery of Eurasian lynx *Lynx lynx* in Finland has had a suppressive effect on red foxes, causing a positive impact on mountain hare *Lepus timidus* abundance (Elmhagen et al., 2010). However, the shape and extent of ecosystem effects of top carnivores can be highly context dependent and are influenced by a range of factors including habitat fragmentation (Wang et al., 2020), human-modified landscapes (Kuijper et al., 2016), landscape productivity (Elmhagen and Rushton, 2007) and food web complexity (Finke and Denno, 2004). In the example of Eurasian lynx recovery, for instance, top-down suppression of red foxes was strongest in more productive ecosystems (Elmhagen and Rushton, 2007).

Shifts in the abundance and behaviours of sympatric species following carnivore declines will necessarily be matched by shifts in the strength of competitive interactions within communities. As competition is a key driving force of ecological niches, including the variety of resources exploited by populations (Roughgarden, 1972), shifts in community structure can be expected to have consequences for niche partitioning at the level of individuals, populations and communities. At the population-level, ecological niches reflect the amount of variation between individuals, whereas at the individual-level they reflect the extent of variation within individual niches (Bolnick et al., 2003;

Roughgarden, 1972). Generalist populations can therefore be composed of individual generalists, or of specialists occupying different areas of the total population niche (Bearhop et al., 2004; Van Valen, 1965). Niche theory, and experimental evidence, suggest that increasing intraspecific competition drives niche expansion at the population-level, generally via increasing variation between individuals as a result of individual specialisation, while interspecific competition constrains population niches (Bolnick et al., 2010; Svanbäck and Bolnick, 2007; Van Valen, 1965). Top carnivore decline could therefore result in shifting niche dynamics among competing species, particularly in niche dimensions that are responsive to short-term ecological changes, such as trophic niche. This mechanism could drive trophic cascades, for example if mesopredator populations have greater reproductive success as a result of ecological release and/or if prey populations respond to increased or decreased predation pressure. However, just as trophic cascades can be context-dependent, trophic niches of populations and individuals may also be driven by bottom-up forces including habitat and ecosystem fragmentation (Layman et al., 2007; Newsome et al., 2015). For example, sea otters *Enhydra lutris* show density-dependent individual specialisation in their trophic niches in rocky habitats but not mixed-substrate habitats, due to differences in resource availability (Estes et al., 2003; Newsome et al., 2009b; Newsome et al., 2015; Tinker et al., 2008). Similarly, in the Great Lakes region of North America, increased availability of anthropogenic food resources in increasingly human-disturbed landscapes has driven increases in trophic niche size and niche overlap among a seven-species carnivore guild (Manlick and Pauli, 2020).

We use a natural experiment to examine the impact of carnivore decline upon the trophic niches of sympatric carnivores in Tasmania, Australia: Tasmanian devils *Sarcophilus harrisii*, spotted-tailed quolls *Dasyurus maculatus maculatus* and, where scarcer data permit, Eastern quolls *Dasyurus viverrinus*. Tasmanian devils are primarily a scavenger and are the largest extant carnivorous marsupial. Devil populations have declined markedly as a result of Devil Facial Tumour Disease (DFTD), a largely fatal transmissible cancer that was first recognised in 1996 (Hawkins et al., 2006). Emerging in north-east Tasmania, DFTD has since spread south and west to cover approximately 80% of the

devil's range, causing populations to decline by an average of 80% in affected areas (Lazenby et al., 2018). The DFTD epizootic has resulted in a spatial gradient of decline which we have used to study the responses of Tasmanian carnivore populations to loss of the top carnivore.

Tasmanian devil decline has caused cascading changes in the density and behaviour of spotted-tailed quoll populations consistent with behavioural mesopredator release. Temporal partitioning of carnivore activity has shifted; where devils are at low densities, devil activity has shifted to later in the evening, presumably due to decreased intraspecific competition (Cunningham et al., 2019b). In response, spotted-tailed quoll activity in areas of low devil density has shifted forward to early evening, suggesting competitive release from temporal avoidance behaviours. In areas where devils have declined, spotted-tailed quolls increase their scavenging behaviour, discovering carcasses sooner and consuming them for longer (Cunningham et al., 2018). Scat contents analyses also suggest quolls eat more large mammals including common wombats *Vombatus ursinus* and Bennett's wallaby *Macropus rufogriseus* (probably scavenged) and fewer small mammals such as antechinus *Antechinus* spp. and rats *Rattus* spp. in drier areas of Tasmania where devils have declined (Andersen et al., 2017a). However, little is known about the impact of devil decline on devil or spotted-tailed quoll trophic niches at the population level, and nothing is known about individual variation in trophic behaviour in response to devil decline.

Increased scavenging behaviour in spotted-tailed quolls could reflect niche expansion or a niche shift with decreasing interspecific competition from devils. Although the identification of fewer small mammals in spotted-tailed quoll scats (Andersen et al., 2017a) suggest a niche shift in areas with low devil density, the niche variation hypothesis predicts that reduced interspecific competition will result in niche expansion, possibly via individual specialisation (Van Valen, 1965). Due to this, and the fact that scavenging is likely to be an opportunistic and flexible behaviour in spotted-tailed quolls, we predict that areas where devils have been in decline longest have seen niche expansion in spotted-tailed quolls. For Tasmanian devils, the niche variation hypothesis would predict population-level niches either remain similar or become larger in high-density

populations, possibly with increased levels of individual specialisation (Van Valen, 1965). Tasmanian devil decline has resulted in trophic cascades, with an increase in feral cat abundance where devils have declined, which may have slowed Eastern quoll recovery from concurrent population declines (Hollings et al., 2014).

Traditional dietary analyses are excellent at identifying the ranges of prey items consumed, but are often snapshots and thus generally less suitable for quantifying variation within and among individual animals' diets. Stable isotope analysis allows for the characterisation of the trophic ecology and niches of both populations and individuals (Bearhop et al., 2004; Newsome et al., 2007). Ratios of heavy to light isotopes in consumer tissues broadly reflect those of their food sources, with predictable changes associated with the processing and incorporation of dietary proteins (Bearhop et al., 2002; DeNiro and Epstein, 1978; Hobson and Clark, 1992b). Sampling consumer tissues for stable isotope analysis can be scaled to generate information on a wide range of individuals, as well as repeat sampling of individuals to build a time series of data. This can either be via repetitive sampling of tissues such as blood, or more often, by subsampling tissues that become inert after formation, such as whiskers or feathers. Ratios of ^{15}N to ^{14}N (expressed as $\delta^{15}N$) within consumer tissues broadly reflect the trophic level of organisms, as the change in $\delta^{15}N$ between food source tissues and consumer tissues is predictably around 3‰ (DeNiro and Epstein, 1981). Variation in carbon ratios (^{13}C to ^{12}C, expressed as $\delta^{13}C$), on the other hand, largely reflects the photosynthetic pathways of vegetation and therefore can provide information on foraging locations and habitats, as well as food sources consumed (Gannes et al., 1998). Isotopic niches are not equivalent to trophic niches, and should not be interpreted as such, however they can provide a robust means of quantifying variation in this niche dimension, given considered application. For example, stable isotope analysis has been applied to demonstrate variation in individual foraging specialisation within European badger *Meles meles* groups (Robertson et al., 2014), and increased individual specialisation with increasing group size in group-living banded mongooses *Mungos mungo* (Sheppard et al., 2018).

We applied stable isotope analysis to characterise the trophic ecology of devils and quolls sampled at 5 study sites across Tasmania, to investigate the potential impact of devil decline on devil and quoll niches at the level of populations and individuals. To investigate isotopic niches at the population-level, we characterised the group isotopic niche breadths of Tasmanian devils and spotted-tailed quolls at each site, alongside the group isotopic niche breadths of Eastern quolls at one site, and quantified the extent of overlap between species at each site. We then analysed the potential drivers of variation in isotopic niche breadth and overlap of these two species, including the time since DFTD arrival (as a proxy for the severity of devil decline). To investigate niche change at the individual-level, we quantified the individual isotopic niche sizes of a subset of devils and spotted-tailed quolls for which we had multiple data points, quantified their relative isotopic niche size as a proportion of their group isotopic niche size, and analysed whether variation in group isotopic niche size between sites is driven by variation in individual specialisation (variation in the relative niche size of individuals between sites), or individual generalism (consistent relative niche sizes of individuals between sites despite variation in absolute individual & group niche size).

Methods

Field sites

Our five field sites each had varying DFTD infection histories and habitat (Figure 5.1). They comprised: Freycinet (-42.107E, 148.277S), the Midlands (the Forest, -41.860E, 147.505S; Oatlands -42.257E, 147.349S), West Pencil Pine (-41.541E, 145.823S), Wilmot (-41.377E, 146.152S), and Arthur River (-41.055E, 144.679S). The Midlands site is a composite of two sites, which we have combined due to low sample sizes related to predator density, as well as geographic proximity and habitat similarity. DFTD was first recorded at Freycinet in 2001, in the Midlands between 2000-2003, at West Pencil Pine in 2006, and at Wilmot in 2008. Arthur River was DFTD-free at the time of fieldwork for this study. Freycinet is a coastal site predominantly of dry eucalypt woodland. The Midlands sites are farmlands with fragmented patches of dry eucalypt woodland. Wilmot is predominantly a commercial eucalypt plantation within a farming landscape, while West Pencil Pine is a commercial eucalypt plantation situated close to a protected area. Arthur River is a coastal site predominantly composed of coastal scrub. Freycinet and Arthur River are largely protected national park or conservation areas.

Data collection

i) Sample collection

We sampled a total of 515 individuals, collecting one whisker each, including Tasmanian devils (n = 384), spotted-tailed quolls (n = 109) and Eastern quolls (n = 22), during three-monthly live trapping periods from 2014-2019 (Table 5.1).

Individual Tasmanian devils, spotted-tailed quolls and Eastern quolls were identified using unique microchip transponders, and individual data including age, sex and weight were recorded. Individuals were aged using tooth wear, which is accurate up to three years of age in Tasmanian devils (M.E. Jones, unpublished data). Whiskers were collected by cutting close to the skin with scissors, and stored in plastic bags at -20°C prior to laboratory preparation.

To enable standardisation of predator isotopic data across sites, samples of muscle tissue from prey species were collected opportunistically. Muscle from the hind leg of road-killed wildlife was sampled from each study site and their immediate surrounding area at least once per season throughout 2018. In the Midlands, muscle samples for brushtail possums *Trichosurus vulpecula*, Bennett's wallabies *Macropus rufogriseus*, Tasmanian pademelon *Thylogale billardierii*, rabbit *Oryctolagus cuniculus* and hare *Lepus europaeus* were also obtained from landowners managing wildlife numbers under Crop Protection Permits. Muscle sample was taken as a representative sample of the bulk material likely to contribute to devil mass, although other tissues will also be ingested. Muscle samples were stored at -20°C, then oven dried at 60°C for 48 hours and stored in sealed Eppendorf tubes.

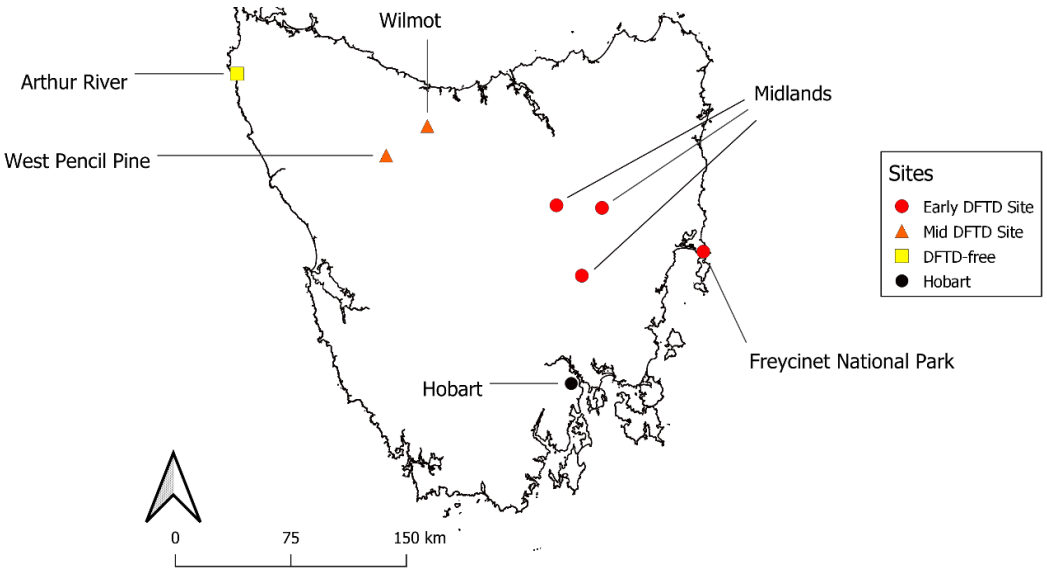

Figure 5.1. Locations of field sites in Tasmania at which Tasmanian devils and spotted-tailed quolls were sampled*. Hobart, the capital city of Tasmania, is shown for reference only (black circle). Study sites are categorised based on the estimated year DFTD arrived at the site, as an 'Early DFTD Site' (red circles), 'Mid DFTD Site' (orange triangles) or 'DFTD-free' (yellow square).*

Table 5.1. Summary of the numbers of individual Tasmanian devils, spotted-tailed quolls and Eastern quolls sampled at each site*. One whisker was sampled per individual. Basal whisker samples from all individuals were used to calculate population-level isotopic niche breadths and overlap. A subset of these individuals' whiskers were fully subsampled and used in our analysis of individual niche breadths. Sites where sample sizes are markedly different from those at other sites (e.g. the number of Tasmanian devils sampled in the Midlands) reflect low population density in these areas.*

Site	Species	Sample size		Sampling period
		Total	Individual study	
Freycinet National Park	Tasmanian devil	106	14	2015-2018
	Spotted-tailed quoll	19	14	
Midlands	Tasmanian devil	17	17	2015-2017
	Spotted-tailed quoll	22	12	
West Pencil Pine	Tasmanian devil	88	14	2014-2019
	Spotted-tailed quoll	15	13	
	Eastern quoll	22	NA	
Wilmot	Tasmanian devil	94	14	2014-2017
	Spotted-tailed quoll	28	7	
Arthur River	Tasmanian devil	79	14	2015-2018
	Spotted-tailed quoll	25	11	
Total	Tasmanian devil	**384**	**73**	
	Spotted-tailed quoll	**109**	**57**	
	Eastern quoll	**22**	**NA**	
	All species	**515**	**130**	

ii) Prey diversity surveys

To assess variation in composition of the prey assemblage, we undertook a remote camera survey of the animal communities at each of our field sites. Cameras (Reconyx PC900) were deployed for 21 days in November-December 2018. Twelve cameras were installed at each site, except for Freycinet National Park, which had 13 cameras, resulting in 273 camera-nights at Freycinet, and 252 at all other sites. Proportional estimates of habitat vegetation coverage of each site were estimated (TASVEG 3.0 GIS layer), and cameras were set in areas of each habitat in proportion to the extent of habitat coverage at the site (e.g. 50% 'agricultural, urban & exotic vegetation', 6 cameras deployed in this habitat type). Camera locations could not be fully randomised due to constraints of track access, so locations were randomly generated from a set of established Tasmanian devil trapping locations. Cameras were then placed at least 200 m away from the devil trap location, to reduce the possibility of predator presence/odour affecting remote camera visitations. Remote cameras were deployed to primarily target small to medium mammals. As such, cameras were placed facing the ground with a vertical field of view; this orientation can improve detection frequencies of medium-sized marsupials and has been previously used to survey Australian mammals (McDonald et al., 2015; Smith and Coulson, 2012). Cameras were tied to trees, approximately 5ft from the ground. Plastic bait canisters were filled with a bait mixture of honey, rolled oats and peanut butter, and tied to the same tree as the remote camera, in view of the camera, approximately 0.6 m off the ground.

Sample preparation

In the laboratory, whiskers were rinsed in distilled water to remove surface contaminants and left to air dry, before being placed in a freeze dryer for 24 hours. Whisker sections were chopped and weighed into tin cups to a weight of 0.7 ± 0.1 mg for isotope analysis. To estimate population isotopic niche sizes and overlap, all whiskers were sampled by taking one section from the base of each whisker, as this is the section grown closest to the sampling occasion and data collection. To quantify variation in individual isotopic niche breadths across

our sites, we chopped and fully sampled entire whiskers of a subset of devils (n = 73) and spotted-tailed quolls (n = 57) (Table 5.1). Fully subsampling devil whiskers resulted in a median of 10 samples per whisker (range 3–30), while quoll whiskers resulted in a median of 4 samples per whisker (range 3–7). Oven-dried muscle samples of prey species were placed in a freeze-dryer for 24 hours to remove any moisture built-up during storage, and then homogenised using a pestle and mortar. Homogenised tissue was then weighed into tin cups for isotope analysis, to a weight of 0.7 ± 0.1 mg.

Stable isotope analysis

Stable isotope analyses were conducted using a Sercon INTEGRA2 elemental analyser-isotope ratio mass spectrometer at the University of Exeter, an Elementar Pyrocube Elemental Analyser linked to a Thermo-Fisher-Scientific Delta XP Plus isotope ratio mass spectrometer at the Stable Isotope Ecology Laboratory at the Scottish Universities Environmental Research Centre, and a Thermoquest EA1110 elemental analyser linked to a Europa Scientific 2020 isotope ratio mass spectrometer at Elemtex Ltd, Cornwall, UK. Stable isotope ratios are expressed as delta (δ) values expressed in parts per thousand, or 'per mil' (‰) relative to international standards, according to:

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

Where X = ^{13}C or ^{15}N, R_{sample} = heavy to light isotope ratio of the sample and $R_{standard}$ = heavy to light isotope ratio of a standard (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). In laboratory settings, materials calibrated against the international standards are routinely used. Our samples were scale corrected using the standards USGS40 and USGS41, and internal standards of bovine liver, alanine, alagel, gel and glygel. Across laboratories and standards, average precision was 0.08 ± 0.01‰ (1 standard deviation ± standard error) for $\delta^{13}C$ and 0.12‰ ± 0.01 for $\delta^{15}N$.

Statistical analysis

Statistical analyses were conducted in R Version 3.5.2 (R Core Team, 2018).

i) Stable isotope data standardisation

To facilitate comparison of predator isotopic data across sites, we standardised predator data according to variation in two common devil prey species. As muscle samples of Bennett's wallabies and Tasmanian pademelon were collected at all sites (n per species at each site > 6, total n = 116), and these species form the bulk of adult Tasmanian devil diets (Jones and Barmuta, 1998), we combined isotopic values for the two species at each site, and calculated mean $\delta^{13}C$ and $\delta^{15}N$ per site. Taking one site, Wilmot, as our baseline, we then adjusted predator and prey $\delta^{13}C$ and $\delta^{15}N$ values at other sites based on the difference between the mean $\delta^{13}C$ and $\delta^{15}N$ of wallabies and pademelons at the relevant site and the mean $\delta^{13}C$ and $\delta^{15}N$ of wallabies and pademelons at Wilmot. This does not fully characterise the isotopic baseline across all of our sites, for example, it is possible the prey assemblage is more isotopically diverse in some sites than others, however this method at least allows us to standardise $\delta^{13}C$ and $\delta^{15}N$ values to a common baseline.

ii) Population niche breadth & overlap

We estimated the isotopic niche breadths of Tasmanian devil, spotted-tailed quoll and Eastern quoll populations using two methods: Standard Ellipse Areas corrected for sample size (SEA_C), and Standard Ellipse Areas calculated using Bayesian inference (SEA_B) (Jackson et al., 2011). These two metrics return similar information, though SEA_B provides uncertainty estimates and is more robust to small sample sizes. While we use SEA_C for visualisation, we rely on SEA_B for further quantitative analysis.

Quoll whiskers are finer in structure than devil whiskers, therefore to achieve the target weight for an isotopic sample (0.7 ± 0.1 mg), a longer section of quoll whisker was generally sampled in comparison to devils. This sampling strategy could result in devil and quoll isotopic niches reflecting diet integrated over different periods of time, particularly if devil and quoll whiskers have different growth rates. To test whether our SEA_B estimates are sensitive to the amount of whisker sampled, we recalculated SEA_B for each site, using the subset of devils and spotted-tailed quolls fully subsampled for individual niche analysis. SEA_B was first calculated using the basal sections of individuals' whiskers

alone, and then again using an average of the two most basal whisker sections per individual. There was little difference between SEA_B estimates calculated using one and two samples per individual for both species, so we proceeded with our population niche estimates using basal samples to compare devil and quoll isotopic niches.

The overlap between isotopic niches of species dyads at each site was calculated using Bayesian methods in SIBER (Jackson et al., 2011), by estimating the overlap between each dyad of ellipses over 500 draws from posterior estimates, which was then expressed as the proportion of the non-overlapping areas of the ellipses.

iii) Drivers of population niche breadth & overlap

To investigate the drivers of isotopic niche breadth of Tasmanian devil and spotted-tailed quoll populations, and niche overlap between the two species, we built two sets of models, using the modal SEA_B estimates of Tasmanian devil and spotted-tailed quolls (n = 10), and the modal overlap between the two species at each site (n = 5) as response variables. We fit simple linear models, separately analysing the effect of: species, as there may be consistent differences in niche breadth between devils and spotted-tailed quolls; the time since DFTD was first recorded at each site (Categorised as Early [2003 and before], Mid [post 2003] and DFTD-free); road cover per km^2, as road cover may increase carrion availability; the percentage of the site area composed of human-modified habitat, as community niche dynamics can be influenced by human disturbance; and Shannon's diversity of functional prey groups, to investigate whether variation in prey diversity and evenness drove variation in niche breadth. We also fitted a linear model regressing the SEA_B of Bennett's wallabies and Tasmanian pademelons (combined) against the predator SEA_B metrics, to assess whether devil & quoll niche sizes are driven by the variation in isotopic baseline within sites, rather than dietary variation. We applied a Bonferroni correction to correct for multiple hypothesis testing and reduce the chance of Type 1 errors.

Road cover (per km^2) and the percentage of human-modified habitat (including farmland, commercial eucalypt plantations and private properties) at each site

were calculated in QGIS using open source LIST Transport Segment and TASVEG 3.0 GIS layers. Home ranges of predators caught towards the edges of our trapping areas may extend beyond the trapping boundaries. To account for this, we added a buffer of 3.22 km (unless extent was restricted by coastline) around our trapping area for each site, based on the radius of the mean 95% kernel density estimate recorded for female devils at Freycinet National Park prior to the first recorded DFTD infection in the area (S. Comte, unpublished data). We used female ranges as female devils have larger home ranges than males. Transport segments were filtered to remove non-road segments and restricted tracks. We then extracted road length and habitat area data from within the area covered by the trapping area and surrounding buffer.

To characterise differences in the prey assemblages of our sites, we calculated Shannon's Diversity Index using the remote camera survey data. Animal detections of the same species were treated as unique and included in the analysis if they occurred over 20 minutes apart. We grouped species into functional groups, as differences in functional diversity rather than species diversity may have the strongest effect on the isotopic and ecological diversity of predator niches. These groups included: large exotics (fallow deer *Dama dama*, sheep *Ovis aries*), large marsupial herbivores (Eastern grey kangaroo *Macropus giganteus*, common wombat), medium macropods (Bennett's wallaby, Tasmanian pademelon), medium arboreal marsupials (brushtail possums, ringtail possums *Pseudocheirus peregrinus*), medium terrestrial marsupials (long-nosed potoroo *Potorous tridactylus*, Eastern bettong *Bettongia gaimardi*, southern brown bandicoot *Isoodon obesulus*), monotremes (short-beaked echidnas *Tachyglossus aculeatus*), small mammals (Eastern pygmy possum *Cercartetus nanus*, sugar glider *Petaurus breviceps*), native rodents (long-tailed mouse *Pseudomys higginsi*, Australian swamp rat *Rattus lutreolus*, broad-toothed rat *Mastacomys fuscus*), non-native rodents (black rat *Rattus rattus*, house mouse *Mus musculus*), birds and reptiles. Birds and reptiles were not further sub-grouped due to low sample sizes, and, as our remote cameras were set up to target mammals, there will likely be biases in sampling of birds and reptiles captured in our survey based on habitat and behaviour.

iv) Individual isotopic niche breadths

To quantify individual isotopic niche breadths of devils and quolls across our sites, we calculated individual standard ellipse areas for 130 individuals where whiskers were fully subsampled (Table 5.1). To quantify variation in the extent of individual specialism between species and sites, we then calculated a relative niche area index for each individual by expressing their individual isotopic niche size (standard ellipse area) as a proportion of the total isotopic niche area of its species at the relevant site, using a function of the R package SIBER, siberKapow (Jackson and Parnell 2017). Here, total group niche size per site was measured as the total area occupied by the individual isotopic niches of all individuals of that species also fully subsampled for individual niche analysis. This measure was positively correlated with the SEA_B mode estimates used to quantify population niche sizes and ecological drivers of niche variation (Spearman's rho = 0.79, p value = 0.01). If relative individual isotopic niche size decreases with increasing total group niche, this would suggest group niche expansion via increased individual specialisation, as individuals occupy a small proportion of the total available isotopic niche space. Conversely, if absolute individual isotopic niches increase with total group niche but the relative individual niche metric remains constant, or if the relative individual niche metric increases due to an increased absolute niche with reduced or constant total group niche width, this would suggest group niche expansion has been driven by increased generalism in individuals.

To analyse whether relative individual isotopic niche breadth varied between sites and with total group isotopic niche size, we regressed relative individual isotopic niche breadths (n = 130) against the fixed variables: species, number of whisker sections used to generate that individual's niche size (as low sample sizes may result in underestimates of isotopic niche size), total group isotopic niche area, and site. As our response variable data were continuous proportional data between 0 and 1, we chose a beta regression model framework (Douma and Weedon, 2019) with a logit link function, using the R package betareg (Cribari-Neto and Zeileis, 2010).

To analyse whether individual absolute isotopic niche size varied between sites and with total group isotopic niche size (n = 130), we regressed individual isotopic niche sizes against the same fixed variables as above: species, the number of whisker samples obtained, the total group isotopic niche area, and site. As our response variable data were zero bounded and positively skewed, we used a Gamma error structure with an identity link function.

Results

i) Population niche breadth & overlap

Combining all standardised data, average $\delta^{13}C$ values were similar for Eastern quolls and spotted-tailed quolls (Eastern quoll $\delta^{13}C$ mean = -23.72; spotted-tailed quoll $\delta^{13}C$ mean = -23.90), but lower for Tasmanian devils ($\delta^{13}C$ mean = -24.80), while average $\delta^{15}N$ values were highest for Eastern quolls ($\delta^{15}N$ mean = 9.26), and similar for spotted-tailed quolls and Tasmanian devils (spotted-tailed quoll $\delta^{15}N$ mean = 8.20; Tasmanian devil $\delta^{15}N$ mean = 8.16) (Table 5.2). Across our five study sites, Tasmanian devils and spotted-tailed quolls had similar isotopic niche breadths (Tasmanian devil SEA_B mode range 0.78-3.28; spotted-tailed quoll SEA_B mode range 1.54- 3.87) (Figure 5.2). Neither species had a consistently larger niche than the other; Tasmanian devils had a larger isotopic niche breadth (SEA_B mode) at the Midlands and West Pencil Pine, while spotted-tailed quolls had larger isotopic niche breadths at Freycinet, Wilmot and Arthur River. At West Pencil Pine, Eastern quolls had a smaller isotopic niche breadth (SEA_B mode = 1.41, 95% Credible Intervals = 0.88-2.15) than devils and spotted-tailed quolls (devil SEA_B mode = 1.86, 95% CI = 1.50-2.28; spotted-tailed quoll SEA_B mode = 1.75, 95% CI = 1.02-3.05). The extent of isotopic niche overlap between devils and spotted-tailed quolls, expressed as a proportion of the total non-overlapping niche area, was lowest at Wilmot (Bayesian modal overlap estimate = 0.30, 95% CI = 0.20-0.41) and largest at Freycinet National Park (Bayesian modal overlap estimate = 0.67, 95% CI = 0.51-0.81). At West Pencil Pine, the isotopic niche of Eastern quolls overlapped slightly more with devils (overlap mode = 0.49, 95% CI = 0.32-0.68) than spotted-tailed quolls (overlap mode = 0.45, 95% CI = 0.23-0.66), and devils and spotted-tailed quolls overlapped the least (overlap mode = 0.37, 95% CI = 0.18-0.60).

Table 5.2. A summary of mean $\delta^{13}C$ and $\delta^{15}N$ of Tasmanian devils, spotted-tailed quolls, Eastern quolls and prey species *(Bennett's wallaby and Tasmanian pademelon) at our five study sites, before and after standardisation. Data were standardised by adjusting prey and consumer data by the difference between the mean $\delta^{13}C$ and $\delta^{15}N$ of prey at the relevant site and those observed at Wilmot (in bold), which was chosen as an isotopic baseline.*

Site	Species	Unstandardised data		Standardised data	
		Mean $\delta^{13}C$	**Mean $\delta^{15}N$**	**Mean $\delta^{13}C$**	**Mean $\delta^{15}N$**
Freycinet National Park	Tasmanian devil	-22.67	8.86	-25.46	7.64
	Spotted-tailed quoll	-21.69	9.07	-24.47	7.85
	Prey	-24.92	4.70	-27.71	3.48
Midlands	Tasmanian devil	-22.82	9.03	-23.94	7.15
	Spotted-tailed quoll	-22.48	9.16	-23.59	7.28
	Prey	-26.59	5.36	-27.71	3.48
West Pencil Pine	Tasmanian devil	-23.97	6.89	-24.34	8.93
	Spotted-tailed quoll	-22.83	7.12	-23.21	9.15
	Eastern quoll	-23.35	7.23	-23.72	9.26
	Prey	-27.34	1.45	-27.71	3.48
Wilmot	Tasmanian devil	-24.75	7.74	-24.75	7.75
	Spotted-tailed quoll	-24.28	8.04	-24.28	8.04
	Prey	**-27.71**	**3.48**	**-27.71**	**3.48**
Arthur River	Tasmanian devil	-24.37	8.40	-25.01	9.07
	Spotted-tailed quoll	-23.20	8.12	-23.85	8.79
	Prey	-27.06	2.81	-27.71	3.48
Combined	Tasmanian devil	-23.72	8.16	-24.80	8.16
	Spotted-tailed quoll	-22.78	8.35	-23.90	8.20
	Eastern quoll	-23.35	7.23	-23.72	9.26
	Prey	-27.71	3.48	-27.71	3.48

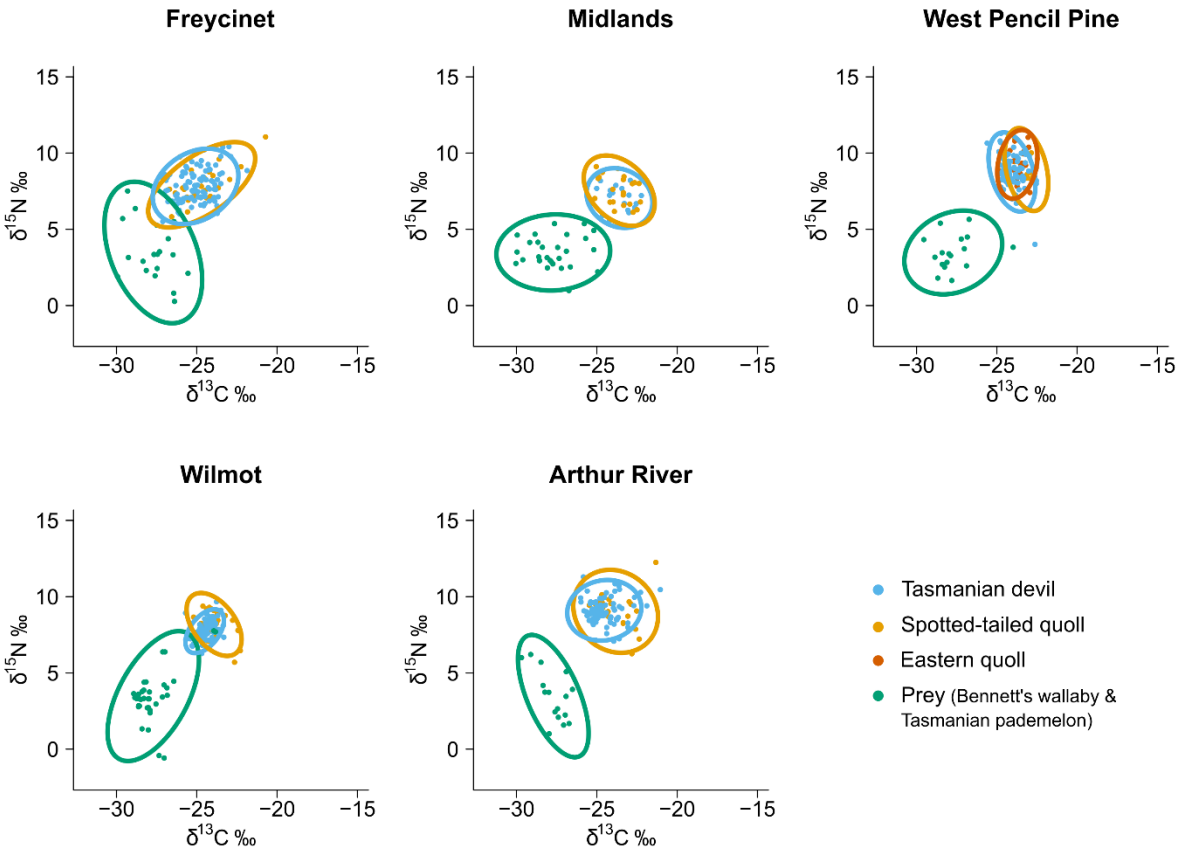

Figure 5.2. Isotopic niches, represented as standard ellipse areas estimated using δ^{13}C and δ^{15}N values, for Tasmanian devils (blue), spotted-tailed quolls (pale orange), Eastern quolls (dark orange at West Pencil Pine only) and their prey species *(Bennett's wallabies and Tasmanian pademelons, green) across five study sites in Tasmania. Sites are presented in order of time since DFTD is estimated to have arrived in the resident population, from Freycinet (2001, top left) to Arthur River (DFTD-free at time of study, bottom middle). Prey standard ellipse areas are provided to allow comparison of carnivore ellipse areas with general isotopic baseline variability.*

ii) Drivers of population niche breadth & overlap

The percentage of human-modified habitat at each site was the most important driver of variation in group isotopic niche breadths in Tasmanian devils and spotted-tailed quolls (p value = 0.007, alpha = 0.008, Table 5.3) but not the extent of overlap between them (p value = 0.08, alpha = 0.008, Table 5.3). At sites with a higher percentage of human-modified habitat, including the Midlands and Wilmot (Table 5.3), both devils and spotted-tailed quolls had smaller isotopic niches than more natural sites such as Freycinet and Arthur River (Figure 5.3).

Contrary to our predictions, time since DFTD arrival, used as a proxy for level of devil decline, had no effect on the isotopic niche breadths or niche overlaps of devils or spotted-tailed quolls (Table 5.3). The SEA_B mode of prey species (Bennett's wallaby and Tasmanian pademelon) at each site also had no effect, suggesting that differences in the isotopic variability of available prey resources does not explain variation in predator niche breadths or overlap, and this effect is therefore likely ecological, rather than an artefact of different isotopic baselines in different environments.

iii) Individual isotopic niche breadths

The total group isotopic niche size was a significant positive predictor of individual absolute niche size (estimate = 0.21, p value = 0.01, Figure 5.4). Relative individual niche size did not vary significantly between devils and spotted-tailed quolls or by site, and did not vary by total group isotopic niche size (Table 5.4). No other terms in this model showed any significance.

Table 5.3. Summary of the ecological variables used to predict group isotopic niche size of Tasmanian devil and spotted-tailed quolls at five sites (SEAB mode) and isotopic niche overlap *of the two species at each site (modal overlap estimate), and the p values related to each test. Ecological predictors included species, the amount of time since DFTD arrived at the site (DFTD category), the percentage of human-modified habitat at each site, the amount of road cover, the functional prey diversity (Shannon's diversity index) and the isotopic niche breadth of prey species (Prey SEA_B mode) at each site. As each predictor was run as a separate model for each of our two response variables (6 models per response variable), our critical p value was 0.008.*

	Species	DFTD category	Human-modified habitat (%)	Road cover (km^2)	Shannon's diversity Index	Prey SEA_B mode
Freycinet	NA	Early DFTD region	13.62	1.48	0.93	6.68
Midlands	NA	Early DFTD region	36.75	0.83	1.20	4.51
West Pencil Pine	NA	Mid DFTD region	25.87	1.18	2.13	4.19
Wilmot	NA	Mid DFTD region	37.59	1.96	1.36	5.07
Arthur River	NA	DFTD-free	16.18	1.60	1.55	3.58
SEA_B mode p value	**0.55**	**0.74**	**0.007**	**0.71**	**0.22**	**0.52**
Ellipse overlap p value	**1.00**	**0.20**	**0.08**	**0.23**	**0.05**	**0.36**

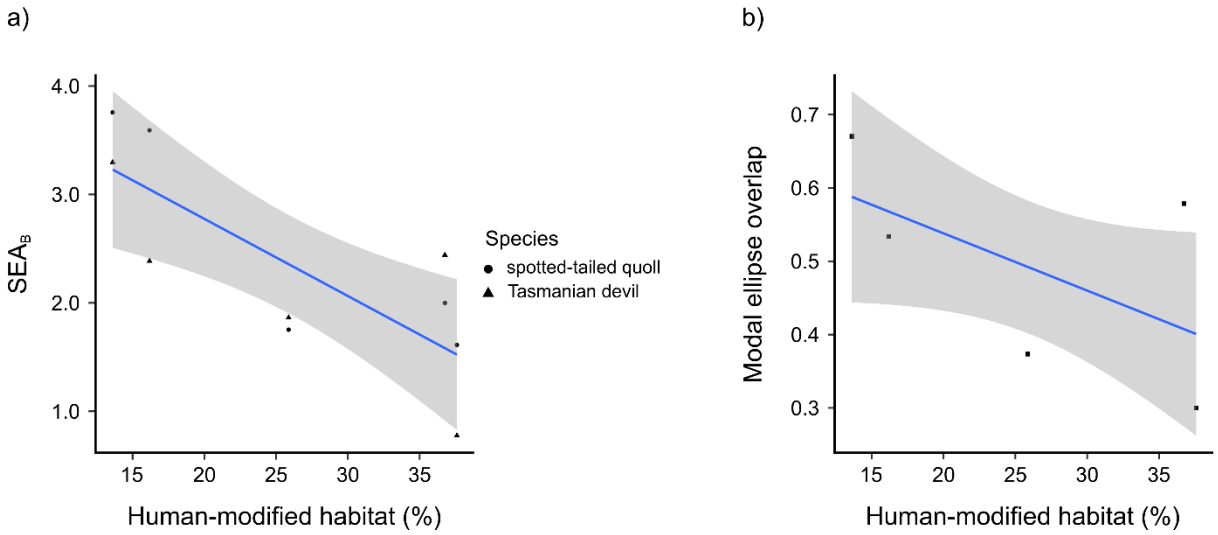

Figure 5.3. Relationships between the amount of human-modified habitat at each site (%) with the isotopic niche breadths of Tasmanian devils and spotted-tailed quoll populations, and the amount of niche overlap between the two species*, at each site. Isotopic niche breadth is represented by modal Bayesian standard ellipse area, of Tasmanian devil (black triangle) and spotted-tailed quoll (black circle) populations at each site (panel a). The amount of niche overlap between devil and quoll isotopic niches at each site was expressed as the mode of the Bayesian-estimated extent of niche overlap (as a proportion of the total non-overlapping area) (panel b).*

Table 5.4. Summary of the results from models investigating the drivers of relative individual niche sizes and individual standard ellipse areas of Tasmanian devils and spotted-tailed quolls*. Relative individual niche size was fitted in a beta regression framework, and individual standard ellipse area was fitted as a generalised linear model with a Gamma error family and identity link function. No fitted predictors had a significant relationship with relative individual niche size (individual ellipse areas expressed as a proportion of the total group niche area). Total group niche area had a significant positive relationship with individual ellipse area.*

Response Variable	Model Variables	Estimate	Standard Error	Lower Confidence Interval	Upper Confidence Interval	z/t value	p value
Relative individual niche size	Intercept	-1.81	0.42	-2.63	-0.99	-4.34	<0.001
	Species (devil)	-0.25	0.24	-0.72	0.21	-1.06	0.29
	Number of whisker sections	0.03	0.02	-0.006	0.07	1.61	0.11
	Total group niche area	0.01	0.02	-0.02	0.05	0.68	0.50
	Site (Freycinet)	-0.13	0.31	-0.73	0.48	-0.41	0.68
	Site (Midlands)	-0.06	0.25	-0.55	0.43	-0.24	0.81
	Site (West Pencil Pine)	0.12	0.23	-0.34	0.58	0.51	0.61
	Site (Wilmot)	0.20	0.29	-0.36	0.76	0.72	0.48
Individual ellipse area	Intercept	-0.66	1.76	-4.22	2.87	-0.37	0.71
	Species (devil)	-0.47	1.09	-2.61	1.72	-0.43	0.67
	Number of whisker sections	0.06	0.10	-0.11	0.29	0.67	0.51
	Total group niche area	0.21	0.08	0.05	0.38	2.47	**0.01**
	Site (Freycinet)	0.01	1.52	-3.18	3.36	0.004	1.00
	Site (Midlands)	-0.23	0.92	-2.21	1.61	-0.25	0.80
	Site (West Pencil Pine)	0.42	0.96	-1.59	2.42	0.44	0.66
	Site (Wilmot)	0.55	1.08	-1.71	2.70	0.51	0.61

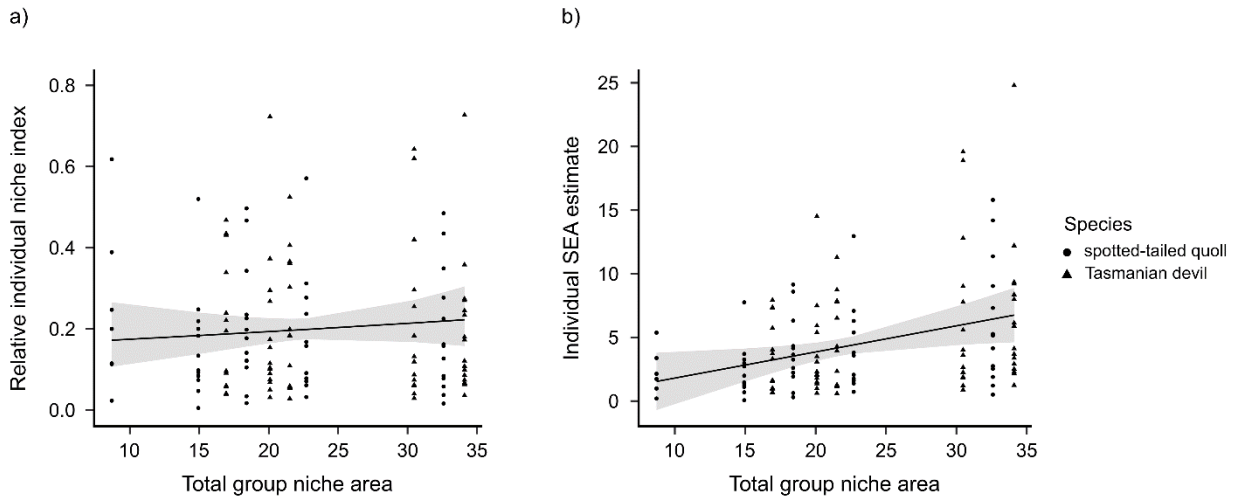

Figure 5.4. The relationship between total group isotopic niche area (the total area of all individual ellipses for each species/site group) against individual devil and quoll relative individual niche index estimates (panel a) and individual standard ellipse area estimates (panel b)*. The model-fitted marginal effects of total group isotopic niche area are plotted against observed relative individual niche index estimates and individual standard ellipse area estimates. Relative individual niche index estimates are individual SEA estimates expressed as a proportion of the total group niche area for the relevant species/site group.*

Discussion

Isotopic niche breadths of Tasmanian devils and spotted-tailed quolls estimated using $\delta^{13}C$ and $\delta^{15}N$ values of whiskers are smaller in increasingly human-modified landscapes (Fig. 5.3). This variation in population isotopic niche breadth appears to be driven by expansion or contraction in the breadth of individuals' isotopic niches among sites, though individuals' niches remain consistent as a proportion of their group niche, suggesting no variation in individual specialisation between populations. Contrary to our initial predictions, the amount of time since devil facial tumour disease is estimated to have arrived in a devil population had no effect on isotopic niche breadths in the carnivore community.

Our results show the multiple ways in which carnivore resource use, diets and trophic niches are altered in human-modified landscapes. Globally, most related studies show that carnivores in human-modified landscapes increase their use of human resource subsidies including livestock, carcasses and food waste (Newsome et al., 2015), resulting in broader population isotopic niches (Magioli et al., 2019), and/or increased dietary overlap between carnivore species (Manlick and Pauli, 2020; Newsome et al., 2014). In contrast, our results suggest that native carnivores, Tasmanian devils and spotted tailed quolls, have smaller isotopic niches in human-modified landscapes. It is possible that in Tasmanian landscapes, carnivorous marsupials do not use, or do not have access to, significant amounts of human resource subsidies, and are therefore competing in a relatively low-quality habitat, driving niche contraction. For example, the breadth of prey resources available to carnivores may be low compared to more natural landscapes, and good farming practise may limit the abundance of carrion. However, our measures of prey diversity were not (negatively or otherwise) correlated with the extent of human-modified habitat, and we did not find an effect of prey diversity on devil and spotted-tailed quoll isotopic niches. While we stratified our camera trap placements according to proportional habitat type within the boundaries of field sites, it could be that these areas are not fully representative of the degree of resource availability within the wider landscape and overestimate prey diversity in human-modified areas. While previous studies into the impact of human disturbance have considered Australian landscapes, these have mostly been on generalist dingoes *Canis lupus dingo* and

domestic dogs *Canis familiaris* (Newsome et al., 2014; Newsome et al., 2015), and the responses of marsupials have received less attention.

The niche variation hypothesis predicts that release from interspecific competition leads to population niche expansion, potentially via increased between-individual variation (Van Valen, 1965), and this has been supported by multiple empirical studies (Araujo et al., 2009; Bolnick et al., 2010; Darimont et al., 2009). However, while we found that Tasmanian devils' and spotted-tailed quolls' individual niche breadths generally contract in human-modified landscapes, driving population isotopic niche contraction, the degree of individuals' specialisation relative to one another remains constant. Several mechanisms could drive reduced isotopic niche breadth of individuals and populations. First, the isotopic niche breadth of prey species may be narrower. However, we did not see an effect of wallaby and pademelon isotopic niche areas upon carnivore population isotopic niches. Therefore, our results are unlikely to be an artefact of isotopic variation in prey species in different landscapes, but a reflection of genuine changes in resource use. Second, the temporal scale at which our chosen tissue, whiskers, integrates may not capture differences in the time scale of dietary variation between sites. If variation occurs over shorter time scales in some sites than others, isotopic variation in whiskers from these sites may be averaged out, appearing less variable. Third, predator isotopic niches could be genuinely constrained or relaxed by variation in ecological opportunity across landscapes with varying degrees of human modification. Even with this variation, competitive conditions within conspecific populations may remain constant enough that the proportional overlap of conspecific individuals with each other does not change. If prey resource diversity reduces in human-modified landscapes, but resources are still abundant, this could constrain population niche breadths but would allow individuals to maintain a degree of differentiation from conspecifics. This could occur either through variation in the proportions of different prey items ingested, or through variation in foraging locations resulting in isotopic variation at a scale we are not able accurately to detect. Although we found that relative individual niche size did not vary with total group isotopic niche size, we did observe increased variability in absolute isotopic niche size with increasing total group niche breadth (Figure 5.4), suggesting there may be

variation in the responses of individuals to different ecological conditions, with a subset of individuals driving the population-level isotopic response.

We found that the time since DFTD arrived at a site did not have an effect on devil or spotted-tailed quoll isotopic niches, despite multiple previous studies demonstrating devil decline has precipitated trophic cascades and changes in spotted-tailed quoll behaviour, including an increase in scavenging in areas devils have declined (Andersen et al., 2017a; Cunningham et al., 2018; Hollings et al., 2014). It is possible that trophic niche changes occur, but are not discernible using isotopic analyses. A further explanation may lie in the range of study sites used. For example, a strong positive effect of devil decline on carcass persistence and mesopredator scavenging behaviour was revealed by comparing environmentally comparable sites of two natural habitat types, with minimal human disturbance (Cunningham et al., 2018); clearly, across these landscapes devils exhibit top-down effects on sympatric competitors. However, our study sites include a range of habitats and levels of human disturbance, and in this case the ecological effects of devil decline could be disrupted by bottom-up influences. For example, devil decline has generally caused trophic cascades in Tasmania via increased feral cat abundance (Hollings et al., 2014), but this effect was dampened in areas with low rainfall and, critically, in areas with relatively high human disturbance, where bottom-up factors had the greatest effect on mesopredators. Our study of varied habitat types and ecological contexts adds further weight to the likelihood that the strength of top-down community effects of devils varies with ecosystem context, whereby Tasmanian devils exhibit top-down controls in natural landscapes, but bottom-up factors play a greater role in human-modified landscapes. A more detailed understanding of the relationship between top-down and bottom-up impacts on carnivore isotopic niches in this system would require a broader range of study sites with replication of multiple habitat types, however our results suggest that across several habitat types, human-modified landscapes affect the realised niches of devils and spotted-tailed quolls to a greater extent than the ecological and competitive effects of devil decline.

Although isotopic niches are related to ecological niches, they are not equivalent and careful interpretation is necessary (Bearhop et al., 2004; Jackson et al., 2012). Our results suggest Eastern quolls have smaller population isotopic niches than

Tasmanian devils and spotted-tailed quolls, which overlap with devil isotopic niches to a greater extent than spotted-tailed quolls but sit in between the two larger species in bivariate δ-space (Figure 5.2). This is contrary to scat contents analyses of the three species conducted at the same site (West Pencil Pine), which reported that Eastern quolls had the most diverse diets, and that dietary niche partitioning in this guild was size-structured, with Eastern quolls overlapping more with spotted-tailed quolls than devils (Jones and Barmuta, 1998). Eastern quoll whiskers are finer, and shorter, than either devil or spotted-tailed quoll whiskers, therefore a longer length of whisker is needed to create one sample for isotope analysis. If all three species' whiskers represent a similar time period, but our isotopic samples are composed of consistently longer lengths of whisker for Eastern quolls, our Eastern quoll samples will represent a longer time period than samples from the other two species, and any isotopic variation along the integration period of each whisker will become averaged out in the process of returning single values for $\delta^{13}C$ and $\delta^{15}N$, resulting in a small isotopic niche breadth even if dietary variation is broad. We were able to test whether our devil and spotted-tailed quoll isotopic niche estimates were sensitive to the length of whisker averaged by varying the amount of whisker averaged to create our estimates, however as our Eastern quoll whiskers generally only yielded one sample each, it was not possible to test this. Furthermore, as we only have Eastern quoll whiskers from one field site, we are unable to test whether the isotopic relationship between Eastern quolls, devils and spotted-tailed quolls is consistent between areas.

The influence top carnivores can have on community structure increases the importance of understanding not just the ecological consequences of carnivore decline, but how these interact with other global phenomena such as anthropogenic habitat change and fragmentation. Here, we have shown that isotopic niche breadths of Tasmanian devils and spotted-tailed quolls are sensitive to human disturbance across a variety of habitat types, but do not vary according to disease history, suggesting bottom-up forces related to habitat have a stronger effect on niche partitioning in disturbed areas than the presence or absence of top-down control by Tasmanian devils. This contributes to our knowledge of the complex interactions between top-down and bottom-up forcing in carnivore guilds. Furthermore, we demonstrate that the directionality of ecological effects of processes such as land-

use change should not be assumed to be consistent, and may vary according to the specific ecologies and opportunities of species, populations and individuals.

Chapter 6

Discussion

Chapter 6: Discussion

Overview

Competition, from conspecifics and from other sympatric species, constrains the ecological opportunities of wild animals. Changes in access to resources, including food, is therefore predicted to change individual and population-level ecological niches (Bolnick et al., 2011; Van Valen, 1965). The current biodiversity crisis, with widespread species and population declines will be causing equally widespread shifts in ecological opportunity, creating winners, including largely generalist invasive species, and losers (McKinney and Lockwood, 1999). In both instances, the ecological niches of many populations and individuals may respond to changing ecological circumstances. In addition, pressures such as infectious disease, and anthropogenic climate change and habitat modification will also impact the ecological opportunities and competitive pressures faced by individuals, populations and communities.

In this thesis, I investigated the ecological effects of Tasmanian devil *Sarcophilus harrisii* decline due to infectious disease. More specifically, I applied stable isotope analysis to investigate the ecological niches of Tasmanian mammalian carnivores, examining the consequences of disease and top scavenger decline for isotopic niche dynamics within individuals, populations and the carnivore community. First, I aimed to quantify variation in the isotopic niches of Tasmanian devil populations, considering both within- and between-individual variation. In **Chapter 2**, I found that age drives the extent of within- and between-individual isotopic variation in devils, with both group and individual isotopic niches reducing in breadth with age. I suggested this was reflective of a transition from an early age diet that is varied and includes omnivorous or carnivorous species, to an adult diet with more consumption of larger herbivores. Second, I aimed to characterise the impact of DFTD progression on the isotopic niches of infected devils, and to consider how tumour volume may affect isotopic signatures and how DFTD infection may alter the course of individual niches. In **Chapter 3**, I found that Tasmanian devils isotopic values generally did not change with increasing tumour volume, except at one field site. DFTD did not cause isotopic changes in individual devils sampled before and after

infection. I then aimed to investigate whether variation in the consumption of major mammalian prey species among and within Tasmanian devil populations can be revealed using Bayesian stable isotope mixing models (BSIMMs). In **Chapter 4**, data for five of my study sites were inappropriate for running BSIMMs, but at one site I revealed Tasmanian devils ate a similar proportion of small mammals and Tasmanian pademelons *Thylogale billardierii*, but fewer Bennett's wallabies *Macropus rufogriseus*. Finally, I aimed to quantify the response of Tasmanian devil and spotted-tailed quoll *Dasyurus maculatus maculatus* isotopic niches to Tasmanian devil declines, at both individual and population scales. I characterised the isotopic niche breadths of individuals and populations of both species across the range of devil decline in **Chapter 5**, and also characterised the population isotopic niche breadth of Eastern quolls *Dasyurus viverrinus* at one of these locations. The extent of human-disturbed habitat at each site had a larger effect on population isotopic niche breadths for both species than the time since DFTD arrival, a proxy for devil decline. Individual isotopic niche sizes generally grew with increasing population niche size, but did not indicate increasing specialisation of individuals.

I will now review and synthesise the key findings of this thesis, and will then go on to discuss the implications of my work and make suggestions for future research under the broad themes I have explored in the thesis, including Tasmanian carnivore ecology, stable isotope analysis and ecological niches in a biodiversity crisis.

Key findings

The dramatic decline of Tasmanian devils on account of devil facial tumour disease (DFTD) has resulted in considerable research efforts to understand the pathology and transmission of DFTD (e.g. Hamede et al., 2013; Pyecroft et al., 2007; Woods et al., 2018), and the ecological impacts of DFTD and related population declines on Tasmanian devils and the wider ecological community in Tasmania (e.g. Cunningham et al., 2018; Hamilton et al., 2020; Hollings et al., 2014; Lachish et al., 2009). Prior to the emergence of DFTD, Tasmanian carnivores had not been the focus of extensive ecological enquiry, with research focussing mostly on Tasmanian devil social organisation and behaviour (Pemberton, 1990; Pemberton and Renouf, 1993), and niche differentiation of Tasmanian mammalian carnivores, based on their

scat contents and skeletal features (Jones, 1997; Jones and Barmuta, 1998). In this thesis, I have aimed to ground questions regarding the impacts of DFTD and disease-related decline on Tasmanian carnivore trophic ecology (**Chapters 3** and **5**) by also considering broader ecological questions regarding ecological niche partitioning within and between individuals and populations (**Chapters 2** and **4**).

The research in this thesis largely supports existing literature suggesting Tasmanian devils eat relatively large herbivores such as Bennett's wallabies and Tasmanian pademelons (Andersen et al. 2017a; Jones and Barmuta 1998; **Chapter 4**). Having said this, the isotopic results of **Chapters 2** and **3** suggest some within-population variation, within this general diet. Young devils appear to eat at a higher trophic level, reflected in higher $\delta^{15}N$ values, and have broader isotopic niches than older devils (**Chapter 2**). Similarly, DFTD-infected devils at one study site appear to feed at a higher trophic level with increasing tumour volume (**Chapter 3**). Taking the findings of both chapters together, it appears that where ecological conditions permit, individuals in a population that are less competitive than others, potentially due to size, experience or sickness, exhibit higher $\delta^{15}N$ values than other devils. In both instances, I suggest this is reflective of a diet of smaller omnivorous species, compared to a healthy adult diet composed predominantly of large herbivores, as these may be consumed quickly or easily without the attraction of other, more competitive, conspecifics. Unfortunately, due to challenges associated with meeting all the requirements of Bayesian stable isotope mixing models across multiple sites, and small sample sizes when one population was broken down by age and disease status, I was not able to explore whether my dietary shift predictions hold true when the proportional compositions of devil diets are estimated (**Chapter 4**).

The use of stable isotope analysis as a means of investigating ecological niches has enabled the exploration of novel areas of Tasmanian devil trophic ecology. A key contribution of this thesis has been the estimation of isotopic variation and niche breadths of Tasmanian devils and spotted-tailed quolls, at the levels of both individuals and populations (**Chapters 2, 3** and **5**), and this has been directly enabled by the use of stable isotope analysis. The total niche width of a population is a product of the variation between individuals, and within individuals (Bolnick et al., 2003). By characterising both of these aspects of niche width, I was able to explore

the trophic niches of Tasmanian carnivores more fully than previous studies, which have examined between-individual variation using scat contents (Andersen et al., 2017a; Jones and Barmuta 1998). My ability to characterise the isotopic niche breadths of individuals was aided by the choice of tissue for analysis, i.e. whiskers. Keratinous tissues are inert after formation and, since they continue growing, whiskers can be divided into multiple sections to obtain multiple isotopic data points from a single sampling event, representing different periods of time in the growth of the tissue (Bearhop et al., 2003; Schell et al., 1989). Using stable isotope analysis of keratinous tissues has been greatly preferable to reconstructing individual niche metrics from multiple scat samples, or collecting samples of other tissues for stable isotope analysis, such as blood, on multiple occasions.

While I have robustly quantified isotopic variation and niche breadths of Tasmanian carnivores, I have often had to infer the actual species being consumed by Tasmanian devils based on the likely isotopic ratios of groups of prey species (**Chapters 2, 3** and **5**). This is not due to my use of stable isotope analysis entirely, as Bayesian mixing models can be used to convert data in δ-space (isotopic values only) to p-space (proportional contributions of dietary sources to consumer isotopic values) (Newsome et al., 2007; Phillips, 2012; Stock et al., 2018), however in the Tasmanian system such models were only feasible at one study site. In **Chapter 4**, we demonstrated the difficulties associated with constructing useful Bayesian stable isotope mixing models for species that are both distantly related from any species with known trophic discrimination factors, and where foraging, although largely revolving around a few herbivorous species, is known to also be opportunistic and occur across a relatively large area.

Generally, I found Tasmanian devil isotopic values and niches were surprisingly robust to the effects of DFTD infection and disease-related declines (**Chapters 3 and 5**). This is despite other studies finding changes in devil behaviour in response to both disease and population decline. With DFTD progression, the likelihood of intraspecific social interaction decreases (Hamilton et al., 2020). With devil-related declines and reduced intraspecific densities, devils appear to have reduced, less overlapping, home ranges (Comte et al., 2020), and devils shift their daily activity, foraging later in the night in areas driven to low densities by DFTD, presumably due

to relaxed intraspecific competition (Cunningham et al., 2019b). Potentially, prey resources for Tasmanian devils living in depleted populations are sufficiently abundant that diet composition can generally be maintained through spatial, social and temporal changes in behaviour, as discussed in **Chapter 5**. This potential importance of prey availability is supported by another key finding of this thesis, that devil isotopic niches, and their robustness to stressors such as disease, are dependent on ecological context. DFTD-infected Tasmanian devils appeared to feed at a higher trophic level with increasing tumour volume at one of our sites, Freycinet National Park, which also showed differences in the relative abundance of common prey species compared to our other sites, where no isotopic changes were found (**Chapter 3**). Devil isotopic niches were also affected by human-disturbance, with smaller niches at both a population and individual level in landscapes with higher proportions of human-modified habitat (**Chapter 5**). In both of these instances, the context of habitat and prey assemblages may create bottom-up trophic opportunities or restrictions for Tasmanian devils to a greater extent than infection status or intraspecific competitive contexts alone.

Implications and future research suggestions

Carnivores in Tasmania: devils, disease and decline

Tasmanian devils are now in decline across almost all of their range (Lazenby et al., 2018). Early concerns that DFTD could drive devils to extinction drove a host of conservation actions and trials, including vaccine research (Pye et al., 2018; Tovar et al., 2017) and the establishment of healthy 'insurance populations' in captivity and in the wild. Wild insurance populations were set up in Tasmania, on the previously devil-free Maria Island and on the fenced Forestier Peninsula following trialled disease suppression via culling of infected individuals (DPIPWE, 2018; Lachish et al., 2010; Save the Tasmanian Devil Program, 2011). The goals of these insurance populations were to maintain disease-free populations, and to be a source for reintroductions to boost population numbers and enhance genetic diversity and adaptive potential (DPIPWE, 2018). However, following evidence of immune responses and even tumour regressions in wild devil populations, there have been warnings that translocations may actually cause outbreeding depression and/or

increase the force of infection in populations receiving translocated individuals (Hohenlohe et al., 2019). Concurrently, as our understanding of the role of Tasmanian devils' in structuring ecological communities has grown (Cunningham et al., 2019a, 2019b; Hollings et al., 2015, 2014), so has interest in the reintroduction of Tasmanian devils to continental Australia to restore native top-down control in mainland Australian ecosystems (Hunter et al., 2015). This has culminated in a 2020 reintroduction of 11 Tasmanian devils into a 400-hectare fenced area of the Barrington Tops, New South Wales, Australia, with further reintroductions of 20 devils per year planned for 2021 and 2022 (Aussie Ark, 2020; Wild Ark, 2020). Eventually, other species may be reintroduced into this area, including Eastern quolls, Rufous bettongs *Aepyprymnus rufescens*, long-nosed potoroos *Potorous tridactylus* and southern brown bandicoots *Isoodon obesulus* (Aussie Ark, 2020; Wild Ark, 2020).

As reintroductions of Tasmanian devils progress, a key area of interest is how populations and individuals respond to relatively novel ecosystem assemblages. The work I have presented on isotopic niches of dasyurid carnivores on Tasmania may serve as a basis for exploring how Tasmanian devils' trophic ecology responds to reintroduction to a novel area, with varying compositions of prey species and competitors. Stable isotope analysis could be used to establish isotopic niche metrics of the newly introduced Tasmanian devils in the Barrington Tops, which could then be reassessed with the addition of further competitors and prey species. As the Barrington Tops devil population is already closely monitored (Aussie Ark, 2020; Wild Ark, 2020), this would be logistically feasible. Although isotopic niche estimates could be robustly estimated, I have demonstrated the difficulty in estimating proportional consumption of dietary items using stable isotopes (**Chapter 4**). To gain specificity in the Barrington Tops population, stable isotope analysis could be used alongside scat contents analyses, which can easily be opportunistically collected during monitoring trapping sessions. Based on the results of thesis, I would predict that niche variation in the initial devil population would be age-structured, with younger devils having larger group and individual isotopic niche breadths and a higher proportion of small to medium mammals, bird and possibly reptiles in their scat contents, compared to adult devils. Greater isotopic variation in adult devils may be observed as new prey species are reintroduced into the

Barrington Tops, however we would expect the potential addition of Eastern quolls to constrain the niches of devils consuming a diet most similar to quolls, which would include younger, less competitively dominant individuals (Van Valen, 1965).

Use of stable isotope analysis in Australia

Stable isotope analysis is a well-established and widely used method of reconstructing animal diets, quantitatively characterising isotopic niche breadths as related to ecological niches, and investigating migrations and large-scale movements of animals and populations (Ben-David and Flaherty, 2012; Crawford et al., 2008; Phillips et al., 2014). However, its application is geographically biased. In Australia, very few studies in terrestrial animal ecology have taken advantage of stable isotope analysis, although the method is widely used in Australian marine and freshwater ecology (Ansmann et al., 2015; Frisch et al., 2014; Jardine et al., 2012; Vanderklift and Wernberg, 2010). Terrestrial examples that do exist include an investigation of seasonal dietary changes in the long-nosed bandicoot *Perameles nasuta* (Thums et al., 2005), niche differentiation between ant communities in North Queensland (Blüthgen et al., 2003), and prey use by dingoes (Morrant et al., 2017). The under-utilised nature of stable isotope analysis in terrestrial Australian ecology presents many research opportunities, and some challenges.

Australia is a region of extreme ecological interest. Its terrestrial fauna is the most phylogenetically distinctive in the world (Holt et al., 2013), and 87% of Australia's terrestrial mammals are endemic (Woinarski et al., 2015). At the same time, the biodiversity of Australia is seriously threatened; for example, 1,760 Australian species are listed as threatened by the IUCN Red List, and over 10% of endemic terrestrial mammal species have become extinct since European colonisation in 1788 (IUCN, 2021; Woinarski et al., 2015). Threats to Australian wildlife include competition and predation by feral species, habitat fragmentation through pastoral encroachment, and climatic changes including drought and changing wildfire regimes (Woinarski et al., 2015). An avenue for investigation which may benefit both our understanding of Australian species and conservationists ability to support those species, is an understanding of how their trophic niches respond, and have responded, to these stressors, and to changing conditions. For example, stable

isotope analysis could be used to compare differences in isotopic niche and estimated diet of native and feral competitors, test the responses of individuals and populations to habitat fragmentation, or investigate diet and habitat use of newly reintroduced species. Ideal candidate species would include those where diet has already been described via faecal, stomach or pellet contents analysis and where their range and habitat use is broadly understood, so that tissue samples of both consumers and their dietary items could be collected in the relevant locations. However, as demonstrated in Tasmanian devils, it can be a challenge to obtain reliable species-specific trophic discrimination factors (TDFs) for marsupials, though these are indispensable where researchers hope to estimate relative dietary contributions using stable isotope mixing models, as there are no published TDFs for any marsupial. Marsupials generally have lower basal metabolic rates than placental mammals (Hayssen and Lacy, 1985; MacMillen and Nelson, 1969), which could therefore result in differing TDF values between the two groups. The application of stable isotope analysis in Australia would therefore benefit from controlled studies, where animals in captivity are fed an isotopically consistent diet, and the TDFs between food source and several tissues, such as whiskers, red blood cells and plasma, could be measured. This would be most achievable in species fed a diet with consistent or easily measurable isotopic input, as opposed to captive Tasmanian devils, which are generally fed a wild-sourced diet.

Stable isotope analysis and scavengers

The prevalence of scavenging across the animal kingdom has often been underestimated (DeVault et al., 2003; Wilson and Wolkovich, 2011). Scavenging enables species to access food resources without the energy expenditure and risk associated with hunting and killing live prey, and many species traditionally perceived as hunters will opportunistically scavenge, including lions *Panthera leo* and wolves *Canis lupus* (Pereira et al., 2014; Selva et al., 2003). Stable isotope analysis and other forms of dietary analysis reflect assimilated or ingested diet, and cannot be used to distinguish predated vs scavenged prey types (Kane et al., 2017). However, stable isotope analysis has been used to investigate the diets and isotopic niches of species known to largely rely on scavenging, including Tasmanian devils (in this thesis; Bell et al., 2021, 2020), within-population isotopic niche variability in

hyaena species (Codron et al., 2016), sexual segregation in the Andean condor *Vultur gryphus* (Perrig et al., 2021), and the contribution of marine subsidies (Blázquez et al., 2016) and landfill waste to diets of vulture species (Tauler-Ametller et al., 2018; Tauler-Ametlller et al., 2019).

In this thesis, we estimated the isotopic niches and consumed dietary proportions of the top mammalian scavenger of the Tasmanian ecosystem. However, in **Chapter 4** we were only able to use BSIMM models at one of our six field sites, as often our devil isotopic values did not sit within the outer boundaries created by isotopic values from our collected dietary sources. While one potential explanation could be inappropriate trophic discrimination factors, another may be our choice of tissue. We collected muscle tissue from potential dietary sources, and Tasmanian devils will certainly consume a large proportion of muscle in their diet. However, Tasmanian devils are one of several scavenging species, including hyaenids and some vulture species, with physiological or behavioural adaptations for consuming bone (Kane et al., 2017; Margalida, 2008; Wroe et al., 2005). As bones grow in sequential layers, bone reflects isotopic values of consumed diet over an individuals' lifetime, while isotopic turnover of metabolically active tissues results in muscle reflecting several weeks of dietary intake (Hobson, 1999; Hobson et al., 1993). This may result in isotopic variation between bone and muscle tissues, which, if bone is eaten in high enough quantities, could shift consumer isotopic signatures significantly enough that muscle tissue collection is not adequate to describe the diet of osteophageous scavenging species. Although most stable isotope studies will analyse carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values, zinc ($\delta^{66}Z$) has recently been used to distinguish between bone and flesh eating species in the Turkana Basin in Kenya (Jaouen et al., 2016), demonstrating osteophagy is significant enough in the bone-eating species, spotted hyaena *Crocuta crocuta*, to leave an isotopic trace. For dietary reconstruction, it may be logistically challenging to sample multiple tissue types from a large number of individuals, and a further challenge may lie in determining the correct tissue types, or combinations of tissue types, to include in BSIMMs. However, studies of scavenging species may benefit from multi-tissue sampling of dietary items. Tissues in general may include muscle, skin and bone, and relative consumption estimates (and hence tissue sampling effort) could potentially be informed by observation of consumption, experiments with deployed carcasses, or

scat contents analysis, although the latter will be skewed towards less easily digested tissue types.

Ecological niche partitioning in a biodiversity crisis

The niche variation hypothesis predicts that the ecological niche breadths of populations and individuals will be influenced by inter- and intra-specific competitive pressures (Van Valen, 1965). Experimental manipulations of three-spine stickleback *Gasterosteus aculeatus* populations offer support the niche variation hypothesis, demonstrating ecological niche release where top predators are removed, and diversifying effects of intraspecific competition (Bolnick et al., 2010; Svanbäck and Bolnick, 2007). The effects of competition on niches have also been demonstrated in wild populations, for example, group-living banded mongooses *Mungos mungo* appear to show greater individual specialisation with increasing group size based on isotopic niche estimations (Sheppard et al., 2018). The potential effects of competition on individual and group isotopic niches, either through age (**Chapter 2**), disease status (**Chapter 3**) or community-level cascades (**Chapter 5**), has been a thread through this thesis. Based on the predictions of the niche variation hypothesis we predicted that the competitive consequences of a trophic cascade, specifically Tasmanian devil decline measured by time since DFTD arrived in a population, would result in changes in spotted-tailed quoll niches, and possibly the extent of individual specialisation within Tasmanian devil populations (**Chapter 5**). However, we found that the extent of human-modified habitat in an area was a greater predictor of population isotopic niche breadths.

Resource availability is clearly a driver of competition in and of itself. Resource availability drives differences in isotopic niche variation between individual European badgers (Robertson et al., 2015), and landscape heterogeneity and marine subsidies drive between-individual niche diversity in grey wolves (Darimont et al., 2009). Human-driven land use change is a major and growing driver of biodiversity loss (Foley et al., 2005), and changes the resources available to wildlife. In North America, carnivore populations had increasing isotopic niches and niche overlap with increasing degrees of human-disturbance, with consequences for competition and potential human-carnivore conflict (Manlick and Pauli, 2020). Trophic cascades are

necessarily top-down and have been considered in opposition to theory suggesting that communities are structured via resource availability, or bottom-up forcing (Ripple et al., 2014). Real-world ecosystems are of course influenced by both top-down and bottom-up forcing, and the same will be true of competitive dynamics in communities. In sea otter *Enhydra lutris* populations, intraspecific competition encourages individual specialisation, but only in some habitats (Newsome et al., 2015). In a cascade context, anthropogenic food subsidies appear to disrupt top-down forcing of grey wolves on wild prey, due to greater consumption of livestock (Ciucci et al., 2020). I suggest that my work in this thesis, specifically **Chapter 5**, encourages the further exploration of both community-level and landscape-level influences on the niche dynamics of populations, particularly given the current simultaneous nature of biodiversity declines and conservation-driven reintroductions. Furthermore, while human disturbance provides ecological opportunity in some systems (Manlick and Pauli, 2020), it appears to constrict carnivore niches in Tasmania (**Chapter 5**). I therefore suggest that as an understanding of the relationship between cascades and carnivores, and land use change, is developed, cross community comparisons may shed some light on the contextual factors that guide population and individual responses to changing competitive dynamics.

Concluding remarks

Understanding the ecological effects of species decline is essential in order to anticipate cascading community-wide implications. This is particularly true for carnivore species. Large carnivores structure communities through fear and predation, but have undergone widespread declines with well-documented impacts on sympatric ecological communities and ecosystems (Estes et al., 2011; Ripple et al., 2014). As communities and ecosystems change in response to carnivore declines, niche theory predicts that the ecological niches of populations and individuals will also change in response. In this thesis, I have used stable isotope analysis to explore the impact of the disease-related decline of a top scavenger, the Tasmanian devil, on the isotopic niches of devils and their competitor, the spotted-tailed quoll. I established age-related niche variation within Tasmanian devil populations and described context-dependent isotopic responses to progression of devil facial tumour disease in infected individuals. I then estimated the proportional

contributions of several dietary items to the diet of Tasmanian devils using Bayesian stable isotope mixing models, and demonstrated some of the challenges in applying this method to a marsupial scavenger. Finally, I revealed that human-modified habitats have a greater impact on Tasmanian devil and spotted-tailed quoll isotopic niches than devil decline, underlining the importance of considering both community-level and landscape-level pressures on wildlife populations. This thesis has laid the ground work for using stable isotope analysis to investigate how individuals, populations and communities respond to change in a highly biodiverse yet ecologically threatened region of the world, Australia. In Australia and globally, carnivores are perceived with both fear and awe, and these charismatic and ecologically influential species are often the focus of both human-wildlife conflict and conservation priority, including reintroductions. Carnivores, including grey wolves and Eurasian lynx *Lynx lynx,* are often seen as instrumental for rewilding Earths' landscapes more generally (Navarro and Pereira, 2012; Seddon et al., 2014). Understanding the ecological dynamics between carnivore species, their competitors, prey and the landscapes they inhabit will therefore be an essential component of broader research aiming to mitigate, halt or reverse our current biodiversity crisis.

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