Exploring the influence of disturbance history and forest type on an arboreal marsupial, the common brushtail possum (*Trichosurus vulpecula*), using a multi-disciplinary approach

Photo by Kirsty Pilkington

Erin Margaret Flynn, BSc (Colorado State University)

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UTAS

School of Zoology
University of Tasmania
Hobart, Tasmania

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To my family,

who taught me to work hard and dream big.

Thank you for your love and support.

Artist unknown 2009

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Statement of originality

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institute, and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

October 18, 2011   Erin Margaret Flynn

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Manuscripts (submitted or intended for submission to peer-reviewed journals) produced as part of this thesis:

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(Head of School)

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Preface

The author collected all of the primary data for this project with the aid of volunteers in the field. Anne Chuter and Adam Pennington (Forest Practices Authority (FPA) botanists) conducted the vegetation surveys used for habitat assessment. Daniel Livingston (FPA GIS, Database, and Systems Support Officer) prepared the maps and the remote habitat assessment data. Chapter-specific contributions are noted in the 'acknowledgements' section of the appropriate chapter. All sample analyses and statistical analyses were performed using the protocols and programs specified. All photographs in this thesis were taken by the author unless noted otherwise. My supervisory panel, specifically Professor Susan M. Jones and Dr. Sarah A. Munks, were instrumental to project design. Dr. Menna E. Jones was integral in incorporation of genetics (Chapter 5) into the study.

With the exception of chapter 1 ('Introduction') and chapter 6 ('General discussion'), this thesis is written as a series of independent, logically connected scientific papers. Due to this, there is some unavoidable repetition between chapters, particularly concerning methods. Papers were written for submission to the journal indicated in a citation on the title page of each chapter and as such, are written in the style and format required by that particular journal. Minor changes have been made to formatting for consistency purposes within the thesis. These minor changes consist of renumbering tables and figures with the chapter number placed before the table and figure numbers for the paper (e.g., Figure 2 in the physiology paper (Chapter 3) becomes Figure 3.2 in the thesis). Co-authors are identified in the citation for each chapter to acknowledge their contribution. Chapters 2 and 4 have been submitted for publication. Chapter 2 has been accepted for publication and Chapter 4 is in review. Chapters 3 and 5 are intended to be submitted for publication in the near future.

Abstract

The level of impact of forest disturbance on mammal communities depends on a species' degree of forest-dependence as well as the intensity and scale (temporal and spatial) of the disturbance. Where land-use practices such as logging, agriculture, and urbanisation alter the habitat characteristics of a forest, a wide range of effects may lead to a reduction in species diversity or the decline of a population. Arboreal marsupials are the faunal group considered to be most at risk from habitat disturbance in Australian forests. Despite this, there have been relatively few studies examining the influence of forest disturbance on arboreal marsupials, particularly in Tasmania.

The overall aim of this thesis is to determine whether habitat disturbance (as a result of harvest and associated activities (and wildlife in one site)) and forest type influence the physiology and population biology of an arboreal marsupial, the common brushtail possum (*Trichosurus vulpecula)*, in Tasmanian forests. A multi-dimensional approach integrating physiological, genetic, and ecological studies was employed to address this aim at both the individual and population levels. In particular, this thesis examines potential impacts of habitat disturbance and forest type on physiological parameters of well-being, reproductive parameters, and population parameters (demographic and genetic) in the brushtail possum.

Trapping was conducted in spring/summer and autumn/winter during 2007–2008 at six dry *Eucalyptus* forest sites (three regenerating after harvest and three in relatively undisturbed forest) in southeast Tasmania and four wet *Eucalyptus* forest sites (two regenerating after harvest and two in relatively undisturbed forest) in northeast Tasmania. Disturbed sites were 4–11 years post harvest. All sites were embedded within a matrix of mature or older-aged regenerating forest. Vegetation and structural attributes of the study site and the land immediately surrounding it were assessed. At each site, data were collected on the characteristics of the faunal community (community composition and biodiversity, species abundance, body mass, sex, and breeding success), which included the brushtail possum. Blood samples, tissue biopsies, and late lactation milk samples were collected from brushtail possums to assess physiological well-being, population genetics, and reproductive

parameters. Plasma cortisol concentration was assessed via radioimmunoassay as a proxy for adrenal status, white blood cell differential was performed on blood smears as a proxy for generalised immune response, and haematocrit was measured as a proxy for body condition. DNA was extracted from tissue for genetic analyses using microsatellites to explore mating system, genetic diversity, and implications of gene flow between brushtail possum populations. Brushtail possum milk was analysed for lipid, solid, carbohydrate, and protein concentrations.

Analysis showed that, in general, habitat structural complexity within sites and age structure of the forest in the surrounding landscape did not vary significantly, indicating that broad resource availability (food and refuge) was equivalent across sites. However, basal area, the number of hollow-bearing trees, and the percentage of mature forest, young forest, and overstorey cover varied with disturbance, while understorey cover and numbers of both tree hollows and hollow-bearing trees varied by forest type. Faunal distribution was dictated by forest type. While most species showed little influence of habitat disturbance, brushtail possums exhibited lower abundance, decreased breeding frequency, and a male-biased adult sex ratio at the disturbed sites.

Comparison of physiological parameters across sites suggest that the brushtail possum is physiologically resilient, with no clear influence of habitat disturbance or forest type on adrenal status, generalised immune response, or body condition. There was a subtle difference in fat content of brushtail possum milk, driven primarily by milk composition at one site; this probably reflects site level differences in maternal diet. However, there were no differences in breeding success, distribution of births, and timing of developmental features, survival, or body condition of young across sites.

Genetic analysis suggests that there are two populations of brushtail possums influenced by geographic distance, with near-random mating and moderate genetic diversity, across eastern Tasmania. There were no effects of disturbance, as examined in this study, on genetic diversity or mating system. However, disturbance resulted in an altered landscape with decreased female-specific resources (e.g., tree hollows which are necessary for reproduction). Results suggest that the male-biased adult sex ratio observed in brushtail

possum populations living in disturbed sites was due to a lower abundance of adult females at these sites, rather than maternal sex allocation, retention of subadult males (e.g., lack of dispersal), or increased immigration of adult males.

The outcomes of this study demonstrate the value of using a multi-dimensional approach that integrates physiological, genetic, and ecological investigations of the potential influences of habitat disturbance and forest type on animal species. Such an approach reduces uncertainty about the relationship between disturbance and the response and subsequent recovery of fauna, which is important for the development of effective forest management strategies. Brushtail possums are resilient and able to cope with habitat disturbance at the individual level. However, subtle population-level responses may have implications for population growth and long-term viability of brushtail possums in areas subject to intensive and extensive forest harvesting. These results also illustrate the importance of retaining mature habitat elements in the landscape to allow forest-dependent fauna to recolonise harvested areas and persist into the long term.

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Chapter 1

Introduction

The Tasmanian subspecies of the common brushtail possum (*Trichosurus vulpecula fuliginosus*)

Habitat disturbance, through resultant fragmentation and loss, has been identified as a primary threat to loss of global biodiversity (Pimm *et al.* 1995; Sih *et al.* 2000; Schipper *et al.* 2008). Disturbance to a particular environment can result in altered resource availability and disrupted community structure (Krebs 2001). Disturbance events may be natural, such as wildfire or a storm, or anthropogenic, such as land clearing for agriculture, urbanisation, introduction of invasive species, or logging for timber. Anthropogenic and natural disturbances may influence the environmental components that are important to fauna in different ways. For example, wildfire clears out the undergrowth, while logging can alter the vertical stratification of forest structure. Both may result in loss of, or decrease in, availability of habitat. Logging has a more direct impact on forest structure (Loyn 1985; Smith and Lindenmayer 1988; Villela *et al.* 2006) while wildfire alters food availability more directly and immediately (Gill 1975). The time it takes for the environment to recover depends on the type of vegetation, and the intensity and frequency of the disturbance event (Krebs 2001).

Where land-use practices result in habitat loss, studies have revealed a wide range of effects that may lead to a reduction in species diversity and the decline of populations of individual taxa in the long-term. These effects include disrupted sex ratios (Martin and Handasyde 2007), increased risk of predation (Kurki *et al.* 1998; Newell 1999), increased immigration of exotic species (Turner 1996), altered social systems (Ims *et al.* 1993; Atwood and Weeks 2003; Banks *et al.* 2005; Walker *et al.* 2008), altered breeding success (e.g., decreased survival or fewer/smaller young) (Wolff 1995; Hinsley *et al.* 1999; Cooper and Walters 2002), and altered patterns of movement (Scheibe *et al.* 2006). While population densities of particular species may increase in the short-term (Hocking 1981; Lambin 1994; Lopez and Ferrari 2000; Atwood 2006), long-term effects of significant habitat loss may result in decline and loss of species. This may result in altered faunal community composition (Lindenmayer and Franklin 2002). For example, clearfelling of mature native forest for timber tends to benefit species that favour early successional forest, while species dependent on habitats provided by mature forest may be disadvantaged (Cork and Catling 1996; Alexander *et al.* 2002).

Habitat loss and fragmentation may also have genetic and physiological implications for individuals or populations. If habitat loss results in population isolation, this can alter gene flow (via decreased immigration), and population structure and differentiation (Cushman 2006). There are few studies that focus specifically on integrating ecological impacts with the genetic and physiological implications of habitat loss in forest fauna. Habitat fragmentation and disrupted spatial structure influenced mating habits and genetic variation between populations of American pika (*Ochotona princeps*) (Peacock and Smith 1997) and resulted in altered population structure via different measures of relatedness and degrees of inbreeding avoidance between populations of southern hairy-nosed wombats (*Lasiorhinus latifrons*) (Walker et al. 2008). Populations of greater gliders (*Petauroides volans*) in fragmented forest habitat had significantly less genetic diversity than both the ancestral population at harvest in 1966 and samples from the surrounding intact forest (Taylor et al. 2007). Suorsa et al. (2003) reported decreased body condition, increased corticosterone (the avian/reptile hormone indicating adrenal responsiveness), and decreased offspring survival in Eurasian treecreepers (*Certhia familiaris*) living in fragmented forest; they related these observations to a decline in food abundance and forest structure. Similarly, in saw-whet owls (*Aegolius acadicus*), individual behaviour and resultant reproductive success were influenced by habitat loss; physiological 'stress' increased while home range size and foraging efficiency decreased, all of which resulted in decreased reproductive success (Hinam and St.Clair 2008).

Forests are a major ecosystem type, covering an estimated 29% of the Earth's land surface (Perry 1994) . Forests throughout the world may be divided into three major groups that are defined by latitude and temperature: tropical (23.5˚N-23.5˚S), temperate (23.5-50˚N and 23.5-50˚S), and boreal (50-65˚N) (Perry 1994; Pidwirny *et al.* 2007). Precipitation, along with more subtle influences of soil type and topography, further determines the division of these broad groups into forest types such as rainforest, swamp, evergreen, and deciduous forests (Whittaker 1975; Perry 1994). For a particular forest type, food and refuge availability and quality, influenced by forest structure and habitat, determine the occurrence of particular faunal species, and thus community composition (Grinnell 1917; MacArthur 1972; Dueser and Shugart Jr. 1978; Mares *et al.* 1986). For example: skunks, rabbits, deer, mountain lions, reptiles, and amphibians are found in temperate forests, while moose,

wolverines, martens, and chipmunks are found in boreal forests (Pidwirny *et al.* 2007). Furthermore, bird community composition in Australian forests is influenced by the vertical stratification and age structure of the particular vegetation type and food availability (Holmes and Recher 1986; Williams *et al.* 2001).

'Disturbance theory', or disturbance ecology, is the broad network of studies that focus on distinct events that disrupt ecosystem function and address the importance and influence of habitat disturbance to biodiversity and ecology of individual species (Connell 1978; Sousa 1984; Petraitis *et al.* 1989; Rogers 1996). Anthropogenic disturbance of forests is a consequence of changes in land use and includes clearing for agriculture, urbanization, and forestry activities (Gibbons and Lindenmayer 2002). With increasing pressure world-wide on forest habitat, it is important to understand the ecology of forest fauna and the impacts of disturbance to their habitats in order to develop effective management actions. The ultimate level of impact of forest disturbance on vertebrate communities depends on the species' degree of forest-dependence (Tyndale-Biscoe and Calaby 1975), as well as the intensity (Kavanagh and Stanton 2005), and spatial (Dumbrell *et al.* 2008) and temporal scale of the disturbance (Fisher and Wilkinson 2005).

Individual species and guilds of species respond very differently to habitat disturbance; they may increase, decrease, or show no observable change in either abundance or community composition (Evans 2001; Kavanagh and Stanton 2005; Dumbrell *et al.* 2008). Species thought to be most at risk from habitat disturbance in forests are those with specialised diet or habitat requirements (e.g., tree hollows), low fecundity, (Michaels 2000; Lindenmayer *et al.* 2003), patchy distribution, or large body size (Turner 1996). Arboreal vertebrates, particularly those that are dependent on standing trees with mature forest elements, are generally strongly affected by structural damage from logging (Laurance 1996). Arboreal vertebrates include species of monkeys, bats, birds, rodents (squirrels, chipmunks, tree rats, and tree voles), and marsupials (koalas, tree kangaroos, possums, and opossums) (McIlroy 1978; Laurance 1996; Carey 1996; Kays and Allison 2001). Populations of some arboreal vertebrates recover slowly after forestry harvesting as they require slow-forming mature forest elements (Tyndale-Biscoe and Smith 1969; Tyndale-Biscoe and Calaby 1975; Cork and Catling 1996; Laurance 1996; Alexander *et al.* 2002). In contrast, ground-dwelling

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mammals, which are dependent on characteristics of the undergrowth rather than the forest canopy, recolonise harvested areas relatively quickly and, unlike arboreal species, may not be significantly affected by forest harvesting in the medium to long-term (Taylor 1991; Cork and Catling 1996; Ferron *et al.* 1998; Moses and Boutin 2001).

Arboreal marsupials are considered to be the faunal group in Australia that is most susceptible to forest harvesting due to loss or decline of refuge sites, primarily tree hollows which are required for breeding (Tyndale-Biscoe and Calaby 1975; McIlroy 1978; Goldingay and Daly 1997). When food supply is adequate, structural components determine habitat quality for such species and have been linked to their abundance and distribution (Pausas *et al.* 1995; Cork and Catling 1996). Availability of nest sites is a crucial habitat component that influences habitat quality as most arboreal marsupial species rely on tree hollows for breeding (Pausas *et al.* 1995; Gibbons and Lindenmayer 2002; Koch *et al.* 2008a). Hollow-bearing trees represent a characteristic component of forest ecosystems across the world, thus their retention and management is of global relevance (Lindenmayer *et al.* 1993; Newton 1994). In Australia, 303 native hollow-using vertebrate species, comprising 15% of terrestrial vertebrates (10% of reptiles, 13% of amphibians, 15% of birds, and 31% of mammals), are considered to be dependent on tree hollows, each species having specific tree hollow requirements (e.g., size, shape, orientation on the tree, and aspect) (Gibbons and Lindenmayer 2002; Gibbons *et al.* 2002; Koch *et al.* 2008a). Removal of hollow-bearing trees during forest harvest has been identified as the most significant threat to wildlife survival (Loyn 1985) and the most significant cause of reduction in biodiversity in production forests of eastern Australia (Lindenmayer *et al.* 1991). Logging operations may impair hollow-bearing tree recruitment, and negatively influence the recovery of the area by fauna dependent on tree hollows (Gibbons and Lindenmayer 1996). Some animals, such as woodpeckers (family *Picidae)*, directly excavate tree hollows, increasing the rate of tree hollow formation (Jackson and Jackson 2004; Losin *et al.* 2006). Australia has no primary hollow excavating species, so tree hollows take more than 140 years to develop (Koch 2008b); formation requires physical injury to the tree that is further exacerbated by fire, termites, or fungus (Gibbons and Lindenmayer 2002). The slow rate of development of tree hollows has long-term implications for hollow-dependent fauna.

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The common brushtail possum (*Trichosurus vulpecula*) is a highly adaptable, nocturnal, arboreal marsupial omnivore, capable of exploiting a large array of food types (Fitzgerald 1984; Cowan and Moeed 1987; Nugent *et al.* 2000; Sadlier 2000) and habitats across Australia (Hocking 1981; Statham 1984; Kerle 1984; Cowan 1990; Bullinski and McArthur 1999). It was introduced to New Zealand in 1858 in an effort to establish a fur trade and is now a well-established invasive pest species (Nugent *et al.* 2001). In its native Australia, it is both common and widespread, with the largest distribution of any Australian marsupial (Cowan 1990). Despite the fact that the brushtail possum is a common laboratory research animal (Clout and Sarre 1997), there are few detailed studies of brushtail possum ecology and physiology in Tasmania where the species is considered to be an agricultural, forestry, and urban pest (Clout and Sarre 1997; Cowan and Tyndale-Biscoe 1997; Bulinski and McArthur 1999; McArthur *et al.* 2000; Kerle 2001). Studies on mainland Australia indicate that habitat disturbance in some areas, through its effect on resource availability, has resulted in a bias in the sex ratio of offspring (Johnson *et al.* 2001; Isaac *et al.* 2005) and a change of mating system in the closely related mountain brushtail possum (bobuck) (*Trichosurus cunninghami*) (Martin and Handasyde 2007).

In Tasmania, Hocking (1981) examined population responses of brushtail possums living in wet forest sites that had been burnt. He found an initial and short-lived male bias in adult sex ratio immediately post-burn that reflected sex-differential survival (favouring males). Furthermore, fecundity, breeding success, survival of pouch young and juveniles, growth of animals, and population density all decreased as the forest regenerated, which he attributed to changes in food availability and quality as a result of burning. In contrast, LeMar and McArthur (2005) in their study of brushtail possum density in a range of habitats, some of which had been subject to production forestry activities but not burning (e.g., grassland, harvested uncleared land (scrub and fallen vegetation), newly planted to 7 year old plantation, and mature forest), found that brushtail possum abundance was higher in mature native forest than in any other habitat. They suggested that when the nutritional value of vegetation between sites was equivalent, mature forest provided more suitable brushtail possum habitat.

This study examines the anthropogenic modification of different forest types by timber harvesting and the impact on brushtail possums at both the individual and population levels. The study was conducted in a forest management context, focusing on comparing impacts of habitat disturbance in wet and dry *Eucalyptus* forest in Tasmania, the island state of Australia. Approximately 48% of Tasmania (3.4 million ha) is forested: 1.5 million ha are reserved forest and 1.9 million ha are unreserved forest, the majority of which is potentially available for timber harvest (DPIW 2007; DPIW 2008). Such forests support diverse forest-dwelling mammalian fauna and need to be managed appropriately if they are to continue to provide habitat for such species. The focal species for this study is the Tasmanian subspecies of the common brushtail possum (*Trichosurus vulpecula fuliginosus*), subsequently referred to as the 'common brushtail possum' or 'brushtail possum' unless otherwise specified. The brushtail possum is of particular interest as it is an arboreal marsupial and part of the hollow-using fauna group, thus recognised as a Priority Species under Tasmania's Regional Forest Agreement (Tasmanian and Australian Governments 2007). It is also partially protected under the *Nature Conservation Act 2002* (Tasmanian Government 2002). The conservation value of the brushtail possum lies in its specialist refuge requirements (tree hollows) and assumed resilience to anthropogenic impacts. This study will contribute to disturbance theory with respect to arboreal mammals and current forest management practices.

This study takes a multi-dimensional approach to understanding the potential impacts of habitat disturbance in two different forest types, focusing on the individual brushtail possum, and integrating knowledge derived from genetic, physiological, and ecological studies (Figure 1.1). To gain a full understanding of the potential impacts of ecosystem disturbance upon a species, we must consider individual, population, and community-level responses. Thus individual animals must be studied in terms of both the population and ecosystem in which they exist. A physiological study allows the potential impacts of disturbance on the individual to be assessed, focusing on measures of well-being that indicate chronic 'stress' responses. Using genetic and ecological approaches, population dynamics are explored in different ways. Genetic analysis is used to explore population diversity and implications of gene flow and mating system on and between populations. An ecological approach is used to address abundance and population demographics.

Multi-disciplinary approaches are the key to more efficient conservation problem solving, providing a more holistic and efficient approach to questions from the perspective of several disciplines (Wildt *et al.* 2003). Each discipline provides information which, when integrated, may inform the mechanism behind what is being observed in other disciplines (Tracy *et al.* 2006).
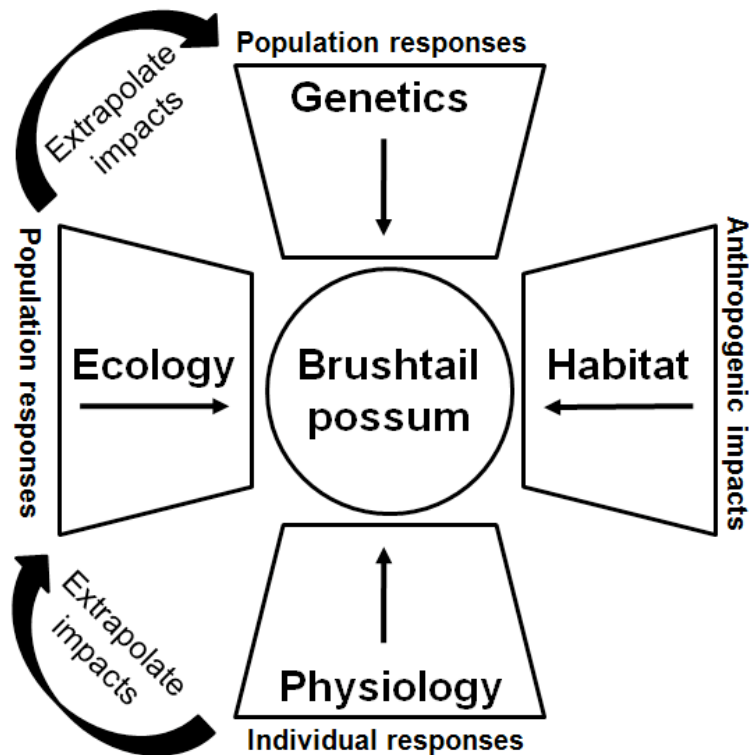
Figure 1.1 The multi-dimensional approach taken in this study integrates genetics, ecology, and physiology to understand the impacts of habitat disturbance in different forest types on the brushtail possum.

Thesis aims

The overall aim of this thesis is to investigate the impacts of habitat disturbance in different forest types on an arboreal marsupial, the common brushtail possum (*Trichosurus vulpecula*). Such studies are important as they can reduce the uncertainty about the relationship between habitat disturbance and response and recovery of forest fauna. They are thus imperative to the development of effective forest management practices. A multi-disciplinary approach is used in this thesis, focusing on integration of ecology, physiology, and genetics, to meet the overall aim at both the individual and population levels. Specific questions address potential impacts of habitat disturbance and forest type on physiological parameters of well-being, reproductive parameters, and population parameters (ecology and population genetics) in brushtail possums.

Thesis structure

Chapters 2–5 of this thesis are written as a series of connected individual papers, which have either been submitted or are intended for submission for publication.

Chapter 2 assesses whether differences in forest type and disturbance history are reflected in the small-medium mammal communities (of which the brushtail possum is a member) of Tasmania's production forests. Field data were collected during 2007–2008 at four dry *Eucalyptus* forest sites in southeast Tasmania and four wet *Eucalyptus* forest sites in northeast Tasmania; half of the sites were logged 4–11 years ago (= disturbed) and half were unlogged (= control). Implications of forest type in regard to species distribution and abundance are addressed. Differences in resource attributes due to forest type and past disturbance regimes are assessed and examined in relation to species composition (occurrence, richness, and diversity), individual body mass, sex ratio, and breeding success.

In order to broaden this study, two additional sites (DD3 and DC3) were incorporated later in the project. To remove sampling bias between sites for Chapter 2, these sites were excluded from analysis and data were analysed from the remaining eight sites that were all trapped the same number of seasons (three). This was not a concern in subsequent chapters as the individual was the unit sampled for the physiology [Chapter

3], reproduction [Chapter 4], and genetic [Chapter 5] studies and results did not directly depend on abundance across sites.

Data for *Chapters 3, 4, and 5* were collected from 10 study sites in eastern Tasmania, five of which had been harvested 4–11 years ago and five of which were in intact forest. Half of the sites were located in dry *Eucalyptus* forest and half were in wet *Eucalyptus* forest. *Chapters 3 and 4* explore impacts of habitat disturbance and forest type on individual brushtail possums. *Chapter 3* documents the physiological parameters of well-being (plasma cortisol concentration, white blood cell profile, and haematocrit values) in brushtail possums living in different forest types subject to disturbance regimes (disturbed and control). This approach assesses the intensity of the disturbance event and the quality of habitat as embodied physiologically by individual brushtail possums. This will determine if one forest type or disturbance regime is more physiologically beneficial, suggesting higher quality or lower environmental stress, with respect to the brushtail possum.

Chapter 4 investigates whether differences in forest type and disturbance history are reflected in milk composition and other reproductive parameters (timing of birth, body condition, and survival of young) in the brushtail possum. Quality and abundance of food and refuge have implications for reproductive capacity of a female, particularly the ability to conceive and rear young. This study addresses a gap in disturbance theory, exploring the relationship between habitat disturbance and female reproduction. Forest type is taken into account as differences in vegetation type may have implications for milk quality and quantity, which may impact the development and survival of offspring.

Chapter 5 explores the mechanism responsible for the male-bias in the adult sex ratio observed in brushtail possum populations living in disturbed areas reported in *Chapter 2*. It also investigates whether habitat disturbance or forest type influence population genetics or the mating system, which has implications for the evolutionary trajectory of a population, in the brushtail possum. Microsatellite analysis is used to assign paternity and determine genetic structure, diversity diagnostics, inbreeding, and immigration between brushtail possum populations.

Chapter 6, the general discussion, offers a synthesis of the findings of this study and addresses the contribution that this study makes to disturbance theory. The value of retaining mature habitat in the landscape surrounding harvested areas and the effectiveness of using an integrated approach for conservation research are discussed.

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Chapter 2

Characteristics of mammal communities in Tasmanian forests: exploring the influence of forest type and disturbance history

Top: spotted-tailed quoll *(Dasyurus maculatus*), long-nosed potoroo *(Potorous tridactylus)*, common brushtail possum *(Trichosurus vulpecula)* (black morph), dusky antechinus (*Antechinus swainsonii*)
Middle: short-beaked echidna *(Tachyglossus aculeatus),* southern brown bandicoot *(Isoodon obesulus)*, eastern quoll *(Dasyurus viverrinus)* (black morph), long-tailed mouse *(Pseudomys higginsi)*
Bottom: Tasmanian bettong *(Bettongia gaimardi),* eastern quoll (fawn morph), common brushtail possum (grey morph), Tasmanian devil *(Sarcophilus harrisii)*

Abstract

With increasing pressure world-wide on forest habitat, it is crucial to understand faunal ecology to effectively manage and minimise impacts of anthropogenic habitat disturbance. This study assessed whether differences in forest type and disturbance history were reflected in small-medium mammal communities found in Tasmania's production forests. Trapping was conducted in spring/summer and autumn/winter during 2007–2008 at four dry *Eucalyptus* forest sites (two regenerating after harvest and two in relatively undisturbed forest) in southeast Tasmania and four wet *Eucalyptus* forest sites (two regenerating after harvest and two in relatively undisturbed forest) in northeast Tasmania. All sites were embedded within a matrix of mature or older-aged regenerating forest. Thirteen mammal species were recorded across all sites. There was no difference in species diversity or richness between forest type or disturbance regime, but species composition differed. Total number of individual animals and captures were influenced strongly by forest type and disturbance history, with most animals and captures found in the dry disturbed forest sites. Abundance of some species (e.g., bettongs and potoroos) was higher in disturbed sites than undisturbed sites. Brushtail possum numbers (adult and offspring), however, were lower in disturbed sites and populations displayed a male-biased adult sex ratio and lower breeding frequency. Habitat structural complexity and vegetation diversity within core sites, and age structure of the forest in the surrounding landscape did not vary significantly, indicating that broad resource (food and refuge) availability was equivalent across sites. In general, the small-medium mammals in this study did not appear to be significantly affected by forest harvesting in the medium-term. Although past harvesting altered the abundance of some habitat features (e.g., canopy cover, basal area of trees, and tree hollow availability), we suggest that the availability of such features in the surrounding landscape may mitigate the potential effects of disturbance on the species for whom such habitat features are important.

Introduction

Studies of the relationships between habitat type and both the occurrence and response of species to habitat disturbance are crucial to understanding faunal responses to landscape heterogeneity, and consequently for conservation. Anthropogenic disturbance of forests is a consequence of a variety of land use activities including agriculture and forestry. Responses of individual species and ecosystems to disturbance vary: while population densities of particular species may increase in the short-term (Charnov and Finerty 1980; Lambin 1994; Lopez and Ferrari 2000; Lambert *et al.* 2005; Atwood 2006), long-term effects of significant habitat loss or alteration may result in decline and loss of species, and thus altered community composition (Lindenmayer and Franklin 2002). Among forest mammals, species richness sometimes increases with disturbance, an effect that may be linked to the creation of new ecological niches (a common byproduct of disturbance) and the presence of ecologically opportunistic and tolerant invasive species (Seagle 1985; McKinney and Lockwood 1999; Simard and Fryxell 2003). This may result in altered faunal community composition as such habitat changes tend to benefit species that favour early successional forest, while species dependent on mature forest may be disadvantaged.

The extent of impact of forest disturbance on mammal communities depends on the species' degree of forest-dependence (Tyndale-Biscoe and Calaby 1975), as well as the intensity (Kavanagh and Stanton 2005), and spatial (Dumbrell *et al.* 2008) and temporal scale of the disturbance (Fisher and Wilkinson 2005). There is increasing recognition of the influence of matrix condition on the effects of disturbance and the value of retaining habitat around 'managed' areas to ensure species persistence (Lindenmayer and Franklin 2002; Eyre and Norman 2003; Anderson *et al.* 2007). Where land-use practices, such as logging, alter the characteristics of a forest at a broad scale, including its structure and the availability of food and refuge, a wide range of effects on fauna may result. These can include: disrupted sex ratios (Martin and Handasyde 2007), altered social systems (Raskja-Jurgiel 1992; Atwood and Weeks 2003; Banks *et al.* 2005; Martin *et al.* 2007), altered breeding success (e.g. smaller young) (Wolff 1995; Hinsley *et al.* 1999), and disruption to foraging and denning areas (Johns 1986; Thiollay 1992).

Species thought to be most at risk from habitat disturbance in forests are those with specialised diets or habitat requirements (e.g., tree hollows) and low fecundity (Michaels 2000; Lindenmayer *et al.* 2003). Tyndale-Biscoe and Calaby (1975) and Goldingay and Daly (1997) identify arboreal marsupials as the Australian mammals most susceptible to anthropogenic habitat disturbance through a reduction in availability of tree hollows necessary for breeding (Gibbons and Lindenmayer 2002). Medium-sized ground mammals (e.g. bettongs (*Bettongia gaimardi*) and potoroos (*Potorous tridactylus*)) are susceptible to disturbance in the short-term (Catling and Burt 1995; Michaels 2000; Kavanagh *et al.* 2004; Kavanagh and Stanton 2005). This may be because they have a limited capacity for dispersal, and their size and reliance on ground cover makes them susceptible to predation (Claridge and Barry 2000). However, Taylor (1991) suggests that such species may recover quickly since the occurrence of many ground-dwelling mammals is dependent on characteristics of the undergrowth rather than the forest canopy. An exception may be species dependent on logs for refuge sites, which could be impacted in the long-term by intensive forest harvesting (Scotts 1991).

Tasmania, the island state of Australia, has a diverse forest-dwelling mammalian fauna, particularly as the fauna has not yet been impacted significantly by introduced predators, although this may change with the recent introduction of the red fox (*Vulpes vulpes*). Approximately 48% of Tasmania (3.35 million ha) is forested, of which 44% (1.48 million ha) is reserved (DPIW 2007; DPIW 2008). Of the 1.87 million ha of unreserved forest, 0.79 million ha is State Forest and 1.09 million ha is private land (DPIW 2007; DPIW 2008). The large majority of unreserved forest is potentially available for timber harvesting and needs to be managed appropriately if it is to provide habitat for forest-dwelling fauna into the future.

In this study, we examine the small-medium mammal community in disturbed (harvested) areas and relatively undisturbed (control) areas within dry and wet *Eucalyptus* forest. Differences in resource attributes due to forest type and past disturbance regimes are assessed and examined in relation to species composition (occurrence, richness, and diversity), individual body mass, sex ratio, and breeding success. We aim to determine if

differences in forest type and disturbance history are reflected in small-medium mammalian community characteristics at the site scale. This work is part of a broader study of the influence of forest type and disturbance on the ecology and physiology of the brushtail possum (*Trichosurus vulpecula*).

Materials and methods

Study sites

This study was carried out at four dry *Eucalyptus* forest sites in southeast Tasmania, Australia, and four wet *Eucalyptus* forest sites in northeast Tasmania (Figure 2.1, Table 2.1). Two sites of both forest types were regenerating after harvest (= disturbed sites) and two were in relatively undisturbed forest (= control sites) dominated by mature forest. All disturbed sites were logging coupes in State Forest that had been harvested between 1997 and 2004. Harvest in wet forest included commercial thinning and clearfell, burn, and sow, while harvest in dry forest included combinations of seedtree retention, advanced growth retention, and shelterwood retention (Appendix A). Dry disturbed sites were between 4–11 years post harvest while the wet forest disturbed sites were both 6 years post harvest. A photograph taken in the centre of each site and an aerial photograph of each site are provided in Appendix B and Appendix C, respectively. Specific location details of each site are included in Appendix D.

Site selection was constrained by the need for access and to ensure that harvested sites had similar pre-harvest conditions (soil type, elevation, and floristics) to the relatively undisturbed (control) sites (Table 2.1). Although not harvested using modern silvicultural methods in recent times (at least not in the past 80 years), the control sites (either designated forest reserves or areas of mature forest not currently planned for logging) were part of the forested landscape that had been subject to various wood extraction activities of settlers for over 100 years.
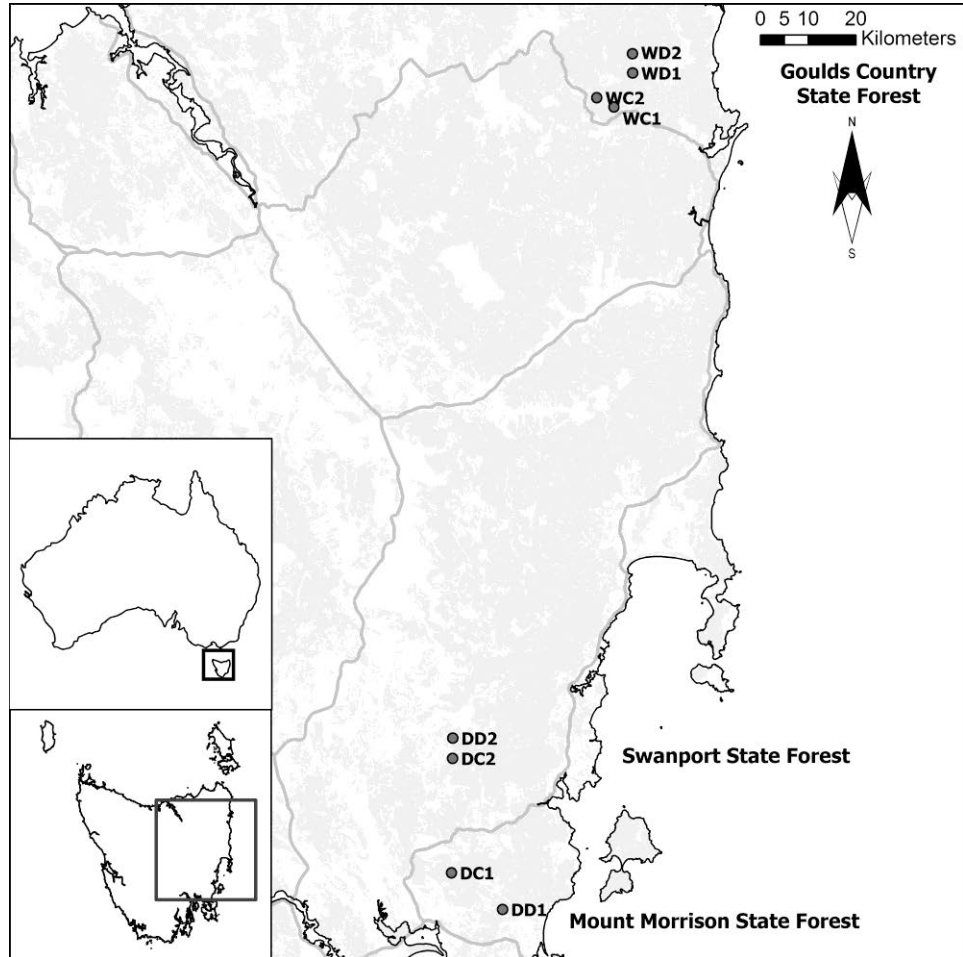
Figure 2.1. Location of study sites across eastern Tasmania, the island state of Australia. Dry *Eucalyptus* forest sites are in the southeast and wet *Eucalyptus* forest sites are in the northeast of the state. Shading indicates forested area while lack of shading denotes non-forest area (agriculture, scrub, lakes, urban areas, etc.). Solid lines denote major roads (DPIW 2001; Harris and Kitchener 2005). (Treatment (forest type/disturbance) is indicated by the first two letters of a site name, followed by the site number: 'DD' = dry disturbed, 'DC' = dry control, 'WD' = wet disturbed, and 'WC' = wet control forest.)

Table 2.1. Environmental and disturbance attributes of study sites used to assess the response of mammal communities to differences in forest type and disturbance history in Tasmania, Australia

Site name	Coupe name	Core area (ha) [1]	Broad forest type[2]	Silvicultural method	Harvest date (yr)[7]	Land tenure[8]	Dominant geology	Elevation (m)	Annual rainfall (mm)
DD1	MM002A & MM004A	62.30	*E. pulchella* - *E. globulus* - *E. viminalis* grassy shrubby forest [Dry]	Partial harvest (SED, AGR)[3]	2000/2004	State	Dolerite	354	651.1
DD2	SW059A & SW059B	34.24	*E. obliqua* forest [Dry]	Partial harvest (SR, AGR)[4]	1998/1999	State	Sandstone	398	478.1
DC1	Reserve	58.72	*E. obliqua* forest [Dry]	N/A	N/A	Formal reserve	Dolerite	333	483.1
DC2	SW051A	26.58	*E. obliqua* forest [Dry]	N/A	N/A	Informal reserve	Sandstone	385	478.1
WD1	GC029B	21.49	*E. regnans* forest [Wet]	Commercial thinning[5]	2002	State	Adamellite	231	884.4
WD2	GC104B	24.54	*E. regnans* forest [Wet]	Clearfell, burn, and sow (CBS)[6]	2002	State	Adamellite	184	884.4
WC1	GC145B	22.93	*E. regnans* forest [Wet]	N/A	N/A	State	Adamellite	206	1190.6
WC2	GC139B	36.68	*E. regnans* forest [Wet]	N/A	N/A	State	Adamellite	529	1095.3

[1] Core area (approximately equivalent to harvested area in disturbed sites) was the physical location of all traps within the research site (logging coupe or reserve as appropriate) plus a 100 m buffer

[2] Broad forest community (DPIW 2001; Harris and Kitchener 2005)

[3] Mosaic of seedtree retention (SED) and advanced growth retention (AGR) followed by a low intensity burn for regeneration (Wilkinson 1994) (Appendix A)

[4] Mosaic of shelterwood retention (SR) and advanced growth retention (AGR) followed by a low intensity burn for regeneration (Wilkinson 1994) (Appendix A)

[5] Stocking reduced to at least 200 well-formed trees ha^{-1}; no regeneration burn (Wilkinson 1994) (Appendix A)

[6] All live trees (> 5 m) are felled, followed by a regeneration burn and seed sowing (Wilkinson 1994) (Appendix A)

[7] From Timber Harvesting Plan for the coupe (Forestry Tasmania, unpublished data)

[8] CAR reserves GIS data layer (DPIW 2007)

All sites (disturbed and control) were embedded in a forested landscape and were subject to natural disturbance (storms and wildfire). Such production forest landscapes in Tasmania include extensive areas of higher productivity forests supporting regeneration of various ages resulting from clearfell, burn, and sow, shelterwood removal, or intensive selective logging silviculture, and most of the lower productivity sites being selectively logged either commercially for pulp or firewood. The forest landscape across Tasmania is also characterised by frequent wildfires, with fire frequency highest in the drier forest. However, only one of the study sites (DD1, Table 2.1), was known to have been subject to wildfire in the last 10 years. DD1 was partially burnt in spring/summer 2006, in the first year of the broader study.

Two zones were recognised at each site: a 'core' and a 'matrix' (Figure 2.2). Care was taken to select sites that were surrounded by forest with similar floristics (disturbed and control sites) and age structure (control sites). This is because the characteristics of forest surrounding a particular site (matrix) may influence animal occurrence within the core of the site. The matrix was the 2 km radius buffer surrounding the core of the site, chosen because this distance has been used to distinguish between 'extreme home range movements' and dispersal in the brushtail possum (the focal species of a concurrent study) (Clout and Efford 1984; Green 1984; Cowan and Tyndale-Biscoe 1997). The age structure of the forest in the matrix was similar across sites (Stone 1998; Forestry Tasmania 2007a; Forestry Tasmania 2007b; Forestry Tasmania 2007c) (Figure 2.3). The core of the site was defined as the physical location of all traps within the research site (logging coupe or reserve as appropriate) plus a 100 m radius buffer (roughly equivalent to the harvested area in disturbed sites). The radial dimension of the buffer was chosen to reflect the maximum distance that brushtail possum usually move from a remnant forest patch (Harper 2005). The area of the core of the sites ranged from 21–62 ha (Table 2.1).
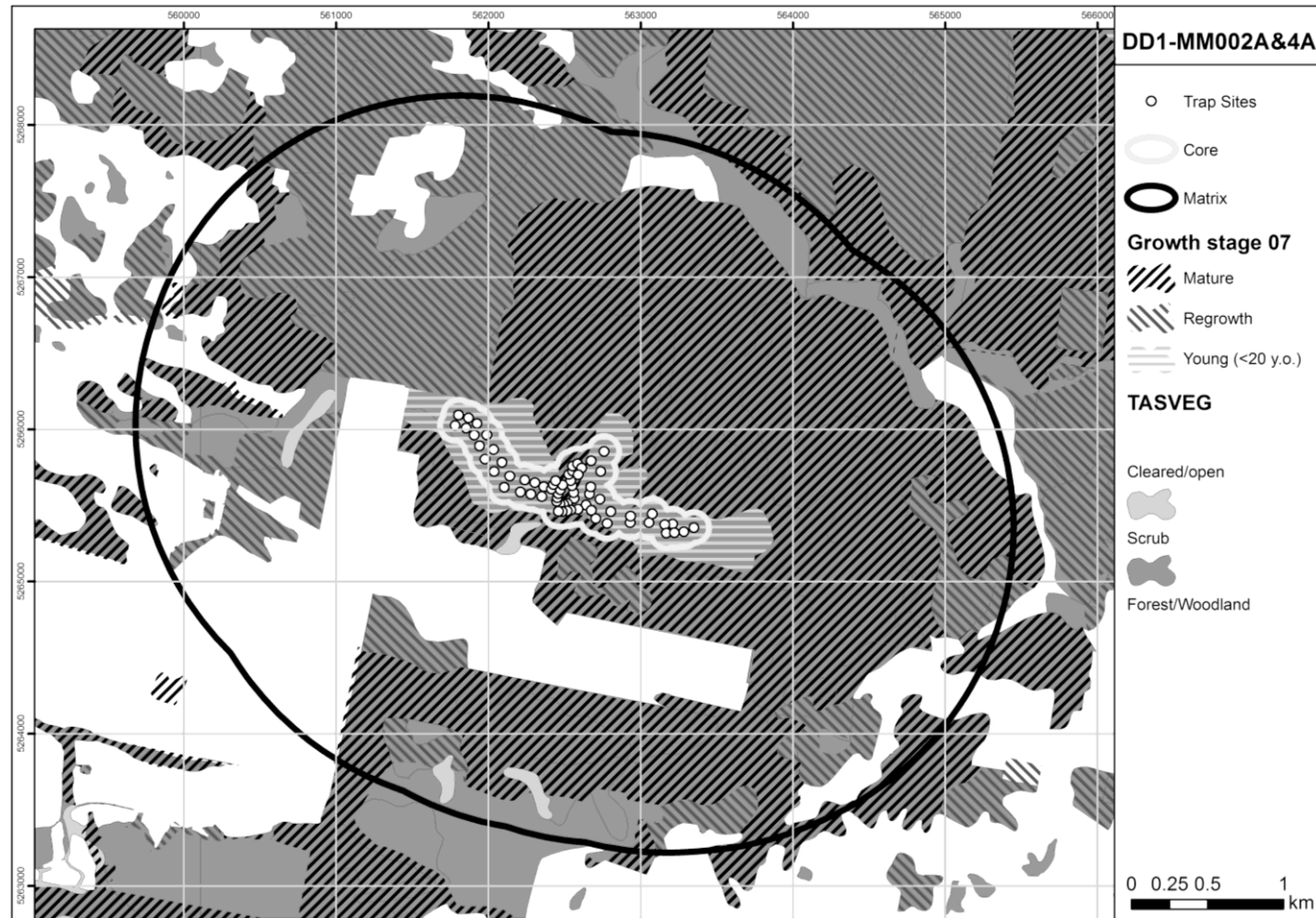
Figure 2.2. Example of core and matrix sites in a landscape context in a disturbed site (DD1). Location of each trap and boundaries of both the core and matrix sites are indicated amongst vegetation type (TASVEG) (DPIW 2001) and the dominant age structure of the vegetation (Stone 1998; Forestry Tasmania 2007a; Forestry Tasmania 2007b; Forestry Tasmania 2007c).
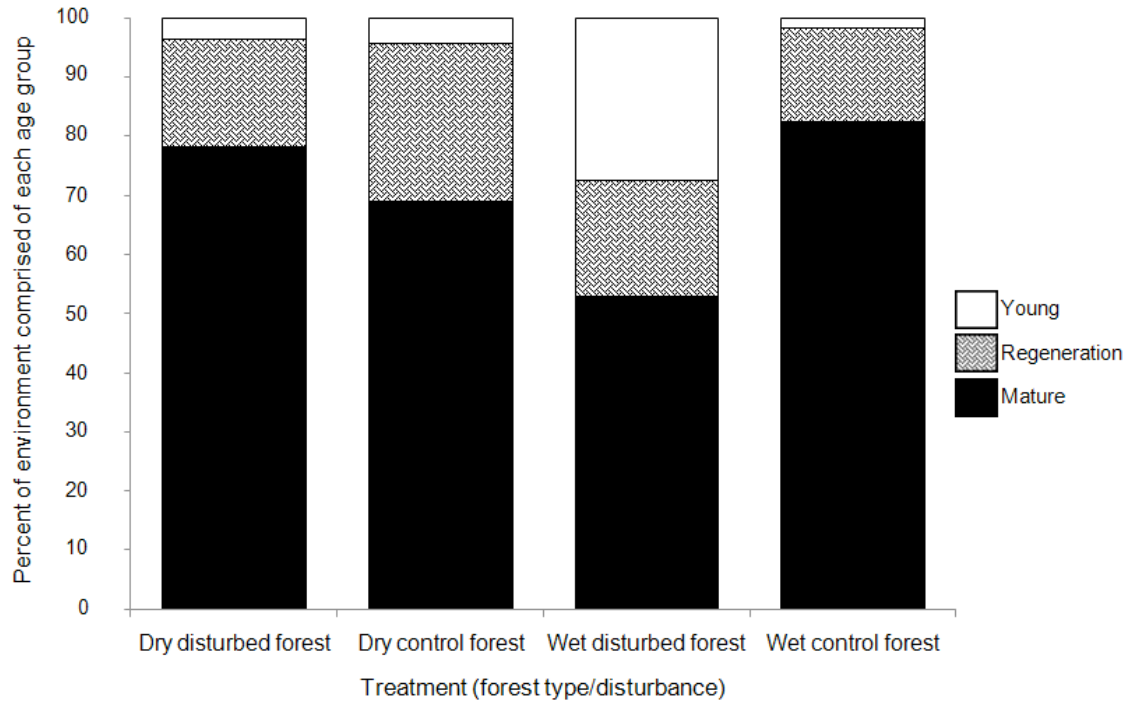
Figure 2.3. Age distribution of the matrix, the forest surrounding core sites, by treatment (forest type/disturbance). Vegetation age structure was derived from GIS spatial data (Stone 1998; Forestry Tasmania 2007a; Forestry Tasmania 2007b; Forestry Tasmania 2007c).

Animal Capture

Animals were captured during 2007–2008 at each site in wire Mascot cage traps suitable for small (< 1 kg) and medium (1–10 kg) mammals. The study covered three trapping seasons (spring/summer 2007, autumn/winter 2008, and spring/summer 2008). One trapping trip was conducted each season, with either 37 (two sites: WD1 and WC1) or 67 traps (the other six sites) deployed at each site, depending on the size and accessibility of the site. The number of traps per site was consistent over the study, resulting in an average spatial trapping effort (number of traps/core area (ha)) of 1.77 traps ha^{-1} (range: 1.1–2.7 traps ha^{-1}).

During each trapping session the hessian-covered 300 x 300 x 660 mm, treadle-triggered Mascot traps (Mascot Wire Works, New South Wales, Australia) were deployed nightly for 4 nights: 1 night of prebaiting (traps were baited and fastened open) and 3 nights of active trapping. Traps were placed at suitable places (e.g., at the base of trees, on stumps, on/under logs, or near refuge areas) at 50 m x 20 m spacing along two transects per site. Traps were checked starting at dawn to minimize capture stress in nocturnal animals (Johnson and McIlwee 1997). Bait was composed of small balls of peanut butter, oats, and vanilla essence. This bait is specifically formulated to target brushtail possums, and is also broadly suitable for herbivores and omnivores. However, it is not the optimal bait type for capturing carnivores such as Tasmanian devils (*Sarcophilus harrisii*) and quolls (*Dasyurus maculatus* and *Dasyurus viverrinus*).

Upon capture, all animals were identified to species. Incidental captures included some birds and reptiles (Appendix E). Rodents, which were in low abundance, were sexed and released without microchipping. All other mammals were transferred to a hessian bag for handling and processing. An ISO FDX-B transponder chip (passive integrated transponder (PIT) tag) was implanted subcutaneously between the shoulder blades for unique identification. Animals were weighed with a Salter 10 kg balance to the nearest 50 g. They were then sexed and, where appropriate, pouches were checked for presence or absence of pouch young (dependent young that reside in the pouch) to assess breeding success. Female macropods were identified to species and the presence of any pouch young was recorded. No further procedures (microchipping or weighing) were carried out on macropods to reduce the chance of loss of pouch young. In the majority of species, only two age classes were recorded: adults and their dependent young (if present). However, in the brushtail possum, subadults were also recorded. Female adults and subadults were separated by body mass: adults weighed ≥ 2.4 kg, the weight of the lightest reproductively active female trapped in this study. Male adult and subadult possums were distinguished by testis size (left testis length and width (excluding the epididymus) were measured with vernier calipers to the nearest millimetre): adults had a testis volume of ≥ 20.0 mm^3 as calculated from the equation for an oblate sphere: $V = \frac{\pi}{6} W^2 L$, where $V =$ testis volume (mm^3), $W =$ width, and $L =$ length (Abbott and Hearn 1978; Hocking 1981).

Habitat variables

Habitat variables considered likely to influence the occurrence of small-medium mammals, including ground-dwelling and arboreal marsupials (Lunney *et al.* 1987; Cork and Catling 1996), and to reflect forest structure/complexity (McElhinny *et al.* 2006) (Table 2.2), were recorded at each study site.

Vegetation age structure of the core and matrix of each site was derived from GIS spatial data (Stone 1998; Forestry Tasmania 2007a; Forestry Tasmania 2007b; Forestry Tasmania 2007c) and broad forest type was derived from TASVEG (DPIW 2001; Harris and Kitchener 2005). Basal area, description of floristic community (dominant and sub-dominant species in each vegetation layer), vegetation cover and height, and occurrence of hollow-bearing trees, tree hollows, and logs were collected during habitat surveys at each core site according to the methods described in Table 2.2. These surveys involved measurement of each variable in 10 evenly spaced 0.25 ha plots per site (Braithwaite *et al.* 1989). Logging history and details were obtained from the Timber Harvesting Plan for each disturbed site (Forestry Tasmania, unpublished data).

Table 2.2. Habitat variables collected at each core site to estimate resource (food and refuge) availability for mammal communities between forest type and disturbance history in Tasmania, Australia

Independent site variables	Description
Vegetation age structure	Vegetation age structure was obtained from GIS spatial data (Stone 1998; Forestry Tasmania 2007a; Forestry Tasmania 2007b; Forestry Tasmania 2007c). Forest was grouped into three age structure categories: mature (mature or senescent (over-mature) forest with structure indicating ecologically mature, undisturbed forest > 80 years old), regeneration (regrowth or regeneration > 20 years old), and young (young native regeneration < 20 years old)*.
Forest type (floristic community)	Dominant broad forest types were grouped into the following categories: dry *Eucalyptus* forest, wet *Eucalyptus* forest/rainforest, and 'other' (agricultural land, non *Eucalyptus* forest, scrubland, native grassland, moorland, highland, wetland, and 'other natural environments') (Harris and Kitchener 2005).
Total basal area (stand density)	The average cross sectional area in square meters of all trees (with wood greater than 10 mm diameter at breast height (DBH) (measured at 1.3 m above ground level on the upward slope)) on the 0.25 ha plot (in a slight modification of Braithwaite *et al.* (1989)) was estimated using the Angle Count Sampling or 'sweep' method (Goodwin 1995) in a circle based around the trap site in the middle of the plot.
Forest type (floristic community)	Finer-scale floristic community following the key in the Forest Botany Manual (Forest Practices Authority 2005).
Vegetation cover and height	Vegetation surveys were conducted in 10 evenly spaced plots per site. Height, percentage of cover, and abundance of dominant and sub-dominant species of each vegetation layer (overstorey, midstorey, understorey, and ground cover) were estimated by a botanist (Dunstan and Fox 1996).
Occurrence of hollow-bearing trees (of use for medium-sized fauna)	Hollow-bearing trees ≥ 500 mm DBH (at 1.3 m), containing at least one hollow for medium-sized fauna (≥ 50 mm diameter entrance that appeared deep enough to be entered) (Koch *et al.* 2008a).
Occurrence of logs	Abundance of logs of ≥ 150 mm diameter that may be of use to fauna.
Annual rainfall	Millimetres of rain per year were obtained from Bureau of Meteorology records.
Logging history and disturbance class	Age since last logging event, type and frequency of past logging events, and frequency of browsing control and sanctioned firewood gathering for each site.
Fire history	Wildfire history (frequency and intensity), and forestry-related burn (fuel-reduction and regeneration burns) history (frequency and intensity).

*As described in Stone (1998), this age classification is not completely accurate due to the patchy nature of native forest and how photographic interpretation (PI typing) is derived. This is, however, a commonly used method that generates the best estimate of age for the area in question based on physical attributes. This is done through three dimensional interpretation of aerial photographs of the forest, in relatively homogenous 3 ha portions. Each of these portions is assigned to the aforementioned age class that dominates the area, though it may include small portions of other age classes.

Data analysis

Species diversity index and richness

Diversity indices for mammal species captured at each site were calculated by applying the Shannon Diversity Index (Magurran 1988) in the form of $e^{H'}$ where $H' = -\sum p_i \ln(p_i)$, where p_i is the relative abundance of each species. Species richness was defined as the number of species. Evenness was assessed via Shannon's Equitability ($E_{H'} = H'/\ln(\text{species richness})$) which can range between 0 and 1; 1 represents completely even species distribution, while lower values reflect increasing uneven distribution. Two-way analysis of variance (ANOVA) with factors disturbance and forest type were performed in SAS 9.1 (SAS Institute Inc. 2003) on overall site scores to assess differences in diversity, species richness, and evenness. Means ± standard error are presented. When no differences were detected between forest type or disturbance history, data were grouped from sites by treatment (forest type/disturbance): dry disturbed ('DD'), dry control ('DC'), wet disturbed ('WD'), and wet control ('WC') forest.

Captures, population sizes, and spatial relationship of community composition between sites

No seasonal or annual differences were detected in preliminary analyses of the data so animal data for each site were pooled across seasons to maximise sample size. When no differences were detected between either forest type or disturbance history, data were grouped by treatment (forest type/disturbance): dry disturbed forest, dry control forest, wet disturbed forest, and wet control forest. Abundance was estimated using minimum number alive (Krebs 1966), derived from capture-mark-recapture methods. Rodent abundance was expressed as number of captures as they were not microchipped; rodents were considered to be unique individuals unless they had distinguishing markings or injuries. Given that the number of nights trapped per trip was identical across sites, capture success was calculated as the total number of captures (including recaptures) divided by the trapping effort (the number of traps multiplied by the number of trips). Two-way ANOVA, with disturbance and forest type as factors, were performed in SAS on overall site scores to assess differences in capture success. Chi-square analysis was used to compare differences in abundance (minimum number of unique individuals alive) of each species between disturbance and

forest type. There were sufficient data for analysis of these parameters for brushtail possums, bettongs, potoroos, eastern quolls (*Dasyurus viverrinus*), swamp rats (*Rattus lutreolus*), and long-tailed mice (*Pseudomys higginsi*). Smaller capture numbers of other species precluded statistical analyses.

For brushtail possums, the species for which there were the most data, the minimum number alive and the proportion of this total that were captured each season were calculated for each site to account for the different numbers of traps set per site. Population size was estimated from the capture history of each brushtail possum using the POPAN option of the open-population Jolly-Seber model (Schwarz and Arnason 1996) in the software Program MARK (White and Burnham 1999). The model with constant survival, capture probability, entry into the population, and population size (phi(.)p(.)pent(.)N(.)) was the most appropriate, with the lowest Akaike information criterion (AIC) of 11 tested models. Two-way ANOVA with factors disturbance and forest type were performed in SAS on overall site scores to compare differences in both the percentage of the minimum number alive captured each season and of the estimated 'total' brushtail population that was captured. Smaller capture numbers and low recapture rate of other species precluded population size estimates.

Non-metric multi-dimensional scaling (MDS) ordination plots assessing the spatial relationship between sites for square root-transformed faunal species abundances were generated in Primer 6 (PRIMER-E Ltd. 2007). These ordinations were based on the Bray-Curtis measure of similarity (Clarke and Warwick 1994).

Sex ratio, breeding success, and body mass

For this study breeding success was defined as the percentage of individual females in the population known to have bred over the duration of the study. Reproductive success was determined by the presence of pouch young which indicated that the mother had successfully given birth and had retained the young, at least in the short-term, regardless of her ability to raise it to independence. It is unlikely that reproduction was missed as marsupials have an extended period of lactation rather than gestation, in contrast with eutherian mammals (Green and Merchant 1988; Tyndale-Biscoe 2005). For the brushtail

possum there were some females that bred in both years; therefore, we were able to calculate breeding frequency and number of offspring for this species. Two-way ANOVA, with disturbance and forest type as factors, were performed in SAS on site means to compare differences in brushtail possum body mass at first capture and on overall site scores to compare differences in breeding success and adult and offspring sex ratio in the brushtail possum. Chi-square analysis was used to compare differences in breeding frequency and number of offspring in the brushtail possum, and adult sex ratio and breeding success in the species with sufficient sample sizes that lacked the replicates suitable for analysis via ANOVA (bettongs, potoroos, and eastern quolls) between disturbance and forest type. Due to the patchy occurrence of bettongs, potoroos, and eastern quolls, body mass at first capture for these species was analysed on site means by one-way ANOVA (factor = site) to compare differences between sites. When no differences were detected between treatments (forest type/disturbance), data were grouped by species.

Habitat diversity index, structural complexity, and site ordination

Diversity indices, evenness, and species richness were calculated for plant species in each site in the same manner as for the fauna. Two-way ANOVA with factors disturbance and forest type were performed in SAS on overall site scores to determine whether these parameters differed significantly. When no differences were detected between forest type or disturbance history, data were grouped by treatment (forest type/disturbance).

An index, modified from McElhinny *et al.* (2006), was constructed to assess habitat structural complexity. Habitat variables to be included in the index were selected such that no variable was strongly correlated (i.e., $r^2 \geq 0.3$) with any other. The variables were: number of logs on the ground, understorey height (m), percentage of understorey cover, midstorey height (m), percentage of overstorey cover of, percentage of rock cover, and number of hollow-bearing trees (Table 2.2). Each variable was divided by the range displayed across the sites (minimum to maximum value) to create a number between 0 and 1. This was done rather than converting the raw data into quadrats as in McElhinny *et al.* (2006) in order to maintain the integrity of variation in the raw numbers. The sum of all the variable numbers was then divided by the maximum overall value (the number of variables

assessed) for the final proportion and index value. This procedure was carried out once for each plot and then averaged to produce the final site habitat complexity score. Variance of habitat complexity score within each site was calculated. Although it is likely that different species will respond to different aspects of habitat complexity, we used a single, unweighted measure of complexity because we aimed to assess overall community responses and wished to avoid ad hoc adjustments to the measure of complexity. Two-way ANOVA with factors disturbance and forest type were performed in SAS on overall site scores to determine whether habitat complexity index or within-site variance differed significantly. When no differences were detected between forest type or disturbance history, data were grouped from sites by treatment (forest type/disturbance).

Non-metric multi-dimensional scaling (MDS) ordination plots assessing the spatial relationship between the habitat variables (floristics and structural complexity, as listed in Table 2.2) of each site were generated in Primer 6. These ordinations were based on the Bray-Curtis measure of similarity (Clarke and Warwick 1994).

Results

Species diversity index and richness

Five hundred and ten individual mammals were trapped, identified, and sexed during the 1428 trap nights of this study. No marked individual was trapped at more than one study site. Thirteen species of small-medium sized mammals (10 native and three introduced) were caught (Table 2.3).

The mean number of species of small-medium sized mammals caught at each site (species richness) was 7.0 ± 0.56 (range: 5.0–9.0). The mean species diversity index was 4.5 ± 0.42 (range: 2.5–5.8) and evenness averaged 0.7 ± 0.03 (range: 0.6–0.8) across sites. There were no significant differences in species richness, species diversity index, or evenness between forest type ($F_{1,4} = 0.42$–1.57, $P = 0.278$–0.552) or disturbance ($F_{1,4} = 0.11$–0.19, $P = 0.688$–0.762) (Figure 2.4). Disturbed sites contained 1.6 times the number of individuals as the

undisturbed sites ($n = 316$ and $n = 194$, respectively). Dry sites contained 1.3 times as many animals as the wet sites ($n = 283$ and $n = 227$, respectively).

Table 2.3. Species caught in core sites trapped during the study

Species		Number of sites in each treatment animals were trapped in			
		Dry disturbed *1	Dry control *2	Wet disturbed *3	Wet control *4
		(2)	(2)	(2)	(2)
Common brushtail possum	(Trichosurus vulpecula)	2	2	2	2
Tasmanian bettong	(Bettongia gaimardi)	2	1	0	0
Long-nosed potoroo	(Potorous tridactylus)	2	1	0	0
Tasmanian pademelon	(Thylogale billardierii)	0	2	1	1
Eastern quoll	(Dasyurus viverrinus)	1	2	2	1
Spotted-tailed quoll	(Dasyurus maculatus)	1	1	1	2
Southern brown bandicoot	(Isoodon obesulus)	1	1	0	1
Short-beaked echidna	(Tachyglossus aculeatus)	1	2	1	0
Swamp rat (native)	(Rattus lutreolus)	1	0	2	2
Long-tailed mouse (endemic)	(Pseudomys higginsi)	1	0	1	2
Black rat*5	(Rattus rattus)	1	1	2	2
Brown rat*5	(Rattus norvegicus)	0	0	2	2
House mouse*5	(Mus musculus)	1	0	1	1

*1 Sites: DD1 and DD2
*2 Sites: DC1 and DC2
*3 Sites: WD1 and WD2
*4 Sites: WC1 and WC2
*5 Introduced species
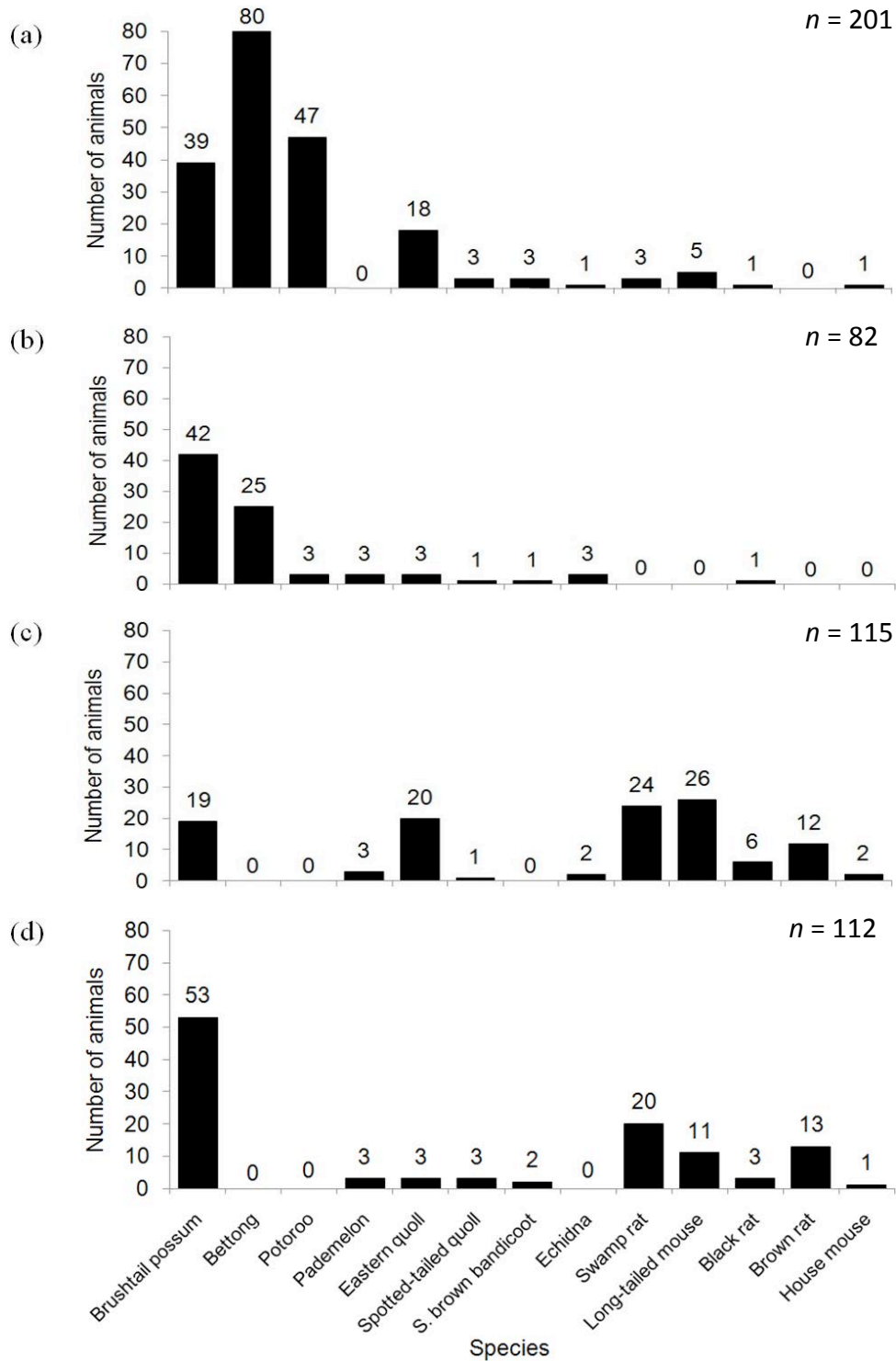
Figure 2.4. Faunal community by treatment (forest type/disturbance): (a) dry disturbed forest (DD1 and DD2), (b) dry control forest (DC1 and DC2), (c) wet disturbed forest (WD1 and WD2), (d) wet control forest (WC1 and WC2). The number of individual animals within each species is given above each bar.

Captures, population sizes, and spatial relationship of community composition between sites

Capture success did not vary by forest type ($F_{1,4} = 0.29$, $P = 0.617$) or disturbance ($F_{1,4} = 1.45$, $P = 0.295$); mean capture success across sites was $37.0 \pm 6.45\%$ (range: 16.4–67.6%). Total number of both captures and minimum number of unique individuals alive of all species combined differed among sites, with higher numbers of both in dry disturbed forests ($\chi^2_3 = 36.97$, $P < 0.001$, $n = 405$ and $\chi^2_3 = 20.78$, $P < 0.001$, $n = 201$, respectively) than in the three other treatments (forest type/disturbance): dry control forest: $n = 211$ and $n = 82$, respectively; wet disturbed forest: $n = 240$ and $n = 115$, respectively; wet control forest: $n = 265$ and $n = 112$, respectively.

The brushtail possum was the only species recorded at all sites, with fewer animals trapped in disturbed sites ($\chi^2_1 = 4.06$, $P = 0.044$, $n = 153$). Bettongs and potoroos were found only in dry forest, with greater numbers found in disturbed forest ($\chi^2_1 = 14.41$, $P < 0.001$, $n = 105$ and $\chi^2_1 = 15.92$, $P < 0.001$, $n = 50$, respectively). Eastern quolls were found most often in disturbed forest ($\chi^2_1 = 11.17$, $P = 0.001$, $n = 44$). Brown rats (*Rattus norvegicus*) were found exclusively in wet forest. Swamp rats were found more often in wet forest ($\chi^2_1 = 17.88$, $P < 0.001$, $n = 47$), while greater numbers of long-tailed mice were found in wet disturbed forest ($\chi^2_1 = 7.11$, $P = 0.008$, $n = 42$). Numbers of pademelons, spotted-tailed quolls, southern brown bandicoots, black rats, and house mice were low and there were no significant differences in numbers found between sites.

There were no differences between forest type or disturbance in the percentage of the minimum number of brushtail possums alive captured each season (mean: $59.9 \pm 1.54\%$, range: 55.1–68.9%) (forest type: $F_{1,4} = 0.55$, $P = 0.500$; disturbance: $F_{1,4} = 0.81$, $P = 0.419$) or between the estimated 'total' brushtail possum population that was captured, calculated using the Program MARK model (mean: $97.1 \pm 1.25\%$, range: 90.8–100.0%) (forest type: $F_{1,4} = 0.57$, $P = 0.493$; disturbance: $F_{1,4} = 0.77$, $P = 0.430$) (Figure 2.5). Trap number did not appear to affect capture success, at least for brushtail possums.
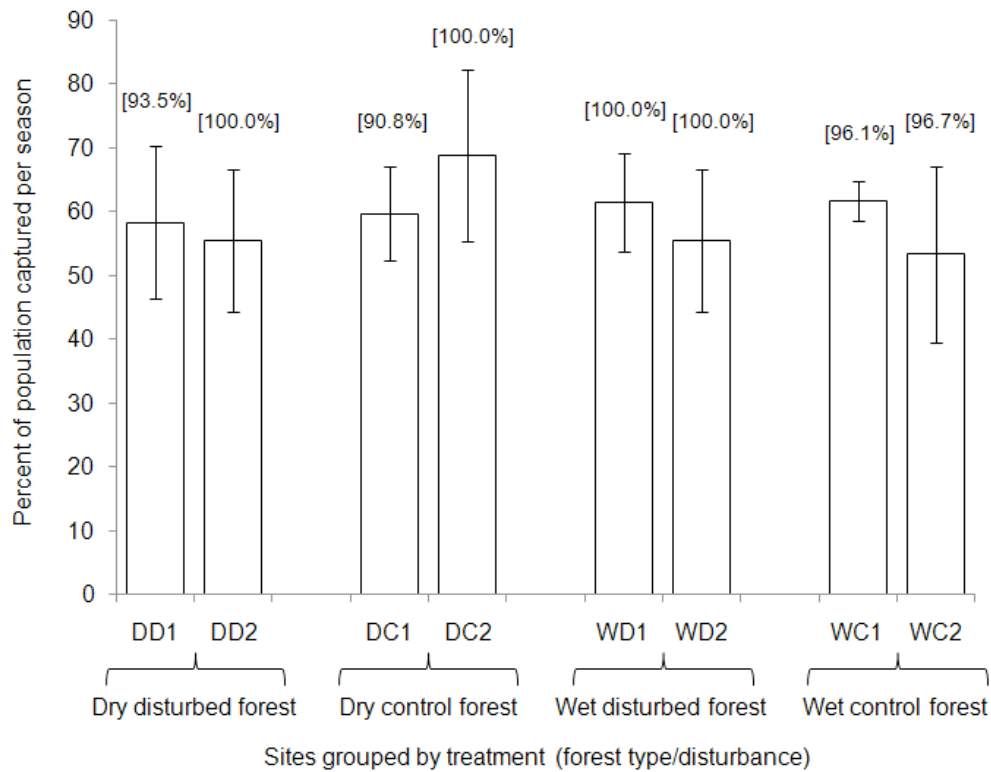
Figure 2.5. Percentage of the known population (minimum number alive (Krebs 1966)) of brushtail possums that was captured per season in each study site. Error bars denote SEM. The percentage of the estimated population that was captured is given in brackets above the error bars. The percentage of the known population that was captured per season and the percentage of the estimated population that was captured were equivalent between all sites. *Note: WD1 and WC1 (constrained by site dimensions) had 37 traps deployed while all other sites had 67 traps.

Non-metric multidimensional scaling ordination of faunal community composition demonstrated that community composition was influenced by broad forest type (Figure 2.6). Communities in the dry sites were separated on the ordination plot according to their level of disturbance: in community composition, disturbed sites (DD1 and DD2) were more similar to each other than to the controls (DC1 and DC2). There was no such relationship in wet forest communities. Within the wet sites, spatial orientation indicated that the clearfell, burn, and sow coupe (WD2) had the most distinctive species composition (and thus was a potential 'outlier' site), with all the other sites nestled closely to each other. The low stress level (0.07), which reflects the relationship between site dissimilarities and ordination

distance, indicates that these are strong relationships with no real risk of misinterpretation (Clarke 1993).
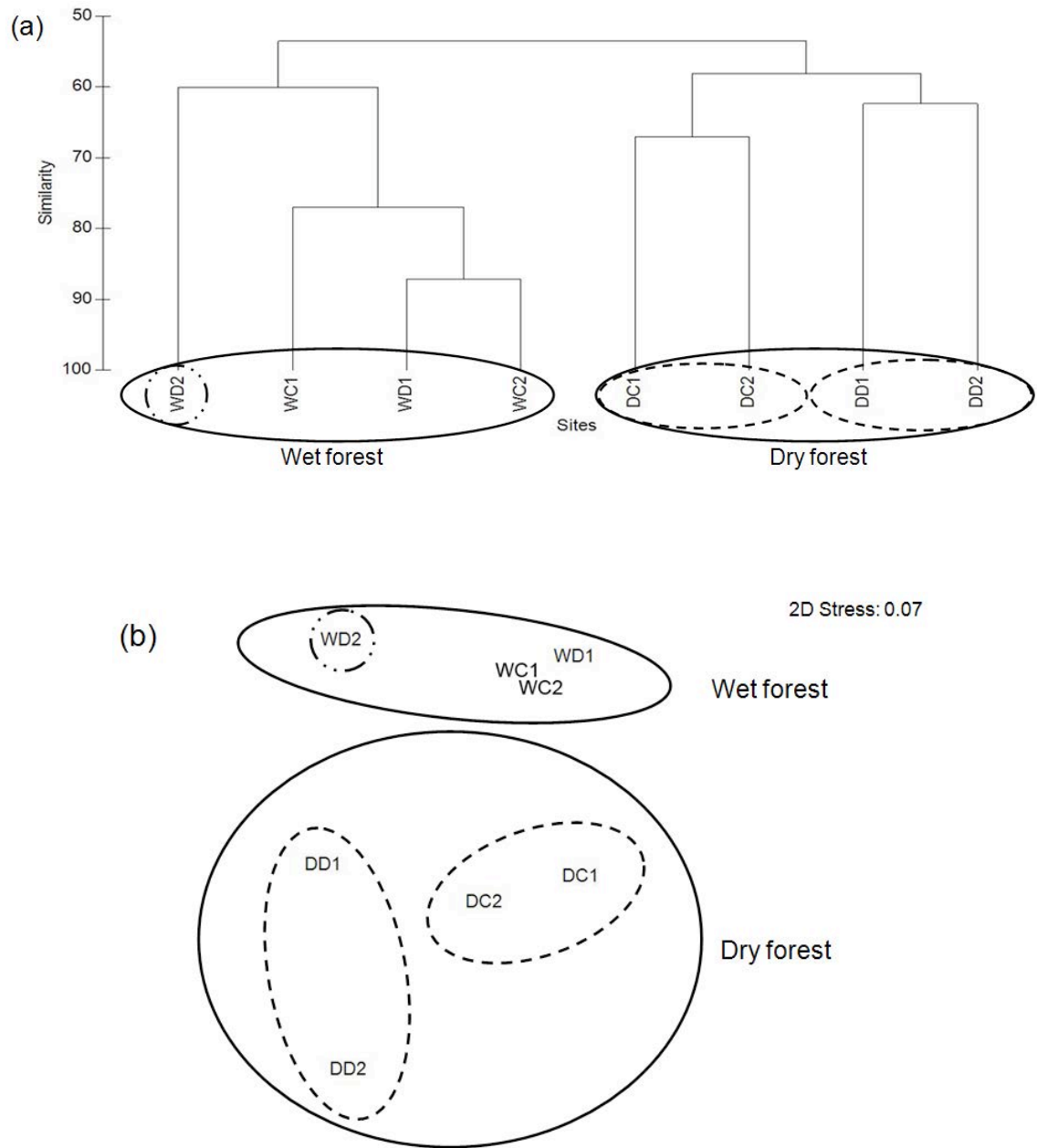
Figure 2.6. Non-parametric multidimensional scaling ordination of faunal community composition by study site (site-specific species presence/absence and abundance (minimum number alive (Krebs 1966)): (a) Cluster analysis indicates two distinct faunal groups based on forest type, (b) MDS plot by site reflects two distinct faunal groups, verifying group assignments from cluster analysis and similarity matrix. *Evenly dashed lines indicate grouping (indicating greater similarity) between control and disturbed sites in dry forest; the unevenly dashed line indicates an 'outlier' site.

Sex ratio, breeding success, and body mass

Brushtail possum populations living in disturbed forest displayed a male-biased adult sex ratio (68.0% male (34:16 male:female), range: 65.4–80.0%, $n = 50$; $F_{1,4} = 9.47$, $P = 0.037$) (Figure 2.7). For populations in control sites, adult sex ratio did not differ significantly from parity (48.7% male (38:40 male:female), range: 47.1–50.0%, $n = 78$). The sex ratio of offspring did not differ significantly from parity in relation to disturbance or forest type (48.6% male (35:37 male:female), range: 42.9–55.2%, $n = 72$; forest type: $F_{1,4} = 0.03$, $P = 0.861$; disturbance: $F_{1,4} = 0.63$, $P = 0.463$). Bettongs in site DD2 displayed a female biased sex ratio (27.3% male (15:40 male:female), $n = 55$; $\chi^2_1 = 5.68$, $P = 0.017$); populations in other sites do not differ from parity (38.0% male (19:31 male:female), $n = 50$; $\chi^2_1 = 1.44$, $P = 0.230$). No other species displayed sex ratios that differed significantly from parity across any treatment (potoroo: 44.0% male (22:28 male:female), $n = 50$; $\chi^2_1 = 0.36$, $P = 0.549$) and eastern quoll: 43.2% (19:25 male:female), $n = 44$; $\chi^2_1 = 0.41$, $P = 0.522$), though there was site-specific variability within species. There were also no significant differences in any species across any treatment in regard to breeding success: 78.8% of brushtail possums bred (forest type: $F_{1,4} = 0.42$, $P = 0.543$; disturbance: $F_{1,4} = 0.10$, $P = 0.758$), 67.5% of potoroos bred ($\chi^2_1 = 1.72$, $P = 0.190$), 59.7% of bettongs bred ($\chi^2_1 = 1.34$, $P = 0.248$), and 43.5% of eastern quolls bred ($\chi^2_1 = 0.21$, $P = 0.646$) across sites with no significant treatment effects. However, significantly more female bettongs bred than did not in site DD1 (12 versus 1, respectively; $\chi^2_1 = 4.65$, $P = 0.031$). Note that this was the site that was partially burnt in a wildfire in 2006. Frequency of breeding of brushtail possums over the study was significantly higher in control than in disturbed forest (i.e., breeding females in the populations in the control sites bred each year and breeding females (as individuals) in disturbed sites did not breed each year) and females in control sites consequently produced more young over the duration of the study (50 versus 22, respectively; $\chi^2_1 = 5.44$, $P = 0.020$).

In brushtail possums, adult females in wet forest were significantly heavier than those in dry forest (3.3 ± 0.07 kg ($n = 27$) and 2.9 ± 0.05 kg ($n = 29$), respectively; $F_{1,4} = 8.41$, $P = 0.044$). There was no influence of disturbance on adult female weight. No differences were detected in body mass of adult males (mean: 3.4 ± 0.50 kg, range: 2.4–4.9 kg, $n = 80$), subadult males (mean: 2.4 ± 0.52 kg, range: 1.5–3.2 kg, $n = 9$), or subadult females (mean:

2.1 ± 0.17 kg, range: 1.8–2.3 kg, $n = 8$) between disturbance ($F_{1,4} = 0.19$–1.22, $P = 0.380$–0.763) or forest type ($F_{1,4} = 0.34$–3.23, $P = 0.147$–0.656). Body mass of eastern quolls did not vary across sites based on one-way ANOVAs (males: $P = 0.141$–0.345, $n = 19$; females: $P = 0.641$, $n = 25$). Female and male eastern quoll body mass averaged 0.9 ± 0.04 kg and 1.4 ± 0.04 kg, respectively, across sites and ranged from 0.7–1.2 kg ($n = 25$) for females and 1.0–1.9 kg ($n = 19$) for males. In bettongs and potoroos, male body mass did not vary across sites based on one-way ANOVAs (bettongs: $P = 0.889$–0.943, $n = 34$; potoroos: $P = 0.967$–0.985, $n = 22$). Mean body mass of male bettongs was 2.0 ± 0.04 kg, ranging from 1.7–2.6 kg ($n = 34$), whereas that of male potoroos was 1.9 ± 0.07 kg, ranging from 1.4–2.3 kg ($n = 22$).
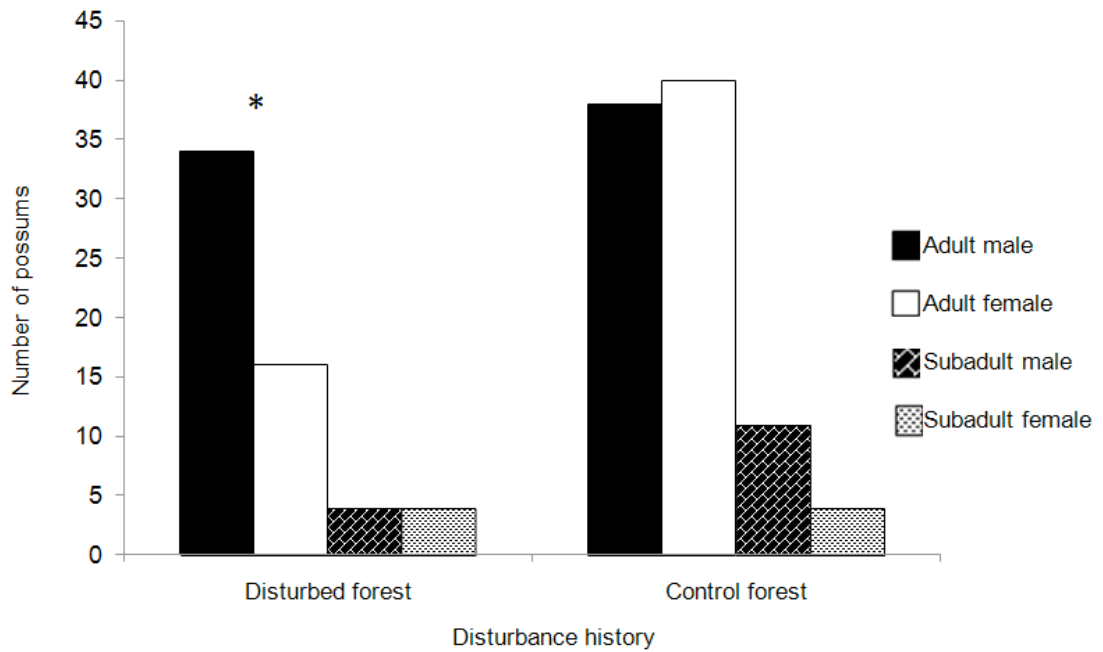
Figure 2.7. Brushtail possum population composition (age class by sex) by disturbance history. Significant deviations from parity in adult sex ratio are indicated by an asterick.

Habitat diversity index, structural complexity, and site ordination

Control sites contained a significantly higher percentage of mature forest (Table 2.2) in the core (mean: 96.7 ± 3.28%, range: 83.6–100.0%) than was seen in disturbed sites (mean: 17.5 ± 10.48%, range: 1.0–58.2%). Disturbed sites contained a significantly high percentage of young forest in the core (mean: 82.1 ± 10.36%, range 41.8–98.4%); control sites contained no young forest in the core (Table 2.4).

Table 2.4. Summary of significant results of habitat variable data. Data were analysed by two-way analysis of variance (factors forest type and disturbance) on site means.

Significant variable	$F_{1,4}$	P	Description
Annual rainfall (mm)	98.81	< 0.001	Higher in wet forest
Mature forest in core site (%)	44.90	0.003	Higher in control forest
Young forest in core site (%)	56.81	0.002	Higher in disturbed forest
Understorey cover (%)*	13.41	0.022	Higher in dry forest
Overstorey cover (%)*	8.25	0.045	Higher in control forest
Basal area (m² ha⁻¹)	7.44	0.048	Higher in control forest
Hollow-bearing trees (average number 0.25 ha⁻¹)*	25.14 / 7.76	0.007 / 0.049	Higher in dry forest / Higher in control forest
Tree hollows (average number 0.25 ha⁻¹)	11.55	0.027	Higher in dry forest

* Habitat variables included in the complexity index

Some habitat variables varied significantly according to either forest type or disturbance history (Table 2.4). This was a reflection of the differences in age and structure of the forest found in particular sites. For example, significantly more hollow-bearing trees were found in dry forest (mean: 21.5 ± 2.32 trees 0.25 ha⁻¹, range: 15–26 trees 0.25 ha⁻¹) than in wet forest (mean: 8.0 ± 3.14 trees 0.25 ha⁻¹, range: 3–16 trees 0.25 ha⁻¹). Similarly, significantly more hollow-bearing trees were found in control sites (both wet and dry) (mean: 18.5 ± 3.50 trees 0.25 ha⁻¹, range: 10–26 trees 0.25 ha⁻¹) than in the disturbed sites (mean: 11.0 ± 4.90 trees 0.25 ha⁻¹, range: 3–23 trees 0.25 ha⁻¹). Also, a higher percentage of understorey cover was

recorded for the dry forest sites, which is a reflection of the well documented differences in floristic structure found between dry and wet forest (Kirkpatrick and Bowman 1982).

Maximum canopy height did not vary significantly by forest type ($F_{1,4} = 0.65$, $P = 0.466$) or disturbance ($F_{1,4} = 0.57$, $P = 0.491$). While the maximum canopy height in both disturbed and control dry forest did not differ significantly (mean: 22.4 ± 3.25 m and 22.6 ± 3.18 m, respectively), the range of heights of the dominant *Eucalyptus* layer were lower in the disturbed than in the control dry forest (range: 4.9–9.6 m versus 12.5–22.5 m). This reflects the dominance of regenerating *Eucalyptus* in the disturbed sites. Similarly, maximum canopy height in wet forest sites were similar, except for the clearfell site WD2 (description in Table 2.1) (32.5–38.8 m versus 8.7 m). Basal area (a proxy for stand density) varied significantly by disturbance history reflecting the 'thinning' effect of harvest; disturbed sites ranged from 9.0–15.5 m^2 ha^{-1} (mean: 9.9 ± 3.62 m^2 ha^{-1}) and control sites ranged from 12.6–34.7 m^2 ha^{-1} (mean: 23.2 ± 4.76 m^2 ha^{-1}).

Although some individual habitat attributes varied (Table 2.4), there was no significant difference in the habitat complexity index between or within sites by forest type (between forest: $F_{1,4} = 1.74$, $P = 0.258$; within forest: $F_{1,4} = 0.15$, $P = 0.722$) or disturbance (between forest: $F_{1,4} = 2.01$, $P = 0.229$; within forest: $F_{1,4} = 0.36$, $P = 0.580$). Habitat complexity indices averaged 47.4 ± 2.81 (range: 44.6–56.1) across sites; within site variance of habitat complexity indices averaged 78.6 ± 15.5 (range: 21.1–95.5) across sites. Plant diversity indices averaged 9.7 ± 0.62 (range: 6.6–12.0) across sites. Species richness averaged 28.2 ± 3.20 (range: 15.0–46.0) and evenness averaged 0.7 ± 0.03 (range: 0.6–0.8) across sites. There were no significant differences for plant species diversity, richness, or evenness between forest type ($F_{1,4} = 3.68$–5.08, $P = 0.069$–0.104) or disturbance ($F_{1,4} = 0.50$–2.95, $P = 0.161$–0.505).

Research sites were distinctly grouped by forest type, as indicated by the ordination plot (Figure 2.8). Furthermore, in wet forest the sites are separated in the ordination plot according to disturbance: habitat (floristics and structure) in disturbed sites (WD1 and WD2) were more similar to each other than to the control sites (WC1 and WC2). There was

no such relationship in the dry forest. The low stress level (0.02) indicates that this interpretation is reliable (Clarke 1993).
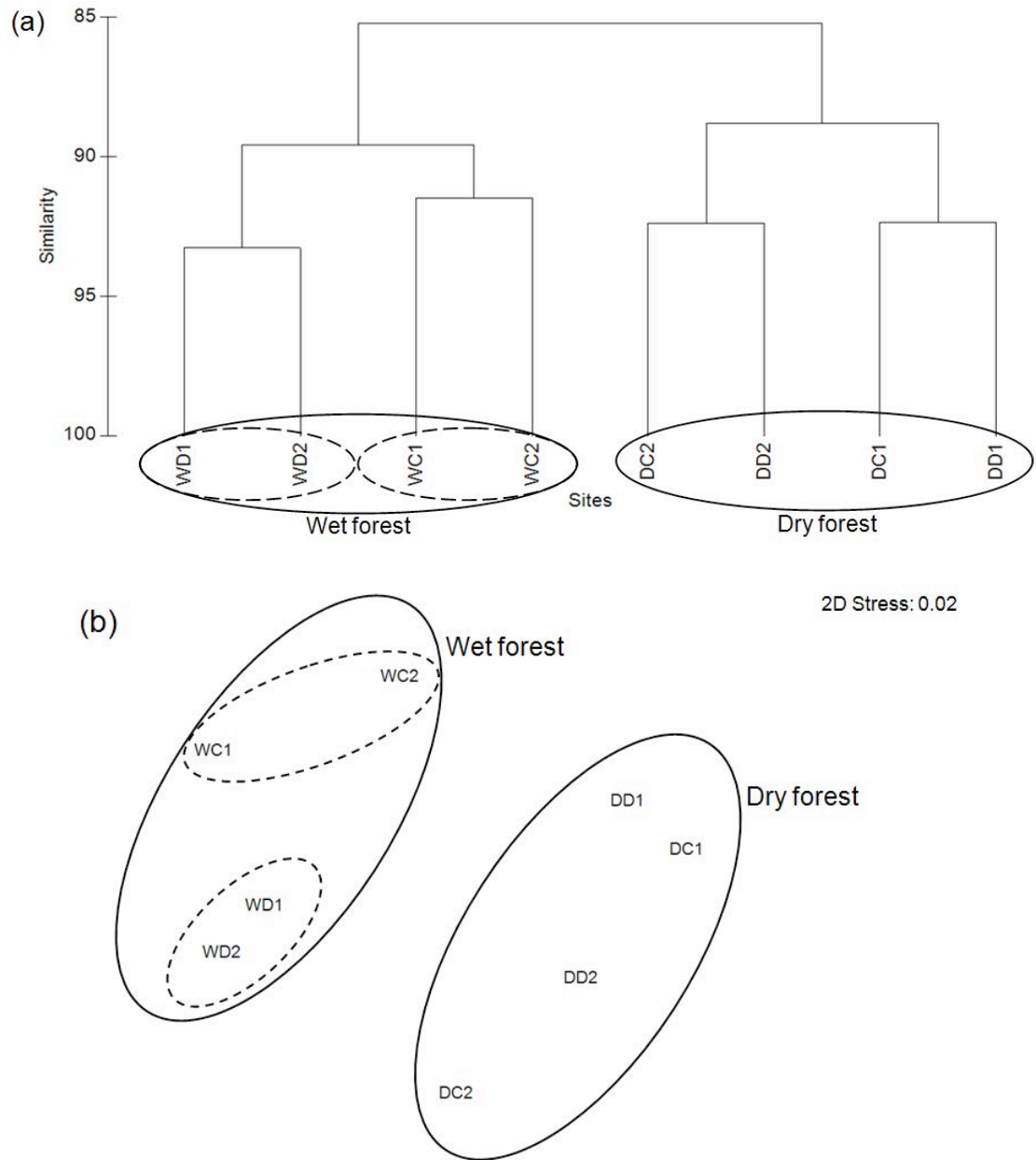
Figure 2.8. Non-parametric multidimensional scaling ordination of habitat by study site (site-specific species presence/absence, diversity indices, and structure indices): (a) Cluster analysis indicates that habitat varies distinctly between forest type, (b) MDS plot by site reflects two distinct groups of habitat, verifying group assignments from cluster analysis and similarity matrix. *Evenly dashed lines indicate grouping (indicating greater similarity) between control and disturbed sites in wet forest.

Discussion

This study addresses how the characteristics of small-medium mammal communities may vary in relation to forest type and disturbance history. Despite the increasing global pressure on forest habitats, such studies remain scarce. In particular, there are few studies focussing on medium-sized ground-dwelling mammals. Despite the low spatial and temporal replication, this study provides some insight into the response of mammal communities 4–11 years following harvest and the contribution of the matrix to recovery. Although core habitat was altered during harvest, significant alteration in the mammal community did not occur at a minimum of 4 years post harvest. We suggest that differences in resource availability between the core sites were muted by the availability of habitat in the surrounding forest (matrix), which buffered the impacts of harvest on the mammalian fauna (see Brockerhoff *et al.* 2008). This supports current ideas that where the matrix provides suitable or even partially suitable habitat, a species will be significantly less affected by habitat alteration at a site scale than where the matrix is lacking in habitat value (Gascon *et al.* 1999; Lindenmayer and Franklin 2002; Brockerhoff *et al.* 2008). A fruitful avenue of future research would be to examine the mammalian community response in disturbed sites within a continuum of degree of matrix disturbance, ranging from a highly altered and cleared landscape (e.g., agriculture) to an unaltered, natural landscape. It is also important to consider the size and connectivity of patches of mature or undisturbed forest in the landscape, as small patches may not support genetically viable populations in the long-term. Patchy spatial arrangement of resources may disrupt gene flow between populations (via restricted immigration) and alter genetic population structure (through genetic drift and inbreeding), particularly if populations become isolated (Lacy 1997; Gibbs 2001).

Mammal community composition, although not diversity or richness, varied between forest type, reflecting the ecologically distinct nature of wet and dry *Eucalyptus* forests (Kirkpatrick and Bowman 1982) (as illustrated in Figure 2.8). The difference in community composition cannot be explained by differences in species ranges, given that all are widespread throughout Tasmania. Species found in each broad forest type were those expected from previous studies (Hocking and Guiler 1983; Rose 1986; Rounsevell *et al.* 1991; Taylor 1993; Johnson and McIlwee 1997; Jones and Barmuta 2000; Jones and Rose 2001), with the exception of potoroos, for which the preferred habitat is generally wet,

dense vegetation (Kershaw 1971; Bennett 1993; Johnson 1995; Claridge *et al.* 1999). In this study, both bettongs and potoroos were found exclusively in dry forest and brown rats were found exclusively in wet forest. Although brushtail possums were the only species captured at all sites, pademelons, eastern quolls, spotted-tailed quolls, southern brown bandicoots, echidnas, long-tailed mice, swamp rats, black rats, and house mice were found in both forest types.

We found no evidence of a significant effect of logging 4–11 years previously at a site scale on the small-medium mammal community in either dry or wet *Eucalyptus* forest. This is concordant with previous studies, which suggest that ground-dwelling mammal populations initially decrease after logging (via a habitat shift or emigration) and recolonise relatively quickly (Cork and Catling 1996; Ferron *et al.* 1998; Moses and Boutin 2001). The response of individual species to disturbance, however, depends on the intensity and degree to which the habitat (refuge, nesting, and foraging areas) on which they rely is altered (Kavanagh and Stanton 2005). Mammals dependent on the forest canopy (including arboreal marsupials) typically experience much slower population recovery as they need slow-forming habitat components such as elements of mature forest and tree hollows (Tyndale-Biscoe and Smith 1969; Tyndale-Biscoe and Calaby 1975; Laurance 1996; Cork and Catling 1996; Alexander *et al.* 2002). Although there were some differences in our study in individual habitat variables (e.g., canopy cover and number of hollow-bearing trees that would affect brushtail possums), overall habitat complexity relevant to the small-medium ground-dwelling mammals (e.g., log abundance, basal area, and percentage of rock cover) in this study did not differ sufficiently between disturbance or forest type to elicit a population response. The higher abundance of some species observed in this study in disturbed areas is most likely attributable to changes in food availability/foraging habitat resulting from disturbance rather than changes to refuge habitat. An increase in the number of individuals in an area following disturbance from harvesting and wildfire has also been found in other studies (Lambin 1994; Evans 2001; Kavanagh and Stanton 2005) and have been attributed to increased food availability, such as insects, fungi, and highly nutritious *Eucalyptus* regrowth (Hocking 1981; Driessen *et al.* 1991; Catling and Burt 1995; Vernes and Haydon 2001).

The only species for which abundance was significantly lower in disturbed sites was the brushtail possum. When food supply is adequate, structural components of the habitat interact to determine habitat quality for arboreal marsupials (Pausas *et al.* 1995; Cork and Catling 1996). Tree hollows are of particular significance because they develop slowly, taking 140 years in Tasmania (Koch *et al.* 2008a), and are in high demand by many vertebrate species (42 in Tasmania) (Koch *et al.* 2008b). In this study, significantly fewer hollow-bearing trees were present in wet compared with dry forest and in disturbed compared with control forest. This could limit the number of possums, particularly breeding females, that the habitat will support (Koch *et al.* 2008b), resulting in smaller populations. A recent study of the use of hollow-bearing trees by fauna in two of the sites (DD1 and DC1) found that brushtail possums, particularly adult females, preferred to den in hollows found in intact mature forest areas surrounding the logged area rather than hollows in trees retained within the harvested area (Cawthen, 2007). However, brushtail possums began to use retained tree hollows 10–17 years post harvest, when forest structure regenerated around them (Cawthen 2007; Cawthen *et al.* unpublished data).

Disturbance is known to influence foraging and denning behaviour by animals in a landscape context (Johns 1986; Thiollay 1992). A change in such behaviours may be why mammal community composition did not change with disturbance and mammal species were able to persist after disturbance in this study. For example, using radiotracking Cawthen (2007) found that possums foraged and denned throughout the mature forest of the core and matrix of the control forest. However, in the disturbed site 95% of dens were located in the mature forest matrix surrounding the site, despite the availability of apparently suitable hollow-bearing trees, albeit without the context of surrounding habitat structure, within the core. Extensive trapping of these individuals throughout the core site (E. Flynn pers. obs.) demonstrated that brushtail possums were still using this habitat. While brushtail possums were no longer denning in the core of the harvested sites, they continued to forage there. A similar pattern of habitat use has been reported in bettongs; animals moved long distances to nest in unburned habitat bordering burned areas, yet returned to the burned area to forage at night due to increased fungi abundance in response to fire (Vernes and Haydon 2001).

In mammals, female abundance is commonly resource-limited while males are female-limited (Clutton-Brock and Harvey 1978). In the brushtail possums in this study, more individual females bred (producing more offspring) in control than in disturbed forest, and sex ratios were male-biased in disturbed forest. This suggests that the disturbed areas were deficient in a necessary habitat requirement for breeding females. Disrupted sex ratios in mammals are reported in the literature as a response to forest disturbance, most likely resulting from intraspecific competition in relation to resource availability (Clark 1978; Silk 1983; Cockburn *et al.* 1985; Martin *et al.* 2007). Brushtail possum social organisation is matriarchal (Jolly and Spurr 1996), and females require and claim the best dens for rearing young (Gibbons and Lindenmayer 2002). Males are able to utilise more marginal den sites (Cawthen 2007), as is evident by the range of structures that male brushtail possums are reported to exploit (Green 1982; Munks *et al.* 2004; Cawthen 2007). This study suggests that hollow-bearing trees are the resource motivating the population-level response observed in the brushtail possums in this study. We believe the availability of hollow-bearing trees is an important factor influencing brushtail possum population size and demographics and is worth further examination. The opposite effect may be seen in the female-biased sex ratio observed in bettongs in site DD2, attributable to a resource in high abundance (i.e., food abundance, particularly fungi which comprise > 80% of their diet (Johnson 1994b)). Bettong abundance is dependent on vegetation patterns (open forest and sparse ground vegetation), thought to be related to abundance of hypogeous mycorrhizal fungi (Johnson 1994a). Female bettongs eat significantly more (1–28%) fungi than males and are able increase their metabolism in response, providing them with a surplus of energy for reproduction that also accelerates growth of pouch young (Johnson 1994b).

This study demonstrates that for the small-medium mammals in Tasmania, species occurrence, and thus community composition, is driven primarily by forest type. Abundance of individual species within broad forest type may be influenced by changes to the physical structure of vegetation resulting from disturbance and associated changes to food and refuge availability. The anticipated difference in community composition as a result of the harvesting was not evident 4–11 years post harvest. This may be because foraging habitat, while altered, was not lost, and because the surrounding forest matrix provided the required

refuge habitat for denning and daytime shelter. Landscape scale habitat availability was deemed equivalent across sites and appeared to mitigate the effects of harvest in the disturbed areas. These results emphasise the importance of retaining networks of mature habitat around harvested sites in production forest landscapes in order to maintain habitat and ensure the presence in, and eventual recolonisation of, the area by breeding animals.

Acknowledgments

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Appendices

Appendix A. Silvicultural treatments encountered in this study (Wilkinson 1994)

[Note: several treatments tend to be applied when harvesting dry *Eucalyptus* forest coupes.]

(a) Advanced growth retention (AGR)

PRE-HARVEST ————————————————————— POST-HARVEST

ADVANCE GROWTH RETENTION   Applied to uneven-aged forests that contain groups of advance growth suitable for retention. All mature trees are harvested other than young stems that have potential for further value increment. Seedbed can be prepared in unstocked gaps by top disposal burning or mechanical disturbance. Retained trees may provide some natural seeding, although large unstocked gaps without a natural seed source may need to be sown.

Basal area reduced to: $< 4 \text{ m}^2 \text{ ha}^{-1}$

(b) Clearfell, burn, and sow (CBS)

PRE-HARVEST ————————————————————— POST-HARVEST

CLEARFELL Applied to forests that have a dense understorey and to other forests that contain little growing stock suitable for retention. All live trees (>5m tall) are felled.   A seedbed must be prepared by burning or by mechanical disturbance. Re-stocking can occur from sowing, natural seeding, release of advance growth (< 5m in height) or planting.
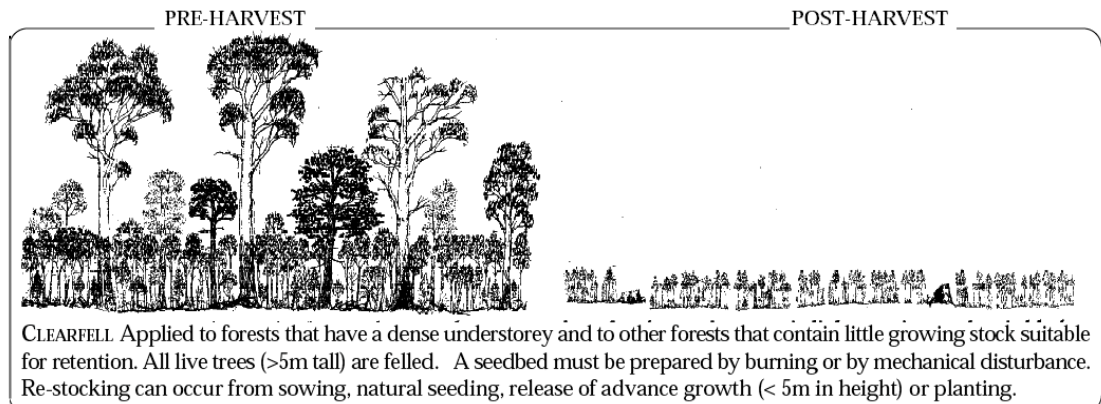
CLEARFELL WITH RETENTION   As above , but where some mature trees (up to 4m² of basal area or about 5-10 trees per ha) are retained because of habitat, lack of markets or other reasons.

(c) Seedtree retention (SED)

SEEDTREE RETENTION   Applied to open forests where a continuing seed source will maximise regeneration opportunities on harsh sites or where the retention of seedtrees is more cost-effective than sowing. Seven to 12 well-spaced trees per ha are retained and receptive seedbed must be prepared by burning or mechanical disturbance. Seedtrees should be removed once the regeneration is established.

(d) Shelterwood (SR)

SHELTERWOOD   Applied to high altitude (>600m) open forests on cold sites which contain little advance growth. Trees with good crowns are retained at 12 - 14 m^2 of basal area on wet sites and 9 - 12 m^2 on dry sites. Seedbed is created by low intensity broadcast or top disposal burns, or mechanical disturbance. Re-stocking can occur from natural seeding, sowing, or release of advance growth. The overstorey should be removed when the regeneration is greater than 1.5m mean height.

(e) Commercial thinning
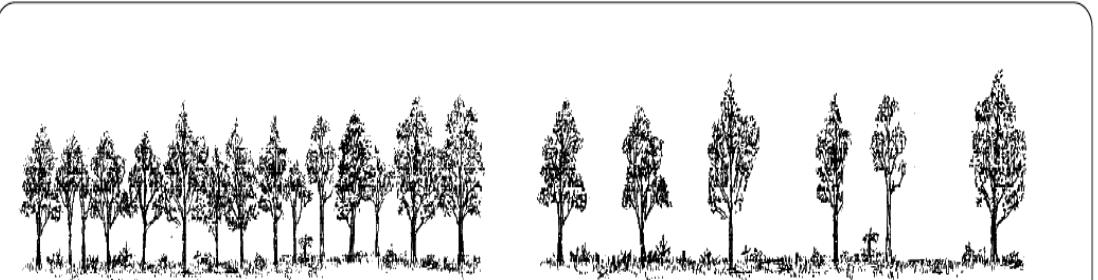
THINNING   Applied to well stocked even-aged regrowth on productive sites. Stocking is reduced to at least 200 well-formed vigorous trees per ha. No further treatment is generally necessary, resulting in the continuation of an even-aged forest.

Appendix B. Photographs from the centre of each core site illustrate broad forest structure and type

(a) Dry *Eucalyptus* forest disturbed sites
(i) DD1

(ii) DD2

(b) Dry *Eucalyptus* forest control sites
(i) DC1

(ii) DC2

(c) Wet *Eucalyptus* forest disturbed sites
(i) WD1

(ii) WD2

(d) Wet *Eucalyptus* forest control sites
(i) WC1

(ii) WC2

Appendix C. Aerial photographs showing trap placement (Google Earth 5 (2009)) of the study sites illustrate broad forest structure and type

(a) Dry *Eucalyptus* forest disturbed sites
(i) DD1

(ii) DD2

(b) Dry *Eucalyptus* forest control sites
(i) DC1

(ii) DC2

(c) Wet *Eucalyptus* forest disturbed sites
(i) WD1

(ii) WD2

(d) Wet *Eucalyptus* forest control sites
(i) WC1

(ii) WC2

Appendix D. Study site locations

Table 1. Grid coordinates and global location of study sites

Site	Coupe name	State forest	Grid coordinates (GDA) Top left corner		Bottom right corner		Longitude	Latitude
DD1	MM002A & MM004A	Mt. Morrison	5,267,000N	561,000E	5,265,000N	564,000E	42°45'31.07"S	147°45'53.78"E
DD2	SW059A & SW059B	Swanport	5,303,000N	551,000E	5,299,000N	554,000E	42°26'14.64"S	147°38'00.13"E
DC1	"Backwoods" reserve	Mt. Morrison	5,275,000N	550,000E	5,272,000N	553,000E	42°41'32.12"S	147°38'06.77"E
DC2	SW051A	Swanport	5,298,000N	552,000E	5,296,000N	553,000E	42°28'30.85."S	147°38'00.28."E
WD1	GC029B	Goulds Country	5,442,000N	589,000E	5,439,000N	591,000E	41°10'56.34"S	148°04'22.86"E
WD2	GC104B	Goulds Country	5,446,000N	589,000E	5,444,00 N	591,000E	41°08'43.18"S	148°04'09.27"E
WC1	GC145B	Goulds Country	5,434,000N	585,000E	5,432,000N	587,000E	41°14'45.53"S	148°01'32.50"E
WC2	GC139B	Goulds Country	5,437,000N	581,000E	5,434,00 N	584,000E	41°13'46.43"S	147°58'53.01"E

Appendix E. Bycatch details

Table 1. Non-mammal incidental bycatch (released after species identification without further processing) trapped in core sites during the study

Species		Number of sites in each treatment animals were trapped in			
		Dry disturbed [1] (2)	Dry control [2] (2)	Wet disturbed [3] (2)	Wet control [4] (2)
BIRDS					
Currawong	*(Strepera graculina)*	1	2	0	1
Forest raven	*(Corvus tasmanicus)*	2	0	1	0
Grey shrike-thrush	*(Colluricincla harmonica)*	0	1	0	0
Scrubtit	*(Acanthornis magnus)*	0	1	0	1
Superb blue wren	*(Malurus cyaneus)*	0	0	1	0
REPTILES					
Black tiger snake	*(Notechis scutatus)*	1	0	1	0
Blotched blue-tongue lizard	(*Tiliqua nigrolutea*)	0	0	2	0

[1] Sites: DD1 and DD2
[2] Sites: DC1 and DC2
[3] Sites: WD1 and WD2
[4] Sites: WC1 and WC2

Chapter 3

A physiological approach to assessing the impacts of habitat disturbance on an arboreal marsupial, the brushtail possum (*Trichosurus vulpecula*)

Citation: Flynn, E.M. and Jones, S.M. (in prep). A physiological approach to assessing the impacts of habitat disturbance on an arboreal marsupial, the brushtail possum (*Trichosurus vulpecula*). *Zoology.*

Photo by Lisa Cawthen

Photo by Laura Flynn

Top left: lateral ear vein of a brushtail possum (*Trichosurus vulpecula*)
Top right: collecting blood from the lateral ear vein
Bottom: making a blood smear

Abstract

Monitoring parameters of physiological status in individuals can be a powerful tool with which to assess population 'health' and capacity to respond to environmental challenges. This study used physiological indicators to assess potential impacts of habitat disturbance on common brushtail possums, *Trichosurus vulpecula*, living in wet or dry *Eucalyptus* forest. Our hypothesis was that animals in disturbed habitats would show significant changes in relevant physiological parameters. Trapping was conducted twice annually 2006–2008 at 10 study sites in Tasmania, the island state of Australia. Control sites (three dry and two wet forest) were unlogged, and disturbed sites (three dry and two wet forest) were 4–11 years post harvest. Upon capture, animals were sexed, weighed, and microchipped: blood samples were analyzed for plasma cortisol concentration, white blood cell (WBC) differential, and hematocrit. There was no significant variation in plasma cortisol concentration between forest type, disturbance, or season when life history stage and temporal factors were taken into account. WBC profiles of males inhabiting dry forest had significantly fewer monocytes, neutrophils, eosinophils, and total WBC count than those inhabiting wet forest; however these animals were healthy and their WBC profiles did not reflect any known pathology. Hematocrit values in females were significantly higher in autumn/winter than in spring/summer, indicating that the greatest constraint on body condition was the metabolic cost of female reproduction. Our results suggest that individual brushtail possums are physiologically resilient, and that animals inhabiting both forest types are able to cope with habitat disturbance. Brushtail possums provide a physiological profile of a species that is able to exploit new habitats due to resilience at the individual level.

Introduction

The overarching goal of conservation management is to preserve biological diversity through the maintenance of ecosystem integrity and both genetic, and species diversity (Clemmons and Buchholz, 1997; Soulé, 1985). Conservation practices are often informed by ecological studies that focus on the population within the landscape as the 'conservation unit' (Opdam and Wascher, 2004). However, there is increasing awareness that individuals within a population may respond differently to environmental pressures. Such variation among individuals may directly influence key demographic processes such as survival and

reproduction that determine population parameters and therefore drive the probability of local extinction (Beissinger, 1997).

Physiological monitoring is a valuable tool that is increasingly used to monitor 'health' and assess the ability of an individual or population to respond to habitat disturbance (Barnes et al., 2008; Creel et al., 2002; Homan et al., 2003; Romero, 2002; Wasser et al., 1997; Wikelski and Cooke, 2006). An individual's physiological profile dictates fitness through impacts on survival (Carey, 2005) and fecundity (John-Alder et al., 2009), so physiological regulation underpins an animal's ability to adapt to environmental challenges (Adolf, 1956). Habitat disturbance constitutes an environmental perturbation, and therefore represents a potential stressor. The hypothalamo-pituitary-adrenal (HPA) axis allows animals to respond to unpredictable environmental, social, or physical challenges (Reeder and Kramer, 2005). When an animal encounters a stressor, homeostasis is disrupted and the HPA axis is activated, triggering a rapid endocrine cascade that ultimately results in production of glucocorticoids (e.g., cortisol or corticosterone) from the adrenal glands (Norris, 1997; Wielebnowski, 2003; Wingfield et al., 1997). Thus in most mammals, plasma cortisol concentrations are a direct measure of adrenal responsiveness (Romero, 2004). The degree of this response is influenced by both the intensity and the duration of the stressor (Busch and Hayward, 2009; McKenzie et al., 2004; Romero, 2004; Wells et al., 2000).

After an acute stress response, physiological parameters rapidly return to normal levels, (Guimont and Wynne-Edwards, 2006). However, chronic activation of the HPA axis can have significant consequences, including immune system suppression, mass protein loss from skeletal muscle, neuronal cell death, complete inhibition of the reproductive system, and suppression of both growth and metamorphosis (Buddle et al., 1992; Davis and Maerz, 2008; Griffin, 1989; McKenzie et al., 2004; Presidente and Correa, 1981; Wingfield et al., 1997). For example, chronically stressed common brushtail possums (*Trichosurus vulpecula)* display aggression, rough pelage, diarrhea, emaciation, dehydration, high hematocrit values, infection, leukocytosis (low WBC count), high cortisol concentration, depression, low glucose concentrations, gastric ulceration and hemorrhage, adrenocorticol hyperplasia, and death (Begg et al., 2004; Presidente, 1978; Presidente and Correa, 1981). In a classic case study of the dusky antechinus (*Antechinus swainsonii*), a small carnivorous

marsupial, glucocorticoids have been implicated in the annual post-mating male mortality (McDonald et al., 1986): plasma corticosteroid concentrations increase, while corticosteroid binding globulin decreases dramatically, resulting in immunosuppression and death.

Generalized immune response and body condition are secondary indicators of health. Adrenal and leukocyte responses are closely related and highly conserved across vertebrates (Davis et al., 2008). The relative proportions of different types of white blood cells (WBCs) to each other may correlate with a specific pathology and can therefore assist in diagnoses such as immune deficiency, cancer, high parasite load, or viral versus bacterial infection (Jain, 1986). Specific WBC profiles also reflect chronic 'stress' (significantly low numbers of lymphocytes and eosinophils and concurrent high numbers of neutrophils (Buddle et al., 1992; Jain, 1986; Presidente, 1978)) and acute or capture 'stress' (high neutrophil to lymphocyte ratio (Baker et al., 1998; Buddle et al., 1992; Davis and Maerz, 2008; Presidente and Correa, 1981)).

Hematocrit is a measure of the percentage of red blood cells (RBCs) in the blood. Its strong relationship with nutritional status and muscle mass makes it an effective indicator of body condition (Brown, 1996; Horak, et al. 1998; Sanchez-Guzman et al., 2004). Hematocrit is also routinely used to diagnose dehydration and anemia (Dawson and Borolotti, 1997; LeResche et al., 1974) and indicate illness, such as infection by a blood parasite (Dawson and Borolotti, 1997). Physiologists therefore have at their disposal a suite of techniques with which to assess the potential impacts of environmental stressors upon individual animals.

Tyndale-Biscoe and Calaby (1975) and Goldingay and Daly (1997) identify arboreal marsupials as the Australian mammals most susceptible to anthropogenic habitat disturbance through a reduction in availability of tree hollows necessary for breeding (Gibbons and Lindenmayer, 2002). The brushtail possum (*Trichosurus vulpecula*), is a nocturnal, arboreal marsupial omnivore, capable of exploiting a large array of habitats (Bulinski and McArthur, 1999; Cowan, 1990; Hocking, 1981; Kerle, 1984; Statham, 1984) and food types (Cowan and Moeed, 1987; Fitzgerald, 1984; Nugent et al., 2000; Sadlier, 2000). It is common and widespread, with the largest distribution of any Australian marsupial (Cowan, 1990). In Australia, its basic physiology, anatomy, and

reproductive biology have been extensively studied from a fundamental perspective, while in New Zealand, where it is an introduced species, there is a strong emphasis on understanding its ecology as a basis for management (Clout and Sarre, 1997; Cowan and Tyndale-Biscoe, 1997). In Tasmania, the island state of Australia, the species is considered to be an agricultural, forestry, and urban pest (Bulinski and McArthur, 1999; Clout and Sarre, 1997; Cowan and Tyndale-Biscoe, 1997; Kerle, 2001; McArthur et al., 2000). Despite this, the brushtail possum is of particular conservation interest as it is part of the tree hollow-using fauna, and is thus identified as a priority species under the Tasmanian *Regional Forest Agreement* (Commonwealth of Australia and State of Tasmania, 1997). The species' responses to habitat disturbance are therefore of interest. The brushtail possum is widely considered to be resilient to human disturbance. It is able to adapt to, and exploit, plantations (Bulinski and McArthur, 1999) and urban areas (Statham and Statham, 1997) and has been reported using a range of structures as makeshift dens (Cawthen, 2007; Green, 1982; Munks et al., 2004). The responses of individual brushtail possums to habitat disturbance have not, however, been specifically explored.

This study explores the premise that individual resilience in species like the brushtail possum may underpin population-level responses to environmental challenge. It complements recent population-level studies of brushtail possums' responses to habitat disturbance. Flynn et al. (2011) [Chapter 2] assessed population-level parameters in brushtail possums living in control (unlogged) and disturbed (logged) *Eucalyptus* forests. They demonstrated that there was a negative influence of habitat disturbance on brushtail possum abundance, with significantly fewer brushtail possums found in disturbed sites. Populations living in disturbed sites displayed a male-biased adult sex ratio. Breeding success was not influenced by forest type or disturbance, but breeding frequency over the duration of the study was lower in females living in disturbed forest than those in control forest. These population-level responses may reflect variation in individual physiology. This study aims to assess the potential impacts of habitat disturbance on brushtail possums living in wet or dry *Eucalyptus* forest via measuring individual-level physiological parameters of well-being. We focus on the following attributes: plasma cortisol concentration (a proxy for chronic adrenal responsiveness or

HPA axis activation); WBC differential (a proxy for generalized immune response); and hematocrit (a proxy for body condition). We hypothesize that brushtail possums living in disturbed habitat (of either forest type) will exhibit significant changes in these physiological indicators of well-being compared with animals living in undisturbed, control, habitat.

Materials and methods

Study sites

This study was carried out at six dry Eucalyptus forest sites in southeast Tasmania and four wet Eucalyptus forest sites in northeast Tasmania (Fig. 3.1). Site selection was constrained by the needs for access and to ensure that harvested sites had similar pre-harvest conditions (soil type, elevation, and floristics) to the relatively undisturbed (control) sites (Table 3.1). A photograph taken in the center of each site and an aerial photograph of each site are provided in Appendix A and Appendix B, respectively. Specific location details of each site are included in Appendix C. As described in detail in Flynn et al. (2011) [Chapter 2], half of the sites in each forest type were subject to harvest (= disturbed sites) 4–11 years ago, and half (= control sites) remained in their natural state. All disturbed sites were logging coupes in State Forest that had been harvested using the silvicultural method appropriate for the forest type: harvest in wet forest included commercial thinning and clearfell, burn, and sow, while harvest in dry forest included combinations of seedtree retention, advanced growth retention, and shelterwood retention (Wilkinson, 1994) (Table 3.1). Control sites remained subject to natural disturbance (wildfire and storms). All study sites were embedded within a surrounding matrix of mature or older-aged regenerating forest.
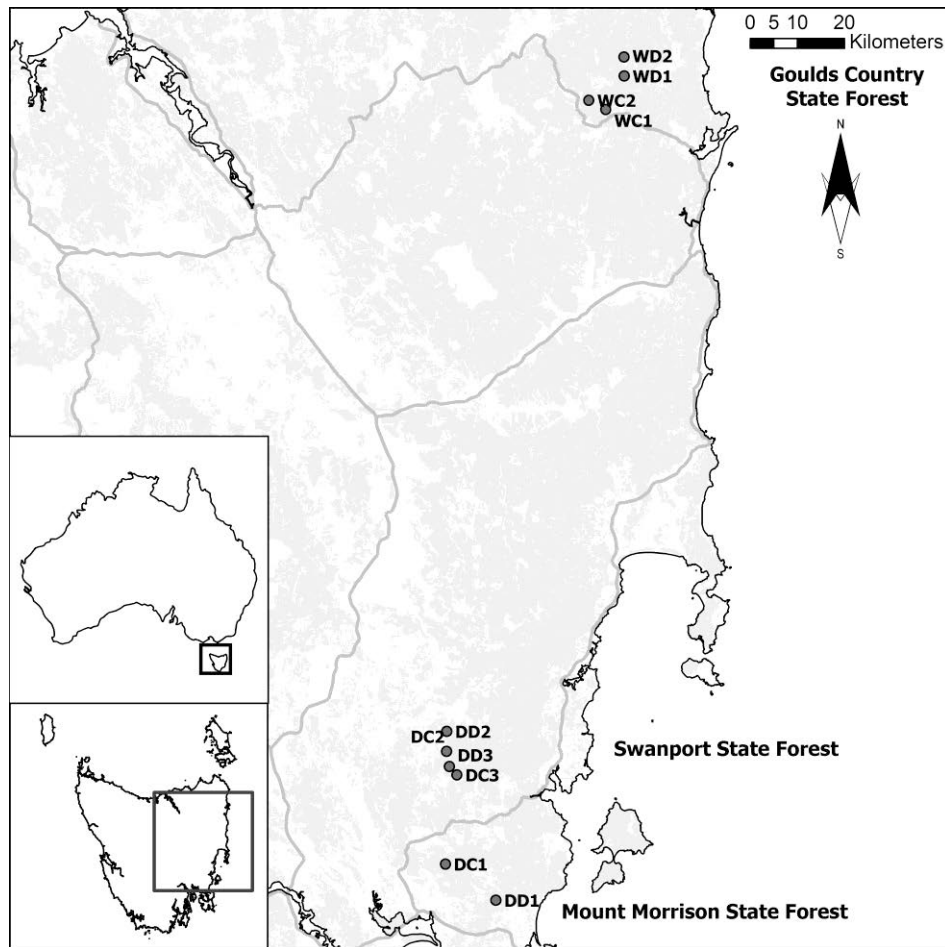
Fig. 3.1. Location of study sites across eastern Tasmania, the island state of Australia. Dry *Eucalyptus* forest sites are in the southeast and wet *Eucalyptus* forest sites are in the northeast of the state. Shading indicates forested area, while lack of shading denotes non-forest area (agriculture, scrub, lakes, urban area, etc.). Solid lines indicate major roads (DPIW, 2001; Harris and Kitchener, 2005). (Treatment (forest type/disturbance) is indicated by the first two letters of a site name, followed by the site number: 'DD' = dry disturbed, 'DC' = dry control, 'WD' = wet disturbed, and 'WC' = wet control forest.)

Table 3.1. Description of study sites including forest type classification and both method and date of harvest of disturbed sites

Site name	Coupe name	Core area (ha)[1]	Broad forest type[2]	Silvicultural method	Harvest date (yr)[8]	Land tenure[9]	Dominant geology	Elevation (m)	Annual rainfall (mm)
DD1	MM002A & MM004A	62.30	E. pulchella - E. globulus - E. viminalis grassy shrubby forest [Dry]	Partial harvest (SED, AGR)[3]	2000/ 2004	State	Dolerite	354	651.1
DD2	SW059A & SW059B	34.24	E. obliqua forest [Dry]	Partial harvest (SR, AGR)[4]	1998/ 1999	State	Sandstone	398	478.1
DD3	SW049A	51.90	E. amygdalina forest on sandstone [Dry]	Partial harvest (SED, SR)[5]	1997	State	Sandstone	337	423.8
DC1	Reserve	58.72	E. obliqua forest [Dry]	N/A	N/A	Formal reserve	Dolerite	333	483.1
DC2	SW051A	26.58	E. obliqua forest [Dry]	N/A	N/A	Informal reserve	Sandstone	385	478.1
DC3	Reserve	37.32	E. amygdalina forest on sandstone [Dry]	N/A	N/A	Informal reserve	Sandstone	301	423.8
WD1	GC029B	21.49	E. regnans forest [Wet]	Commercial thinning[6]	2002	State	Adamellite	231	884.4
WD2	GC104B	24.54	E. regnans forest [Wet]	Clearfell, burn, and sow (CBS)[7]	2002	State	Adamellite	184	884.4
WC1	GC145B	22.93	E. regnans forest [Wet]	N/A	N/A	State	Adamellite	206	1190.6
WC2	GC139B	36.68	E. regnans forest [Wet]	N/A	N/A	State	Adamellite	529	1095.3

[1] Core area (approximately equivalent to harvested area in disturbed sites) was the physical location of all traps within the research site (logging coupe or reserve as appropriate) plus a 100 m buffer

[2] Broad forest community (DPIW, 2001; Harris and Kitchener, 2005)

[3] Mosaic of seedtree retention (SED) (reduced to 7–12 trees/ha) and advanced growth retention (AGR) (basal area reduced to < 4 m^2/ha) followed by a low intensity burn for regeneration (Wilkinson, 1994)

[4] Mosaic of shelterwood retention (SR) (basal are reduced to 12–14 m^2 in wet sites and 9–12 m^2 in dry sites) and advanced growth retention (AGR) (basal area reduced to < 4 m^2/ha) followed by a low intensity burn for regeneration (Wilkinson, 1994)

[5] Mosaic of seedtree retention (SED) (reduced to 7–12 trees/ha) and shelterwood retention (SR) followed by a low intensity burn for regeneration (Wilkinson, 1994)

[6] Stocking reduced to at least 200 well-formed trees/ha; no regeneration burn (Wilkinson, 1994)

[7] All live trees (> 5 m) are felled, followed by a regeneration burn and seed sowing (Wilkinson, 1994)

[8] From Timber Harvesting Plan for the coupe (Forestry Tasmania, unpublished data)

[9] CAR reserves GIS data layer (DPIW, 2007)

Brushtail possum sampling (trapping regime, animal processing, and blood collection)

Study sites were trapped twice yearly (once during spring/summer and once during autumn/winter) from spring/summer 2006–2008. In the brushtail possum, autumn/winter corresponds to breeding and early pouch life, and spring/summer corresponds to late pouch life and independence of the young of the year (Hocking, 1981). As described in Flynn et al. (2011) [Chapter 2], hessian-covered 300 x 300 x 660 mm wire Mascot cage traps were baited with peanut butter/oat/vanilla essence and ¼ apple, and deployed in each site for an average spatial trapping effort of 1.77 traps ha^{-1} (range: 1.08–2.73 traps ha^{-1}). Trips consisted of 4 nights: 1 night of prebaiting and 3 nights of trapping. Checking traps and processing animals began at dawn to minimize stress in captured animals (Johnson and McIlwee, 1997). Capture and handling protocols were conducted with adherence to the *Australian code of practice for the care and use of animals for scientific purposes* (Australian Government National Health and Medical Research Council, 2004) and were approved by the University of Tasmania Animal Ethics Committee (approval A0009118) and the Department of Primary Industries and Water, Parks and Wildlife (permits: FA 06558 (2006–2007), FA 07202 and TFA 07227 (2007–2008), and FA 09209 (2008–2009)). All juvenile, subadult, and adult brushtail possums were sexed, tagged with a subcutaneous ISO FDX-B transponder chip (passive integrated transponder (PIT) tag), and weighed to the nearest 50 g. Left testis length and width (excluding the epididymus) were measured with vernier calipers to the nearest millimeter. At the time of processing, general observations were made of the condition of each brushtail possum: animals were examined for signs of diarrhea, emaciation, dehydration, rough pelage, aggression, lethargy, external parasite load, or obvious problems with range of motion or ability to move.

Field protocols were standardized to ensure that all animals received an equivalent experience. In free-living mammals, capture results in rapid activation of the HPA axis, impacting baseline glucocorticoid concentrations (Boonstra et al., 2001; Ortiz and Worthy, 2000). However Fletcher and Boonstra (2006) assert that the impact of trapping on the HPA axis may be considered a constant bias within such studies. Meadow voles (*Microtus pennsylvanicus*) held in live-traps up to 16.5 hr displayed a trap-induced increase in baseline cortisol concentration that was not impacted by the duration of time spent in the trap

(Fletcher and Boonstra, 2006). Meadow voles remained able to mount an acute stress response after trap confinement, displaying further increase in plasma cortisol concentrations when subject to handling or restraint (Fletcher and Boonstra, 2006). In addition, the impact of trapping on the HPA axis does not obscure natural patterns of seasonal variation in glucocorticoids (Kenagy and Place, 2000; Place and Kenagy, 2000) nor the effects of experimental treatments on plasma glucocorticoid concentrations (Boonstra and Singleton, 1993; Hik et al., 2001). In contrast to glucocorticoids, which respond to a stressor within minutes, leukocytes (i.e., WBC parameters) react slowly, taking hours to days to respond (Davis et al., 2008). Hematocrit, particularly in the brushtail possum, is very responsive to stress (Dawson and Denny, 1968); values of > 50% in the brushtail possum are indicative of capture stress (Parsons et al., 1971), allowing us to assess the response of captured animals to our trapping regime and sampling protocol. In this study, blood samples were taken from adult animals once only, at first capture. All blood samples were taken within 3 min of first contact with the animals. Any samples that took longer than 3 min to obtain were discarded, and that animal was sampled at the next capture. 100 μL of blood was collected from the lateral ear vein using heparinized microhematocrit capillary tubes, directed into an Eppendorf tube, and kept on ice. Upon return to field camp, the blood was centrifuged and the plasma was separated off and frozen at -20°C until assay.

Brushtail possums are most active, and thus most likely to enter the trap, between 10:00 pm and 2:30 am (Kerle, 2001; Than and Mcdonald, 1973). Plasma cortisol concentrations are known to fluctuate temporally (seasonally or diurnally) in various species (Bonier et al., 2009; Romero, 2002). Though we could not accurately plan the time that each sample was taken, all animals were processed during the daytime, when they were least responsive and plasma cortisol concentrations were likely to be at their lowest concentrations. The mean time at which blood samples were taken was 10:31 am ± 11.6 min (n = 178), with 65% of sampling occurring during the window when cortisol concentrations are at their lowest (8:00 am–12:00 pm). The remaining 35% of sampling occurred well outside of the nocturnal period of activity and the corresponding increase in plasma cortisol concentrations (peak 10:00 pm–1:00 am) for this species (Than and Mcdonald, 1973).

Hematocrit was measured over three field seasons: upon first capture in spring/summer 2007, autumn/winter 2008, and spring/summer 2008. Triplicate samples of up to 75 µL of blood were collected in heparinized microhematocrit capillary tubes and one end of each tube was plugged with plasticine. Tubes were spun in a portable hematocrit centrifuge (LW Scientific Inc. ZipOCRIT, Georgia, USA) at 11,000 RPM for 5 min to separate the RBCs from the plasma and the lengths of tube containing RBCs and plasma were measured to the nearest millimeter with vernier calipers. The percent hematocrit was calculated as the percentage of the total blood volume (i.e., length of capillary tube) represented by RBCs.

For each blood sample, one drop of whole blood (~10 µL) was used to make a blood smear. The droplet was drawn across a base slide using a second slide held at a 45˚ angle. The resulting smear was air dried for 30 min, fixed in methanol for 2–3 min, and allowed to air dry again. Slides were stained with 'Diff Quik' (modified Wright's stain) (Lab Aids Pty. Ltd., New South Wales, Australia) upon return to the field camp.

Determining relative age and survival of brushtail possums

Ageing brushtail possums to the year by enamel wear on the teeth (as in Winter, 1980) was not possible because our possums were not sedated. Thus, we assigned each animal a relative age of: adult, subadult, dependent juvenile (trapped alone), back young, or pouch young. We defined pouch young as still residing in their mother's pouch, while back young were found either on her back or in her direct vicinity. Dependent juveniles were trapped without their mother and had not yet developed a sternal gland; subadults had pronounced sternal glands and female pouches were invaginated (Hynes, 1999). Female adults and subadults were separated by weight: females were considered adult if they weighed ≥ 2.4 kg, the weight of the lightest reproductively active female trapped in this study. Male adults and subadults were distinguished by testis size. Testis size in brushtail possums does not increase with body weight during growth; rather, the testes grow dramatically in a short time at puberty (up to 300% in two months) (Hocking, 1981). Males were considered adult when their testis volume was ≥ 20.0 mm^3 as calculated from the equation for an oblate sphere: $V = \frac{\pi}{6} W^2 L$, where $V =$ testis volume (mm^3), $W =$ width, and $L =$ length (Abbott and Hearn, 1978; Hocking, 1981).

Survival over the duration of this study was calculated from the capture history of each individual using the POPAN option of the open-population Jolly-Seber model (Schwarz and Arnason, 1996) in the software Program MARK (White and Burnham, 1999). The model with constant survival, capture probability, entry into the population, and population size (phi(.)p(.)pent(.)N(.)) was the most appropriate, with the lowest Akaike information criterion (AIC) of 11 tested models.

Cortisol radioimmunoassay

Plasma cortisol concentration was analyzed by radioimmunoassay as in Jones et al. (2005) with the exception that cortisol was extracted from 25 µL of plasma into 1 mL of absolute ethanol. Duplicate aliquots of 100 µL of extracts of female plasmas and 200 µL of extracts of male plasmas were carried forth into the assay. The standards were 0-800 pg/tube of authentic cortisol (Sigma-Aldrich, Missouri: Cat. No, H-0135) in absolute ethanol. Radiolabelled cortisol (~4,000 CPM/50 µL ^{3}H-Cortisol (Amersham Laboratories, Buckinghamshire, UK)) and a 1:20,000 antibody solution (Sirosera cortisol antiserum, Total Reproduction, New South Wales, Australia; Cat. No. C-3368) were used in this assay. Extraction efficiency was 83.5%; intra-assay variation was 4.2%, and assay sensitivity was 12.5 pg cortisol. Control plasmas (simulated human plasma cortisol of known concentrations (Con4 = 30 ng/mL, Con5 = 114 ng/mL, Con6 = 300 ng/mL (DPC, California, USA)) were run in each assay to assess inter-assay variation (18.9%).

White blood cell differential

White blood cell differentials (WBC Diffs) were conducted as in Clark (2004). The monolayer of the blood smear, where cells were only one row of cells thick in depth, was examined under a compound microscope at 400X magnification. Each WBC was identified by its distinct histological characteristics as a one of the five types of white blood cells: monocyte, lymphocyte, neutrophil, eosinophil, and basophil (Appendix D). For a WBC Diff, the first 100 WBCs encountered were identified, tallied, and the percentage of each group in the total was calculated. The total number of WBCs (regardless of type) was counted in 10 consecutive fields of view, and the total concentration of WBCs was calculated as follows:

WBC x 10^9/L of blood = mean(# WBCs in 10 consecutive fields of view)*2 (Fudge, 1997).

The total number of WBCs and the percentages by cell type were multiplied together to calculate absolute values for each cell type. Absolute numbers of WBCs (cells x 10^9/L) were used in the statistical analyses.

Statistical analysis

All statistical analyses were carried out using SAS (SAS Institute Inc., 2003) and all results are reported mean ± standard error (SEM). Three-way analysis of variance (ANOVA) with factors disturbance, forest type, and season was performed on site means to assess variation in physiological parameters: plasma cortisol concentration, WBC parameters (absolute numbers of the five types of WBCs and total number of WBCs (cells x 10^9/L)), and hematocrit at first capture. Data for adult males and females were analyzed separately due to intrinsic differences in physiology between the sexes (Barnes et al., 2008; Barnett et al., 1979a; Bonier et al., 2009; Presidente, 1978; Wells et al., 2000). Differences in survival were analyzed by two-way ANOVA with factors disturbance and forest type performed on overall sites scores. When no differences were detected between forest type or disturbance history, data were grouped from sites by treatment (forest type/disturbance): dry disturbed ('DD'), dry control ('DC'), wet disturbed ('WD'), and wet control ('WC') forest. When no differences were detected between seasons, all results were pooled across seasons. Differences in plasma cortisol concentration, WBC parameters, and hematocrit were assessed between reproductively active (e.g., lactating) and quiescent female brushtail possums via one-way ANOVA (factor = reproductive status) performed on site means.

Seasonal variation in hematocrit from the same animal was assessed by a repeated measures mixed model ANOVA using animal as subject, with a two-way factorial of disturbance and forest type as fixed effects and site nested within disturbance*forest type as the random effect. The repeated measure was season, and separate analyses were done for each sex. The repeated measures analysis controlled for any effect of individual animals on the data. Repeated measures and single capture data sets were separate from each other.

The influence of treatment on physiological parameters measured concurrently (plasma cortisol concentration, WBC parameters, hematocrit, and body mass) was assessed via two-way ANOVA, with disturbance and forest type as independent variables and the physiological parameters as covariates. Correlation between physiological parameters was assessed via a correlation matrix.

Results

During this study, 209 brushtail possums were trapped: 178 adults, 29 subadults, and two dependent juveniles. The Program MARK model estimates that 93.8% (range: 85.2–99.7%) of the 'total' population was sampled across all sites. Survival of animals between field seasons over the course of the study (which remained constant under the employed model) did not differ with forest type ($F_{1,6} = 1.03$, $P = 0.350$) or disturbance ($F_{1,6} = 0.02$, $P = 0.880$) (mean: $82.0 \pm 3.95\%$, range: 66.4–100.0%).

A summary of the physiological results for the adult possums is presented in Table 3.2. Mean plasma cortisol concentration was 22.5 ± 1.47 ng/mL (range: undetectable to 51.1 ng/mL) in females and 15.0 ± 0.96 ng/mL (range: undetectable to 48.8 ng/mL) in males. Plasma cortisol concentrations showed no significant differences between disturbance (male: $F_{1,12} = 0.77$, $P = 0.400$; female: $F_{1,12} = 0.23$, $P = 0.639$) or forest type (male: $F_{1,12} = 0.43$, $P = 0.729$; female: $F_{1,12} = 0.04$, $P = 0.845$) (Fig. 3.2). There was no effect of season on plasma cortisol concentration in either sex (male: $F_{1,12} = 0.03$, $P = 0.877$; female: $F_{1,12} = 0.47$, $P = 0.508$).

Table 3.2. Physiological profiles (white blood cell (WBC) parameters, hematocrit values, and plasma cortisol concentration) of adult brushtail possums by sex, pooled across all seasons and treatments (forest type/disturbance) (± SEM).
Note: no animals showed signs of severe dehydration. 11 animals across all sites showed signs of mild dehydration and one female was acutely anemic (at site DC1).

Blood parameter	Adult male	n	Adult female	n
Total WBC count (x 10^9/L)	6.5 ± 0.31	100	6.7 ± 0.42	72
Neutrophils (x 10^9/L)	1.6 ± 0.09	100	1.7 ± 0.11	72
Lymphocytes (x 10^9/L)	3.4 ± 0.17	100	3.6 ± 0.26	72
Monocytes (x 10^9/L)	1.1 ± 0.07	100	1.0 ± 0.08	72
Eosinophils (x 10^9/L)	0.3 ± 0.03	100	0.3 ± 0.04	72
Basophils (x 10^9/L)	0.1 ± 0.01	100	0.1 ± 0.01	72
Neutrophil : Lymphocyte	1 : 2.14	100	1 : 2.12	72
Hematocrit (%)	42.8 ± 0.49	96	40.6 ± 0.63	73
Plasma cortisol concentration (ng/mL)	15.0 ± 0.96	103	22.5 ± 1.47	75
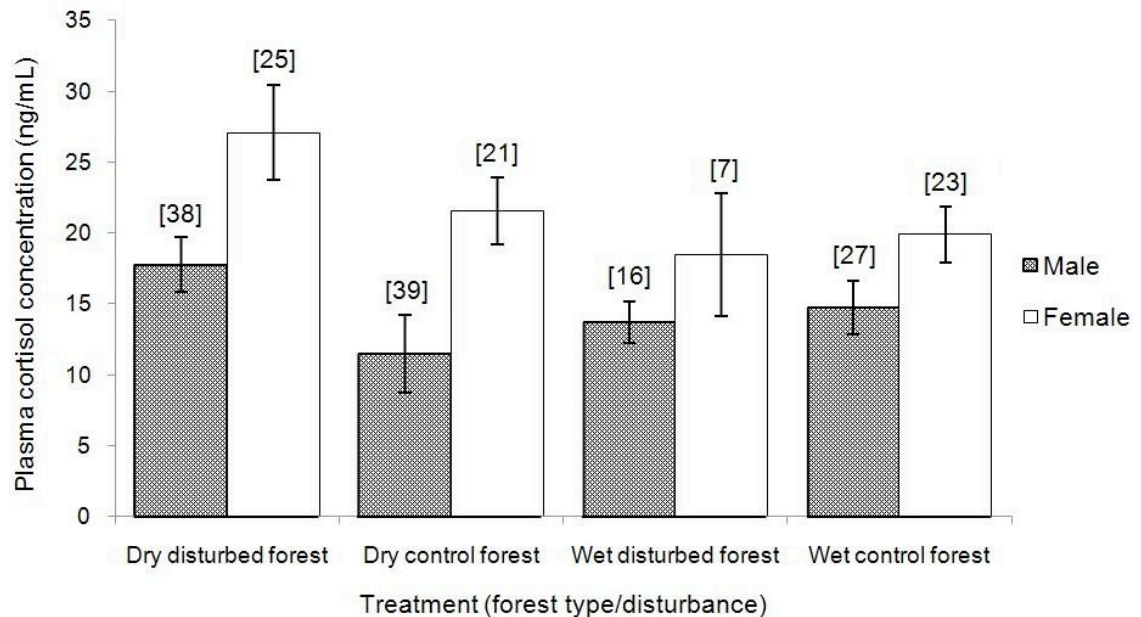
Fig. 3.2. Plasma cortisol concentration of adult brushtail possums by sex and treatment (forest type/disturbance): dry disturbed, dry control, wet disturbed, and wet control forest. Results were pooled across all seasons. Error bars denote SEM. The number of animals is given in brackets above the bars.

There was no significant difference in WBC profile (abundance of the different types of WBCs) in females between any treatment (disturbance: $F_{1,12} = 0.08$–1.08, $P = 0.325$–0.785; forest type: $F_{1,12} = 0.14$–0.84, $P = 0.382$–0.714) or in males between disturbance histories ($F_{1,12} = 0.01$–0.36, $P = 0.564$–0.984). There were significant differences between four WBC parameters in males between forest type (Table 3.3). Males living in dry forest had significantly lower numbers of neutrophils ($F_{1,12} = 9.48$, $P = 0.013$), monocytes ($F_{1,12} = 8.13$, $P = 0.026$), eosinophils ($F_{1,12} = 6.56$, $P = 0.034$), and total WBC count (influenced by the low number of three of the five cell types) ($F_{1,12} = 5.12$, $P = 0.043$) than did those living in wet forest. There was no seasonal effect on WBC profile in either sex (male: $F_{1,12} = 0.03$–2.66, $P = 0.134$–0.859; female: $F_{1,12} = 0.04$–1.84, $P = 0.208$–0.840), nor did any white cell profiles provide any indication of a 'stress response' (significantly high numbers of neutrophils with low numbers of lymphocytes and eosinophils (Buddle et al., 1992; Jain, 1986; Presidente, 1978)), or of capture stress (high neutrophil to lymphocyte ratio (Baker et al., 1998; Buddle et al., 1992; Davis and Maerz, 2008; Presidente and Correa, 1981)).

Table 3.3. White blood cell (WBC) parameters in adult male brushtail possums living in different forest types (wet forest n = 39, dry forest n = 71) pooled across all seasons and treatments (forest type/disturbance) (± SEM). [* indicates significance ($P < 0.05$)]

WBC parameter	Males living in wet forest (cells x 10^9/L)	Males living in dry forest (cells x 10^9/L)
Lymphocyte	3.6 ± 0.31	3.4 ± 0.22
Neutrophil*	2.0 ± 0.18	1.4 ± 0.09
Monocyte*	1.4 ± 0.18	0.9 ± 0.05
Eosinophil*	0.4 ± 0.08	0.3 ± 0.03
Basophil	0.1 ± 0.02	0.1 ± 0.01
Total WBC count*	7.5 ± 0.61	6.0 ± 0.53

Mean hematocrit values were 42.8 ± 0.49% (range: 31.3–48.1%) in males and 40.6 ± 0.63% (range: 25.3 (displayed in one animal in this study in particularly poor condition) –48.4%) in females. There were no significant differences in hematocrit values between disturbance (male: $F_{1,12} = 0.19$, $P = 0.666$; female: $F_{1,12} = 0.33$, $P = 0.575$) or forest type (male: $F_{1,12} = 0.74$, $P = 0.406$; female: $F_{1,12} = 0.20$, $P = 0.762$) for either sex. No seasonal effect was demonstrated in males ($F_{1,12} = 0.58$, $P = 0.459$), but in females there was a significant difference between seasons ($F_{1,12} = 6.45$, $P = 0.025$) (Fig. 3.3). In females, hematocrit values

were higher (indicating better body condition) in autumn/winter when they have pouch young, than in spring/summer when they have back young and an associated increased metabolic demand (Hocking, 1981; Kerle, 1984). Only one animal, a female at site DC1, showed signs of acute anemia (20–25% hematocrit (Jain, 1986)). No animals showed signs of severe dehydration (> 12% above the average range of hematocrit (Jain, 1986)) but 11 animals showed signs of mild dehydration (5–12% above the average range of hematocrit (Jain, 1986)): one female at DC1 (autumn/winter), four males in site DC2 (two in autumn/winter and two in spring/summer); three males at site DD2 (two in autumn/winter and one in spring/summer), one female at DD3 (spring/summer), and two males at WC2 (spring/summer). Hematocrit values greater than 50% indicate acute 'stress' (Parsons et al., 1971); such samples would indicate capture stress and we would have excluded them from the statistical analysis but none were encountered. Analysis of repeated measures of hematocrit from the same individuals over time showed no significant differences between forest type (male: $F_{1,11} = 0.16$, $P = 0.696$; female: $F_{1,11} = 0.23$, $P = 0.821$), disturbance (male: $F_{1,11} = 0.16$, $P = 0.986$; female: $F_{1,11} = 0.66$, $P = 0.524$), or season (male: $F_{1,11} = 0.00$, $P = 0.696$; female type: $F_{1,11} = 0.03$, $P = 0.979$) in either sex.
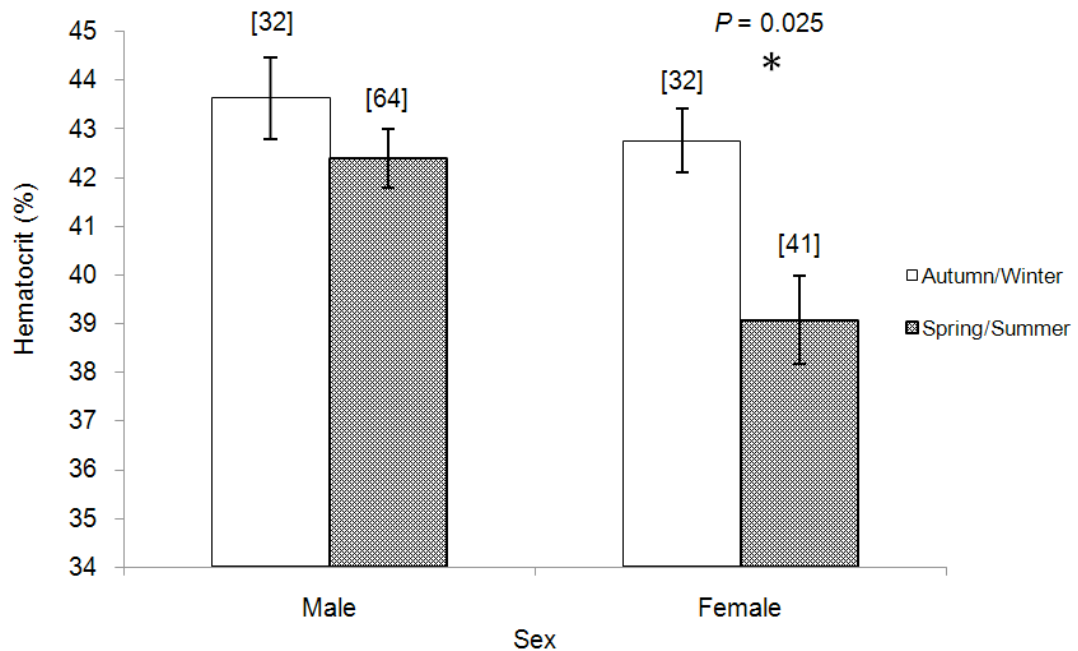
Fig. 3.3. Hematocrit values of adult brushtail possums by sex and season (autumn/winter and spring/summer). Results were pooled across treatments (forest type/disturbance). Error bars denote SEM. The number of animals is given in brackets above the bars.

There were no significant differences between reproductively active (e.g., lactating) and quiescent female brushtail possums in any parameter except hematocrit (plasma cortisol concentration: $P = 0.569$, $n = 75$; WBC parameters: $P = 0.166–0.313$, $n = 72$). Lactating females had significantly lower hematocrit values (suggesting poorer body condition) than reproductively quiescent females, based on one-way ANOVAs (39.9 ± 0.70% and 42.1 ± 0.85%, respectively: $P = 0.048$, $n = 73$).

The correlation matrix revealed that while the numbers of the different WBC types were correlated with the total WBC count ($P < 0.001$), no other physiological parameters (plasma cortisol concentration, WBC parameters, hematocrit, or body mass) were significantly correlated with each other (i.e., $r^2 \geq 0.3$). There was no significant effect of either forest type (males: $F_{1,6} = 0.90–4.76$, $P = 0.117–0.867$; females: $F_{1,6} = 0.05–0.4.17$, $P = 0.087–0.832$) or disturbance (males: $F_{1,6} = 0.11–2.40$, $P = 0.172–0.996$; females: $F_{1,6} = 0.01–2.47$, $P = 0.179–0.913$) on any of the physiological covariates sampled concurrently (plasma cortisol concentration, hematocrit, WBC parameters, and body mass) in either sex.

There was no sign of diarrhea, emaciation, rough pelage, aggression, or lethargy detected in any brushtail possums in this study. Most animals carried < 5–10 ticks. Six possums carried a higher external parasite load (four individuals with > 20 ticks and two with > 50 seed ticks) but the physiological profiles of these animals did not indicate chronic adrenal activation.

Discussion

The results of this study indicate that habitat disturbance due to forestry activities does not present a significant physiological challenge to individual brushtail possums living within either dry or wet *Eucalyptus* forest in Tasmania. None of the key physiological parameters of plasma cortisol concentration, WBC profile, and hematocrit varied significantly between our study populations. This 'physiological resilience' may underpin the success of the brushtail possum as an invasive species, and its ability to exploit anthropogenically altered habitats such as plantations and agricultural areas.

The physiological parameters we employed provide a set of technically simple indices with which to assess the health of individuals or populations (Barnes et al., 2008; Romero, 2002; Wikelski and Cooke, 2006) across all vertebrate taxa, in both captive and wild animals (Baker, 2003; Davis et al., 2008; Hesser, 1960; Hillman, 1980; Munday and Blane, 1961; Romero, 2002). These indicators are sensitive to, and reflect, environmental factors such as habitat quality and change, capture or captivity, nutritional condition, disease, and breeding status (Busch and Hayward, 2009; Wells et al., 2000; Wingfield et al., 1997). For example, fence lizards (*Sceloporus occidentalis*) (Dunlap and Wingfield, 1995) and northern spotted owls (*Strix occidentalis caurina*) (Wasser et al., 1997) living on the periphery of the species' range display physiological profiles indicating adrenal activation: in such species, habitat fragmentation may make individuals more susceptible to 'stress', with consequent impacts at the population level. Specific habitat variables such as food availability may be altered by disturbance. For example, Eurasian treecreepers (*Certhia familiaris*) living in disturbed forests in which habitat fragmentation had decreased food abundance exhibited decreased body condition and increased plasma corticosterone concentrations, resulting in lower offspring survival (Suorsa et al., 2003). Similarly, black howler monkeys (*Aloutta pigra*) living in fragmented forest displayed smaller body size, altered locomotion patterns (making them more vulnerable to predation), and higher glucocorticoid production than monkeys living in continuous forest (Martínez-Mota et al., 2007). In the long term, such differences may result in decreased fitness and population viability. In our study, we used both direct (plasma cortisol) and indirect (WBC profiles and hematocrit) measures of physiological well-being or adrenal responsiveness. This integrated suite of parameters provides information on different time scales: WBCs provided a long-term indicator of physiological stress (Davis and Maerz, 2008), while plasma cortisol concentration provides an indicator of health at the time of sampling (Bonier et al., 2009; McKenzie et al., 2004).

There was no evidence of a significant impact of handling on plasma cortisol concentrations in the brushtail possums sampled in this study. Capture and handling can activate an acute stress response: in many mammals, physical restraint results in significantly increased plasma glucocorticoid concentration (e.g., brush-tailed rock-wallaby (*Petrogale pencillata*) (Barnes et al., 2008), platypus (*Ornithorhynchus anatinus*) (Handasyde et al., 2003), fruit bats (*Pteropus vampyrus, Pteropushypomelanus,* and *Rousettus aegyptiacus*) (Widmaier and

Kunz, 1993), and many African mammals (Morton et al., 1995)). Mindful of this, we performed blood sampling as quickly and efficiently as possible in order to minimize any impact on the physiological parameters of interest. The mean plasma cortisol concentrations we measured were within the range of those in captive brushtail possums (Presidente and Correa, 1981; Baker and Gemmell, 1999), and similar to those reported in healthy brushtail possums by Presidente and Correa (1981) (22.8 ng/mL in males and 29.1 ng/mL in females). In contrast, maximal plasma cortisol concentrations in brushtail possums have been reported as 86.9 ng/mL in males and 63.8 ng/mL in females (Baker et al., 1998), while Than and McDonald (1973) reported 70 ng/mL as the maximum plasma cortisol concentration in response to administration of adrenocorticotropic hormone. These comparisons suggest that our blood samples were taken before the onset of any significant acute stress response to removal from the trap. Furthermore, the hematocrit values and WBC profiles we observed were not in the ranges typically associated with acute stress, namely, hematocrit values of > 50% (Parsons et al., 1971) or a high neutrophil to lymphocyte ratio (Baker et al., 1998; Buddle et al., 1992; Davis and Maerz, 2008; Presidente and Correa, 1981). The neutrophil to lymphocyte ratios observed in this study were similar to those reported in healthy, unstressed brushtail possums by Presidente and Correa (1981) (1:2.27 in males and 1:1.68 in females) and Presidente (1978) (1:1.88 in both males and females together). Similarly, in other free-living mammals, capture and handling does not influence these physiological indicators of acute stress: plasma cortisol concentration in the koala (*Phascolarctos cinereus*) (McDonald et al., 1990) and Cape buffalo (*Syncerus caffer*) (Morton et al., 1995); hematocrit in the common brushtail possum and the short-eared possum (*Trichosurus caninus*) (Barnett et al., 1979a); and WBC profiles in Weddell seals (*Leptonychotes weddellii*) (Mellish et al., 2010).

Plasma cortisol concentrations did not differ between populations at control and disturbed sites. This suggests that the brushtail possums in our study populations were not experiencing prolonged stress due to habitat disturbance. Indeed, Baker et al. (1998) found that the capture and transfer to captivity of wild brushtail possums did not result in either chronic adrenal activation or changes in WBC profile. McKenzie et al. (2004) found a similar lack of adrenal or WBC response in captive tammar wallabies (*Macropus eugenii*) that were subject to disturbance via isolation from conspecifics and an altered feeding

regime. Some mammalian species may, therefore be relatively resilient to some forms of habitat alteration.

The WBC values for all populations in our study were within the normal ranges previously reported for brushtail possums (Presidente, 1978; Presidente and Correa, 1981; Wells et al., 2000). Other studies have found that WBC profiles associated with the adrenal status indicating 'stress' (i.e., high neutrophil to lymphocyte ratio) may be detected even if plasma cortisol concentrations remain within the normal range of the species (Baker and Gemmell, 1999; Hajduk et al., 1992). The WBC profile generated for the Tasmanian subspecies in this study reflects those reported for Australian and New Zealand populations (Baker and Gemmell, 1999; Presidente, 1978; Presidente and Correa, 1981; Wells et al., 2000). There was a subtle, but subclinical, difference between WBC profiles of male brushtail possums who lived in dry forest as compared to those in wet forest; however, they were still within normal range for the species (Presidente and Correa, 1981; Winter et al., 2000), and the males living in dry forest did not show any significant differences in plasma cortisol concentration or hematocrit.

Hematocrit values can indirectly indicate the abundance or quality of food resources, and reflect subtle changes in body condition, body mass, and nutritional status (Sanchez-Guzman et al., 2004). Hematocrit values reported in this study are within the normal range for the brushtail possum (Barnett et al., 1979a, 1979b; Presidente, 1978; Wells et al., 2000). However, hematocrit values were significantly higher (indicating better body condition (Sanchez-Guzman et al., 2004)) in females in autumn/winter, when they had pouch young, than in spring/summer, when they had back young and milk production was highest (Grand and Barboza, 2001; Kerle, 1984). This pattern indicates that the energetic demands of late lactation may compromise body condition in female brushtail possums.

There was no effect of season on either plasma cortisol concentration or WBC profile in either sex. This was unexpected as both these parameters generally show annual cycles of variation in mammals (Romero, 2002; Jakubów et al., 1984). For example, in the degu (*Octodon degus*), a caviomorph rodent, plasma cortisol concentrations are lowest in the

mating season and peak during lactation when metabolic demand and energy consumption are high (Kenagy et al., 1999). However, there are exceptions. Spotted hyenas (*Crocuta crocuta*) exhibit no seasonal pattern of plasma cortisol concentrations, although this may be due a high level of variation in plasma cortisol concentrations between individuals that is not related to sex, age, social status, or immobilization procedure (Van Jaarsveld and Skinner, 1992). The brushtail possums we sampled did not exhibit marked variation between individuals, and there was a significant difference in mean plasma cortisol concentration between the sexes. We do acknowledge that, for logistic reasons, we carried out sampling over only two extended periods each year, so any annual cycle of plasma cortisol may have been obscured.

Seasonal variation in hematological profiles generally reflect seasonal differences in food availability, energetic demand, or immune challenges (Griffin, 1989; Hellgren et al., 1993; Jakubów et al., 1984) and may be absent in captive animals with a constant food supply and a mediated environment (Wenberg et al., 1973). In brushtail possums, WBC responses may be determined by habitat and resource abundance rather than seasonally variable parameters, and may be population-specific (Wells et al., 2000). Wells et al. (2000) found that brushtail possums in New Zealand showed no seasonal effect on hematocrit, hemoglobin concentration, or RBC count, and suggested that temperature and rainfall may not be significant environmental stressors in habitats with adequate food supply. Similarly, Barnett et al. (1979a) found no seasonal response in RBC count and plasma glucose and protein in brushtail possums, and attributed this to the species' capacity to cope with environmental stress. If brushtail possums are able to compensate for any seasonal disparities in food or tolerate weather changes, then we would expect no seasonal signal in hematological parameters (Wells et al., 2000). Our results suggest that this is the case for our study populations in Tasmania.

Contrary to our hypothesis, we found that brushtail possums living in disturbed habitat (of either forest type) did not exhibit significant change in physiological indicators of well-being compared to animals living in undisturbed, control, habitat. Our results suggest that current forestry practices in Tasmania do not change the habitat sufficiently to present an

environmental challenge that alters physiological status in brushtail possums. Such information is valuable for the development of scientifically based conservation management actions that will inform landscape planning for the conservation of local biodiversity.

Acknowledgements

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Appendices

Appendix A. Photographs from the center of each core site illustrate broad forest structure and type

(a) Dry *Eucalyptus* forest disturbed sites

(i) DD1

(ii) DD2

111

(iii) DD3

(b) Dry *Eucalyptus* forest control sites
(i) DC1

(ii) DC2

(iii) DC3

(c) Wet *Eucalyptus* forest disturbed sites
(i) WD1

(ii) WD2

(d) Wet *Eucalyptus* forest control sites
(i) WC1

(ii) WC2

Appendix B. Aerial photographs showing trap placement (Google Earth 5 (2009)) of the study sites illustrate broad forest structure and type

(a) Dry *Eucalyptus* forest disturbed sites
(i) DD1
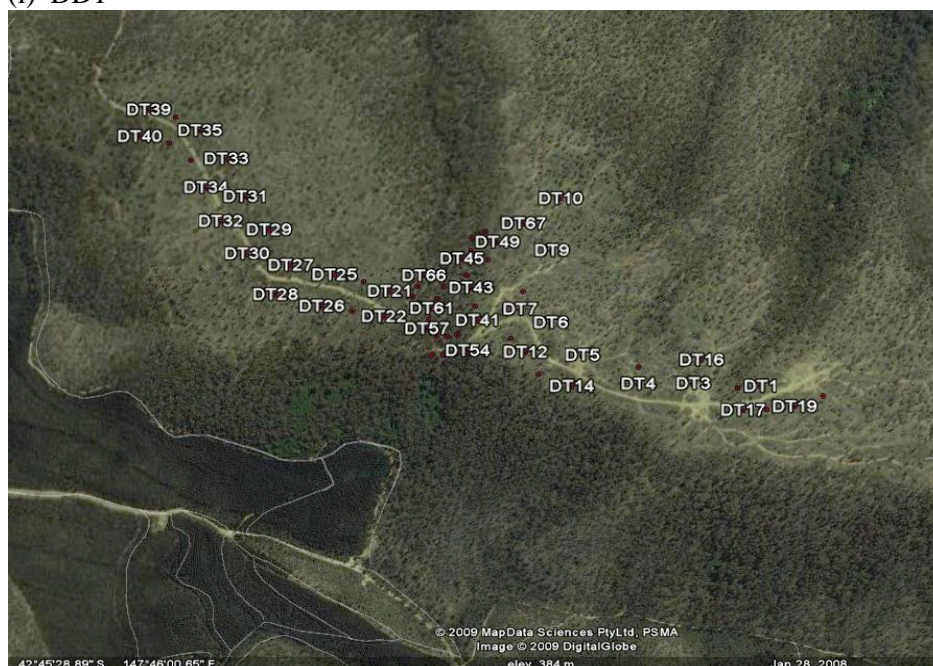
(ii) DD2
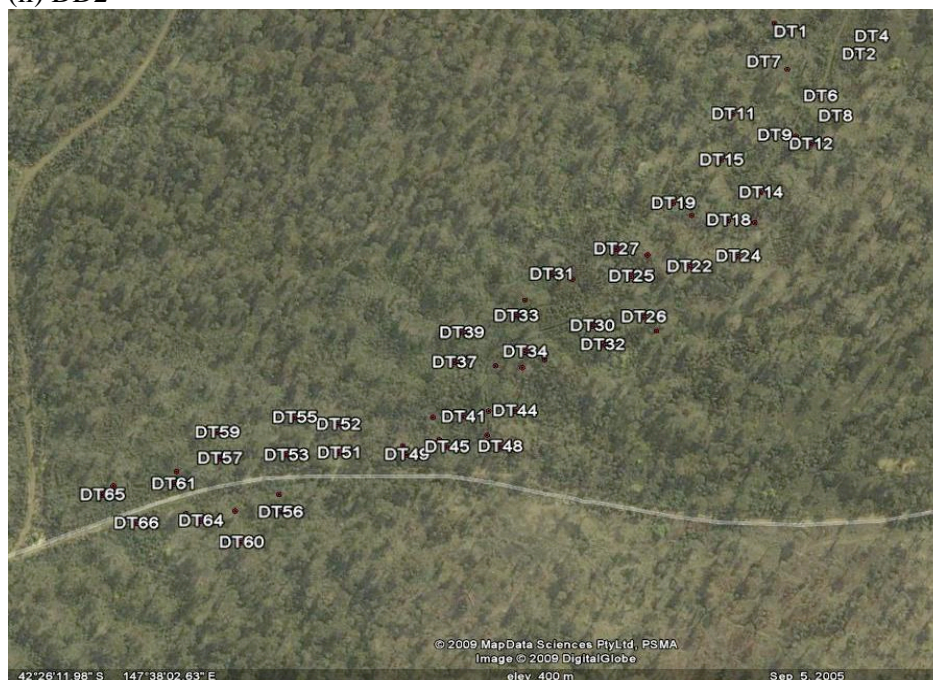
(iii) DD3
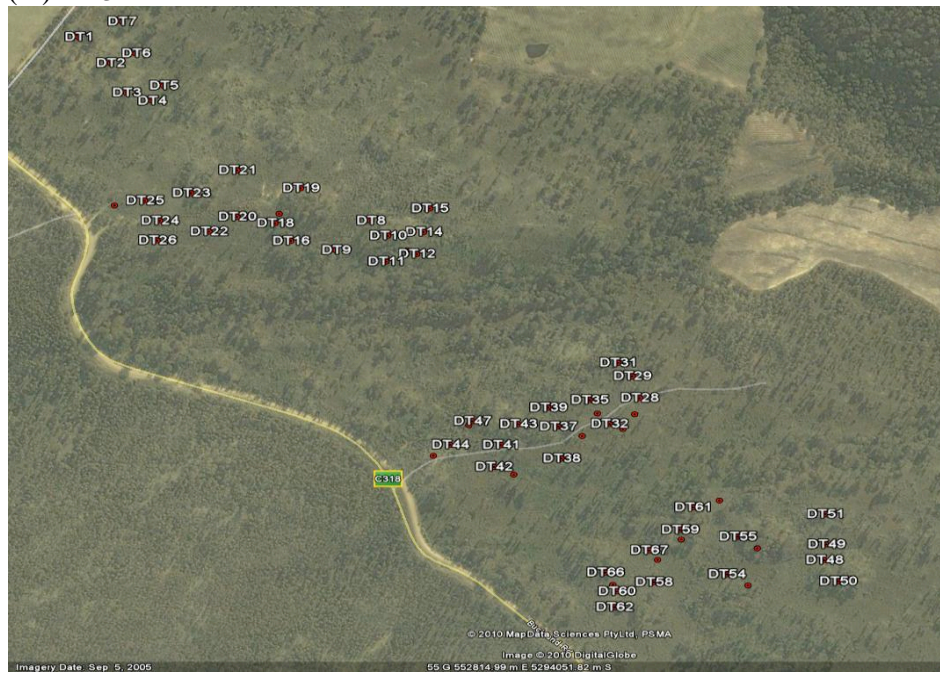
(b) Dry *Eucalyptus* forest control sites
(i) DC1

(ii) DC2
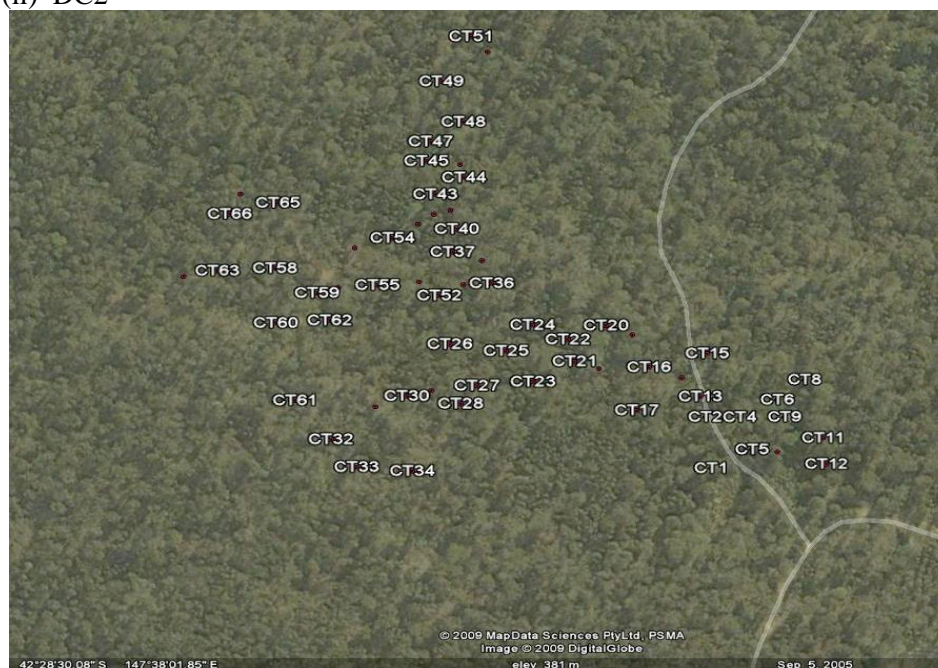
(iii) DC3
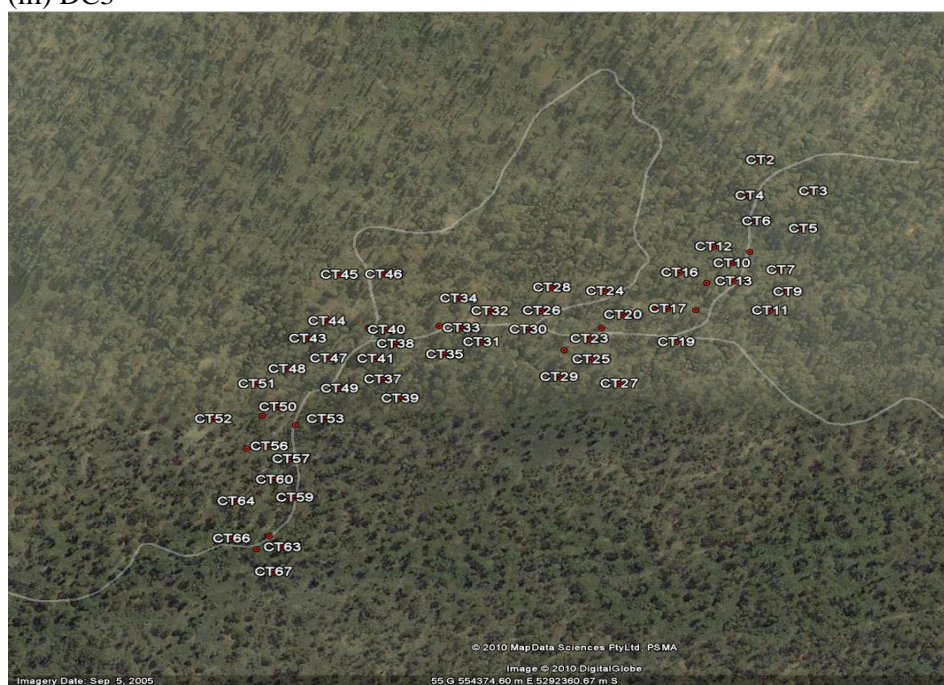
(c) Wet *Eucalyptus* forest disturbed sites
(i) WD1
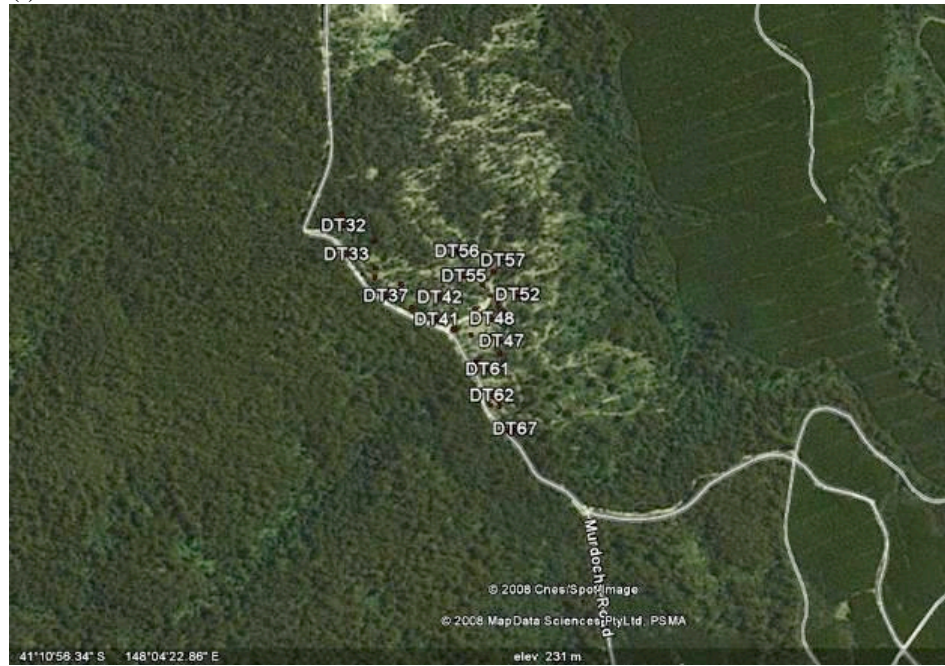
(ii) WD2
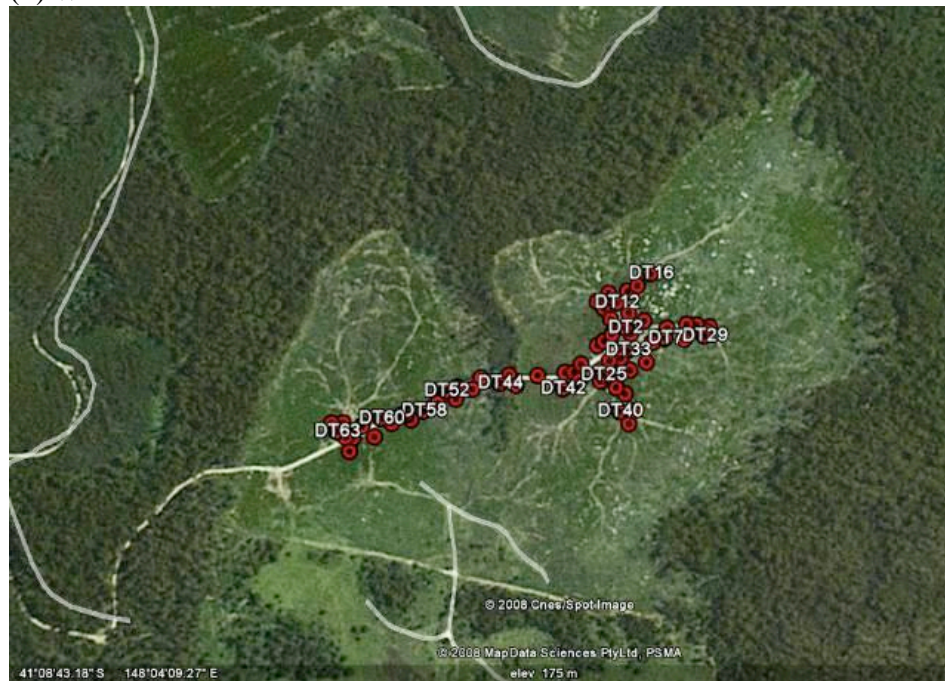
(d) Wet *Eucalyptus* forest control sites
(i) WC1
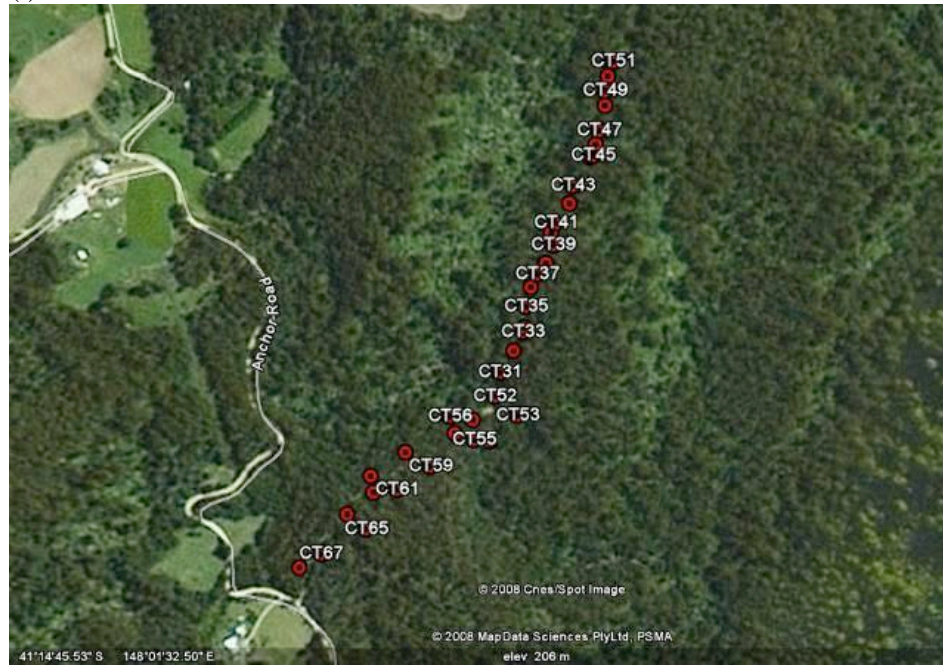
(ii) WC2
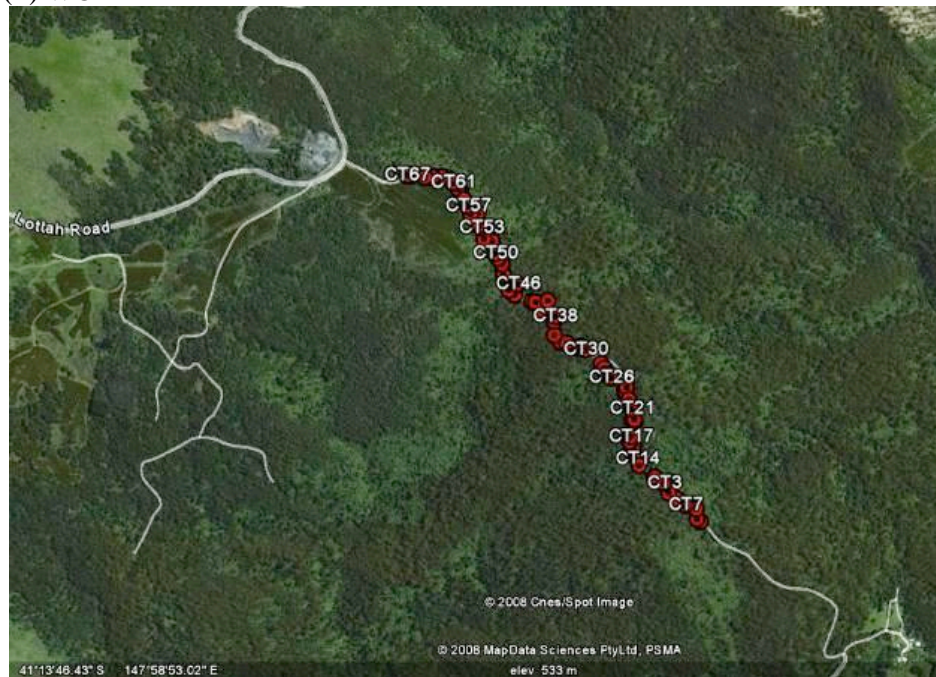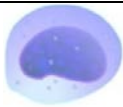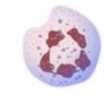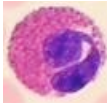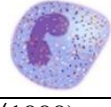
Appendix C. Study site locations

Table 1. Grid coordinates and global location of study sites

Site	Coupe name	State forest	Grid Coordinates (GDA)				Longitude	Latitude
			Top left corner		Bottom right corner			
DD1	MM002A & MM004A	Mt. Morrison	5,267,000N	561,000E	5,265,000N	564,000E	42˚45'31.07"S	147˚45'53.78"E
DD2	SW059A & SW059B	Swanport	5,303,000N	551,000E	5,299,000N	554,000E	42˚26'14.64"S	147˚38'00.13"E
DD3	SW049A	Swanport	5,296,000N	551,000E	5,291,000N	556,000E	42˚50'41.34" S	147˚64'06.67"E
DC1	"Backwoods" reserve	Mt. Morrison	5,275,000N	550,000E	5,272,000N	553,000E	42˚41'32.12"S	147˚38'06.77"E
DC2	SW051A	Swanport	5,298,000N	552,000E	5,296,000N	553,000E	42˚28'30.85."S	147˚38'00.28."E
DC3	Reserve	Swanport	5,296,000N	551,000E	5,291,000N	556,000E	42˚52'01.79" S	147˚66'05.18"E
WD1	GC029B	Goulds Country	5,442,000N	589,000E	5,439,000N	591,000E	41˚10'56.34"S	148˚04'22.86"E
WD2	GC104B	Goulds Country	5,446,000N	589,000E	5,444,00 N	591,000E	41˚08'43.18"S	148˚04'09.27"E
WC1	GC145B	Goulds Country	5,434,000N	585,000E	5,432,000N	587,000E	41˚14'45.53"S	148˚01'32.50"E
WC2	GC139B	Goulds Country	5,437,000N	581,000E	5,434,00 N	584,000E	41˚13'46.43"S	147˚58'53.01"E

Appendix D. Description of white blood cells (WBC) for white blood cell differential (WBC Diff)

Table 1. Mammal WBC identification

Cell name	Group	Dye Colour	Distinction	Appearance*
Monocyte	Non-granulocytes	Blue	Large, lots of cytoplasm	
Lymphocyte	Non-granulocytes	Blue	Not much cytoplasm	
Neutrophil	Granulocytes	Blue	Large "fragments", granules	
Eosinophil	Granulocytes	Red	Colour, granules	
Basophil	Granulocytes	Blue	Granules	

*Images were modified from RnCeus Interactive, LLC. (2006); Rubbelke (1999)

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Chapter 4

Influences of forest type and disturbance on reproduction of the brushtail possum (*Trichosurus vulpecula*)

Top: mother brushtail possums (*Trichosurus vulpecula*) with their back young of various ages
Bottom: milking under anaesthesia and mother with back young

ABSTRACT

The effects of forest type and disturbance on specific reproductive traits are unknown for many forest-dwelling mammals. We assessed whether differences in forest type and disturbance influenced key reproductive traits of an arboreal marsupial, the common brushtail possum (*Trichosurus vulpecula*) in Tasmania, Australia. Trapping was conducted in spring/summer and autumn/winter during 2007–2008 at 6 dry *Eucalyptus* forest sites (3 regenerating after harvest and 3 in relatively undisturbed forest) in southeast Tasmania, and 4 wet *Eucalyptus* forest sites (2 regenerating after harvest and 2 in relatively undisturbed forest) in the northeast. We aged pouch young and assessed the body condition of back young. We milked females captured during the late stage of lactation and compared the nutritional composition of milk among sites. Births occurred slightly earlier at the northern sites than those in the south. Female body mass and offspring body condition and survival were similar across all sites. Milk composition was also similar across all sites, but possums in dry, undisturbed forest, primarily at 1 site, produced milk with significantly higher fat content; however, there was no evidence of a difference in body condition or survival of young that could be attributed to a difference in milk quality. Although forest type and disturbance influence demography and breeding frequency in the brushtail possum populations we studied, these population-level differences are not linked to variations in the reproductive traits examined here. We conclude that the brushtail possum is a physiologically resilient species, and the ability of individual females to reproduce successfully is relatively insensitive to forest type or habitat disturbance.

INTRODUCTION

Understanding the relationship between the reproductive capacity of a species and its environment is important for predicting that species' ability to adapt to habitat loss and alteration. In mammals reproductive success of females is related to their nutritional status, which is influenced by habitat type (Eiler 1981; Sadlier 1969). Potential consequences of habitat loss or alteration include decreased ability to attract mates (de la Torre et al. 2000), reduced breeding success (e.g., decreased survival or fewer or smaller young—Hinsley et al. 1999; Wolff 1995), disrupted parturition (Bleicher 1962; Newton et al. 1996), and decreased female reproductive output due to the higher energy demands of searching for and defending new territories after habitat disturbance (Brigham and Fenton 1987; White et al.1999). If

disturbance leads to food shortage when females are pregnant, prenatal or postnatal mortality of offspring can increase, birth mass and postnatal growth can decrease, and the capacity for females to raise young to independence can be compromised (Labov et al. 1986; Sadlier 1969). Habitat disturbance that results in chronic 'stress' in pregnant females may have long-term behavioral and physiological effects on the offspring that may carry over into future generations (Braastad 1998).

Alterations to maternal nutritional resources may lead to changes in lactational traits such as milk quantity and composition, and the duration of lactation (Blackburn 1993). Milk composition is influenced by the nutritional and metabolic demands of both mother and young, as well as by environmental and habitat-related factors (Gitleman and Oftedal 1987; Griffiths et al. 1988; Munks and Green 1997; Munks et al. 1991; Rose et al. 2003; Rose and Flowers 2005; Sadlier 1969). The relationship between lactation and environmental factors is particularly important in marsupials, in which the majority of offspring development occurs during a prolonged period of lactation, rather than during gestation as in eutherian mammals (Green and Merchant 1988; Tyndale-Biscoe 2005). Milk composition has been documented for a range of marsupial species, including arboreal marsupials such as the common brushtail possum (*Trichosurus vulpecula*—Cowan 1989; Gross and Bolliger 1959; Sharman 1962), common ringtail possum (*Pseudocheirus peregrines*—Munks et al. 1991), sugar glider (*Petaurus breviceps*—Green 1984a), and koala (*Phascolarctos cinereus*—Krockenberger 1996). However, the effects of habitat disturbance and forest type on milk composition and subsequent development of marsupial young have not been well studied. We investigated these key reproducrive traits in the common brushtail possum (*T. vulpecula*), an arboreal marsupial inhabiting forest in Tasmania that is used extensively for wood production and is therefore subject to habitat disturbance.

The brushtail possum is a nocturnal omnivore and a physiologically resilient species (Barnett et al. 1979) capable of exploiting a large array of habitats (Bulinski and McArthur 1999; Kerle 1984; Statham 1984) and foods (Cowan and Moeed 1987; Fitzgerald 1984; Nugent et al. 2000). However, arboreal marsupials that depend on tree cavities (hollows) are susceptible to anthropogenic habitat disturbance that reduces the availability of tree hollows necessary for breeding (Gibbons and Lindenmayer 2002; Goldingay and Daly 1997;

Tyndale-Biscoe and Calaby 1975). For example, forest type and disturbance influence population traits and breeding frequency in the Tasmanian brushtail possum (*Trichosurus vulpecula fuliginosus*). Hocking (1981) found that in disturbed wet forest habitats representing different stages of regeneration post-harvest and fire, brushtail possums living in burned 1–to 5-year-old sites matured at a younger age and had higher breeding success than those in mature wet forest (60–80 years old). These observations were attributed to the high nutritional content of *Eucalyptus* regrowth after fire (Hocking 1981). However, a more recent study by Flynn et al. (2011) [Chapter 2] that considered both wet and dry *Eucalyptus* forest sites found significantly fewer brushtail possums in areas disturbed 4–11 years previously by intensive forest harvesting and regeneration burns than in mature, relatively undisturbed (control) sites. Additionally, populations living in disturbed sites displayed a male-biased adult sex ratio. While the percentage of adult females that bred during the study did not differ significantly across sites, females bred less frequently in the disturbed sites; females living in control forest bred each year, but those in disturbed forest did not. These population-level differences were attributed to a shortage of resources, principally hollow-bearing trees, at the disturbed sites. We tested the hypothesis that differences in resource availability (food and refuge) associated with forest type and disturbance history affect reproduction of individual brushtail possums living in dry or wet forest by examining milk composition, timing of births, offspring survival, and body condition of young.

MATERIALS AND METHODS

Study design and sites.—Three broad types of forest exist in Tasmania: austral montane, temperate rainforest, and sclerophyll forest (Jackson 1999). Sclerophyll forests are divided further into wet and dry forests, which are ecologically distinct forest types reflecting differences in rainfall and water availability (Jackson 1999; Kirkpatrick and Bowman 1982). Wet forest is dominated by *Eucalyptus*, with an understory of broad-leaved shrubs or ferns, or both. Dry forest also is dominated by *Eucalyptus* but has an understory of prickly and small-leaved shrubs or grasses (Jackson 1999).

This syudy was conducted at 10 sites in eastern Tasmania, the island state of Australia. Six dry *Eucalyptus* forest sites (3 regenerating after harvest and 3 in relatively undisturbed

forest) were located in the southeast, and 4 wet *Eucalyptus* forest sites (2 regenerating after harvest and 2 in relatively undisturbed forest) were located in the northeast (Fig. 4.1, Table 4.1). Geology, which influences soil type, was classified as adamellite in wet forest sites. In dry forest, sites DD1 and DC1 (Table 4.1) were located on dolerite, and DD2, DD3, DC2, and DC3 (Table 4.1) were located on sandstone.
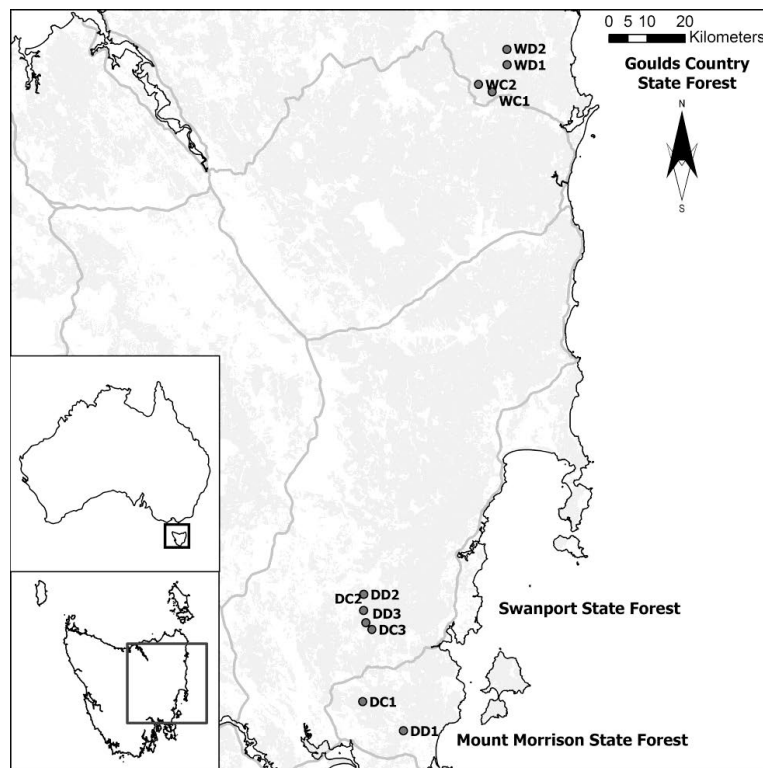
FIG. 4.1.—Location of study sites in eastern Tasmania, Australia. Dry *Eucalyptus* forest sites are in the southeast and wet *Eucalyptus* forest sites are in the northeast of the state. Shading indicates forested area while lack of shading denotes non-forest area (agriculture, scrub, lakes, urban area, etc.). Solid lines indicate major roads (Tasmanian Department of Primary Industries and Water 2001; Harris and Kitchener 2005). (Treatment (forest type/disturbance) is indicated by the first 2 letters of a site name, followed by the site number: 'DD' = dry disturbed, 'DC' = dry control, 'WD' = wet disturbed, and 'WC' = wet control forest.)

Table 4.1.— Details of study sites in eastern Tasmania, Australia, including forest type classification and both method and date of harvest of disturbed sites. See Fig. 4.1 for location of sites and explanation of site names. N/A = not applicable.

Site name	Broad forest type[1]	Silvicultural method	Harvest date (yr)[7]
DD1	*E. pulchella - E. globulus - E. viminalis* grassy shrubby forest [Dry]	Partial harvest (SED, AGR)[2]	2000/ 2004
DD2	*E. obliqua* forest [Dry]	Partial harvest (SR, AGR)[3]	1998/ 1999
DD3	*E. amygdalina* forest on sandstone [Dry]	Partial harvest (SED, SR)[4]	1997
DC1	*E. obliqua* forest [Dry]	N/A	N/A
DC2	*E. obliqua* forest [Dry]	N/A	N/A
DC3	*E. amygdalina* forest on sandstone [Dry]	N/A	N/A
WD1	*E. regnans* forest [Wet]	Commercial thinning[5]	2002
WD2	*E. regnans* forest [Wet]	Clearfell, burn, and sow (CBS)[6]	2002
WC1	*E. regnans* forest [Wet]	N/A	N/A
WC2	*E. regnans* forest [Wet]	N/A	N/A

[1] Broad description of *Eucalyptus* forest community (DPIW 2001; Harris and Kitchener 2005)

[2] Mosaic of seedtree retention (SED) (resulting in 7–12 trees/ha) and advanced growth retention (AGR) (basal area reduced to < 4 m^2/ha) followed by a low intensity burn for regeneration (Wilkinson 1994)

[3] Mosaic of shelterwood retention (SR) (basal area reduced to 12–14 m^2/ha in wet forest and 9–12 m^2/ha in dry forest) and advanced growth retention (AGR) (basal area reduced to < 4 m^2/ha) followed by a low intensity burn for regeneration (Wilkinson 1994)

[4] Mosaic of seedtree retention (SED) (resulting in 7–12 trees/ha) and shelterwood retention (SR) (basal area reduced to 12–14 m^2/ha in wet forest and 9–12 m^2/ha in dry forest) followed by a low intensity burn for regeneration (Wilkinson 1994)

[5] Stocking reduced to at least 200 well-formed trees/ha; no regeneration burn (Wilkinson 1994)

[6] All live trees (> 5 m) are felled, followed by a regeneration burn and seed sowing (Wilkinson 1994)

[7] From Timber Harvesting Plan for the coupe (M. Miller, V. Thompson, and A. Walls, Forestry Tasmania, pers. comm.)

Sites had a mean area of 38.57 ha (range: 21.49–62.30 ha) and were at elevations of 184–529 m (dry forest sites were 301–398 m and wet forest sites were 184–529 m). During our study dry forest sites received 490 mm of rainfall per year and wet forest sites received 1,014 mm of rainfall per year. Both northeastern and southeastern Tasmania experienced serious (experienced 1 in 10 years) to severe (experienced 1 in 25 years) droughts during this study, including some of the lowest rainfall recorded historically (Australian Bureau of Meteorology 2009a).

All disturbed sites had been harvested 4–11 years ago. Each site was harvested using the silvicultural technique appropriate for the forest type. In the wet forest sites this included commercial thinning and clearfell, burn, and sow (Wilkinson 1994). In the dry forest sites seedtree retention, advanced growth retention, and shelterwood retention were used (Wilkinson 1994)). The relatively undisturbed sites (controls) were dominated by mature forest and were unlogged but subjected to storms and wildfire. All study sites were embedded within a surrounding matrix of mature or older-aged regenerating forest.

Although multiple-control before–after-control-impact (MBACI)-type designs (Stewart-Oaten and Bence 2001; Underwood 1997) are a preferred experimental approach for assessing land-use impacts using analysis of variance (ANOVA), they require a long-term commitment of sites and funding. An alternative is to use a retrospective ('space-for-time') inferential approach (Pickett 1989) and to compare sites under different current land uses. We used a retrospective approach because resources for a long-term experiment were not available. We made every effort to select study sites that were likely to have been similar before harvesting operations began.

Trapping procedures.—Study sites were trapped twice yearly (spring/summer and autumn/winter) during 2007–2008. In the brushtail possum autumn/winter corresponds to breeding and early pouch life, and spring/summer corresponds to late pouch life and independence (Hocking 1981). Hessian (burlap)-covered, treadle-triggered, wire Mascot cage traps (300 x 300 x 660 mm; Mascot Wire Works, Homebush West, New South Wales, Australia) were baited with ¼ apple and small balls of peanut butter/vanilla essence

(extract)/oats. Either 37 (2 sites: WD1 and WC1) or 67 traps (the other 6 sites) were deployed at each site, depending on the size and accessibility of the site (Flynn et al. 2011). The number of traps per site was consistent over the study, resulting in an average spatial trapping effort of 1.77 traps/ha (range: 1.08–2.73 traps/ha). Traps were placed 50 x 20 m apart in 2 transects per site. Trapping sessions consisted of 4 nights: 1 night of pre-baiting and 3 nights of active trapping. Checking traps and processing animals began at dawn to minimize stress in captured animals (Johnson and McIlwee 1997).

Upon capture, all adult, subadult, dependent juvenile, and young possums were processed, and relative age was assigned. Pouch young resided in their mother's pouch; back young were found either on the mother's back or in the mother's direct vicinity; and dependent juveniles were trapped without their mother and had not yet developed a sternal gland. Subadults had pronounced sternal glands and female pouches were invaginated (Hynes 1999). Female adults and subadults were separated by body mass; adult body mass was $\geq$ 2.4 kg (the body mass of the lightest reproductively active female we trapped). Male adults and subadults were distinguished by testis size; adults had testis volume ≥ 20.0 mm^3 (Hocking 1981). Animals were sexed and tagged with a subcutaneous ISO FDX-B transponder chip (passive integrated transponder tag; Allflex Australia, Capalaba, Queensland, Australia). Animals were weighed and head, leg, pes, and tail length were measured to the nearest mm with vernier calipers. Animals > 1 kg were weighed with a Salter 10 kg spring balance (Salter Australia, Springvale, Victoria, Australia) to the nearest 50 g. Back young < 1 kg were weighed with a Salter 1 kg spring balance (Salter Australia) to the nearest 5 g. Pouch young were sexed, and head and snout-rump length were measured (Lyne and Verhagen 1957).

Female reproductive status was assessed via pouch condition (Hocking 1981; Sharman 1962) (Table 4.2) (Appendix A). Females were classified as: nulliparous (never bred), currently reproductively quiescent (non-breeding) but have bred previously, breeding, pregnant, or reproductively active (having produced young).

Table 4.2.—Classification of the reproductive status of female brushtail possums determined from pouch condition (derived from field observations from our study, Hocking (1981) and Sharman (1962)).

Reproductive status of female	Pouch characteristics
Nulliparous	No red, waxy residue in pouch; nipples and mammary glands are inactive.
Currently reproductively quiescent but has bred previously	Presence of dried red, waxy residue in pouch; inactive nipples and mammary glands. The last nipple to be active may be distinguishable by shape/size.
Breeding	Waxy residue produced in pouch without distinction between nipples.
Pregnant	Pouch condition is comparable to a nulliparous pouch, but both nipples are swollen.
Reproductively active	
(a: Young in pouch)	Presence of young (may be attached to the nipple depending on stage of lactation and growth of young) and red, waxy residue in pouch; enlarged nipple and mammary gland. Pouch increases in size with the age of the young.
(b: Pouch vacation)	Pouch is closed around nipples so that young may not enter the pouch but suckling continues.
(c: Weaning)	Presence of dried red, waxy residue in an active pouch indicates that weaning is in progress.

Milk collection.—Marsupial milk composition changes during 3 distinct phases of lactation that correspond with the needs of the young. Early lactation is characterized by low-volume milk production and high carbohydrate and low lipid content, whereas late lactation is characterized by high-volume milk production and high lipid and low carbohydrate content (Green and Merchant 1988). We collected milk only from females in late lactation, defined as beginning at day 141–149 (4.7–5.0 months) of lactation (Cowan 1989; Crisp et al. 1989). Females in late lactation (with their dependent back young if present) were transferred from wire cage traps to a hessian sack where they were gently

restrained. Young were separated only after their mothers were anesthetized for milking and returned before she recovered. Anesthesia was induced using gaseous Isoflurane (Isorrane; Baxter Healthcare Pty Ltd, Old Toongabbie, New South Wales, Australia)) and an Ohmeda veterinary anesthesia machine with an Isotec-3 vaporizer (number MQA11000; Mediquip Pty Ltd, Meadowbrook, Queensland, Australia). To minimize the impact of anesthesia and assist in rapid recovery, the mother was placed on a heat pad, and anesthesia was induced slowly and maintained at a low level (0.5–1.5% Isoflurane). Induction took 3–4 min, while complete recovery took approximately 10 min. Once the mother was anesthetized, the young possum was secured in a pillow case. The mother was weighed and injected intramuscularly with oxytocin (a dose of 0.1 mL/kg of 10 i.u./mL Ilium Syntocin; Troy Laboratories, Glendenning, New South Wales, Australia) to assist with milk ejection. The active teat was cleaned with alcohol, and milk ejection was stimulated further by gentle manual manipulation of the teat and mammary gland. Up to 1 mL of milk was expressed manually and collected into a 1 mL Eppendorf tube. Milk samples were refrigerated in the field, and frozen at -20˚C until analysis.

While the mother was recovering on oxygen, the young was sexed, weighed, measured, and then placed with its mother in a hessian sack before the mother regained full consciousness. The mother and young were left in an unsecured sack in a sheltered spot (such as a hollow log) for self release. The bag was checked first thing the next morning to make sure that both mother and young had left.

Age and survival of pouch young and body condition of dependent young.—Pouch young were aged using the nomogram of Lyne and Verhagen (1957). Hocking (1981) suggested that the age estimates of Lyne and Verhagen (1957) were accurate until 120 days of age, so for young estimated to be older than 120 days at capture, only body masses are reported. Developmental landmarks of pouch young by age are provided in Appendix B. Survival of pouch young was calculated as the percentage of the total number of pouch young known to be born over the 2-year duration of our study that were known to survive to late lactation (i.e., become back young) based on presence of young in the pouch. Mortality of pouch young was distinguished by absence of the pouch young in the pouch and a quiescing teat and mammary gland. Body condition of back young was calculated as the

ratio of observed to expected body mass (Krebs and Singleton 1993; Lohr et al. 2009). A linear regression between head length (in mm) and body mass (in g) of back young was used to generate the equation for expected mass: body mass = -1762.2 + 34.938 x head length (R^2 = 0.644, n = 36) (Appendix C).

All animal capture and handling protocols were conducted with adherence to the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (Australian Government National Health and Medical Research Council 2004), were approved by the University of Tasmania Animal Ethics Committee (approval A0009118) and the Department of Primary Industries and Water, Parks and Wildlife (permits: FA 06558 (2006–2007), FA 07202 and TFA 07227 (2007–2008), and FA 09209 (2008–2009)), and were consistent with guidelines approved by the American Society of Mammalogists (Gannon et al. 2007).

Milk composition.—Total solid, lipid, carbohydrate, and protein content were measured as in Munks et al. (1991) and Rose and Flowers (2005) (protocols are provided in Appendix D). Total solids were measured to the nearest 0.1 mg by freeze-drying weighed quantities of whole milk. Total lipids were measured using the creamatocrit procedure (Fleet and Linzell 1964) and standardized using a microanalysis modification (K. Newgrain, Commonwealth Scientific and Industrial Research Organization, *pers. comm.*) of the Roese-Gottlieb ether extraction method (Horwitz 1980) to calculate grams of lipid. Results from the creamatocrit and ether extraction methods were compared by regression analysis to derive the equation: lipid (g/100 mL) = 1.159 creamatocrit - 0.9124 % (R^2 = 0.929, n = 64). Total carbohydrates were measured as hexose using the modified phenol-sulfuric method of Messer and Green (1979): β-lactose was used as a standard, and a 1:600 dilution of milk in distilled water was carried forth into the assay to maximize assay efficiency. Total proteins were measured using the Bradford assay (Bradford 1976), using Coomassie blue protein-binding dye. Bovine serum albumin was used as a standard, and milk was diluted 1:100 in distilled water. For these 2 assays, 6 milk samples from different sites were randomly selected from each year to generate a 'milk turbidity blank', which controls for and cancels out the matrix effects of milk turbidity in spectrophometric assays. To do this, the dilution of milk appropriate for each assay was added to distilled water in place of the reagent used

in each assay. The resulting absorbancies were averaged to create the milk turbidity blank absorbance, which was subtracted from all sample values.

All analyses were conducted in triplicate on whole milk. Samples were analyzed within 1 year of collection (in most cases, < 6 months), which is important for preservation of lipids (Munks 1990). Samples were aliquotted and subjected to no more than 3 freeze-thaw cycles to preserve the integrity of the milk. The milk was slowly thawed over several hours to maintain its integrity, kept cold on ice, and well mixed manually before assay.

Statistical analysis.—To assess differences in milk composition, a restricted maximum likelihood (REML) mixed model with factors disturbance, forest type, and disturbance*forest type as fixed effects and factor site as a random effect was performed on milk traits: solids (% weight/weight (% w/w)), proteins (g/100 mL), carbohydrates (g/100 mL), lipids (% w/w), and lipids (g/100 mL). Site was used as the error term for tests of the fixed effects. Differences in maternal body mass were analyzed in 2 ways. Differences in maternal body mass in relation to habitat were analyzed using two-way ANOVA with factors disturbance and forest type, performed on site means. Differences in maternal body mass in relation to reproductive status and reproductive output were analyzed using one-way ANOVA (factor = reproductive status (breeding versus quiescent) or number of young produced (1 or 2), respectively) performed on site means. To assess differences in timing of births (i.e., birth date), survival of pouch young, and body mass of back young two-way ANOVA with factors forest type and disturbance were performed on site means. Post hoc comparisons among means were performed using Tukey's studentized range (honestly significant difference) test. All statistical analyses were carried out using SAS 9.1 (SAS Institute Inc. 2003) and all results are reported ± 1 standard error (*SEM*).

Milk samples taken from the same female in different years were considered independent of each other. No inter-annual differences were detected in preliminary analyses of the data, so animal data for each site were pooled across years. Where no significant differences in a particular trait between forest type or disturbance were found, data were grouped from sites by treatment (forest type/disturbance): dry disturbed ('DD'), dry control ('DC'), wet disturbed ('WD'), and wet control ('WC').

RESULTS

Reproduction in females.—Over the 2-year duration of our study, 75 individual adult female brushtail possums were trapped across all sites; 24 of these females were caught in both years. Overall, 78 females bred; 18 of these individuals bred in both years of the study. No females bred twice in the same year, and no females that failed to breed in autumn/winter bred later in spring/summer.

Mean body mass of breeding (mean: 3.1 ± 0.03 kg, range: 2.4–4.0 kg, $n = 78$) and reproductively quiescent females (mean: 3.0 ± 0.06 kg, range: 2.7–3.7 kg, $n = 21$) did not differ ($F_{1,97} = 2.83$, $P = 0.096$, $n = 99$). Also, maternal body mass between disturbed (mean: 3.2 ± 0.05 kg, range: 2.4–4.0 kg, $n = 26$) and control sites (mean: 3.1 ± 0.05 kg, range: 2.4–4.0 kg, $n = 52$) did not differ ($F_{1,6} = 1.67$, $P = 0.244$, $n = 78$). Maternal body mass was greater ($F_{1,6} = 25.61$, $P = 0.002$, $n = 78$) in wet forest (mean: 3.4 ± 0.05 kg, range: 2.7–4.0 kg, $n = 32$) than in dry forest (mean: 2.9 ± 0.04 kg, range: 2.4–4.0 kg, $n = 46$). We found no evidence that heavier females produced more young ($F_{1,58} = 1.38$, $P = 0.246$, $n = 60$); females that produced 2 young had a mean body mass of 3.2 ± 0.09 kg (range: 2.8–4.0 kg, $n = 18$) and females that produced 1 young had a mean body mass of 3.1 ± 0.06 kg (range: 2.4–3.95 kg, $n = 42$).

Milk composition.—During our study, 64 milk samples (32 in 2007 and 32 in 2008) were collected from 54 individual females in late lactation. Total milk solids, proteins, and carbohydrates did not differ significantly between forest type (solids: $F_{1,10} = 0.74$, $P = 0.406$; proteins: $F_{1,10} = 0.01$, $P = 0.915$; carbohydrates: $F_{1,10} = 0.19$, $P = 0.680$) or disturbance (Table 4.3) (solids: $F_{1,10} = 0.64$, $P = 0.451$; proteins: $F_{1,10} = 1.24$, $P = 0.295$; carbohydrates: $F_{1,10} = 0.04$, $P = 0.849$).

Table 4.3.—Milk composition of brushtail possums in Tasmania by treatment (forest type/disturbance). Mean values are presented ± SEM. Asterisks indicate significant differences ($P < 0.05$) between treatments in ANOVA. Fat content varied and was highest in milk from females living in DC forest (differing significantly from milk of females from DD and WC forest, but not from milk of females in WD forest). w/w = weight/weight.

Treatment (forest type/disturbance)	n	Milk solids (% w/w)	Proteins (g/100 mL)	Carbohydrates (g/100 mL)	Lipids (% w/w)*	Lipids (g/100 mL)**
Dry control (DC) forest	21	28.4 ± 2.14	6.0 ± 0.75	5.0 ± 0.70	10.4 ± 1.59	10.9 ± 1.32
Dry disturbed (DD) forest	17	28.7 ± 2.99	6.5 ± 0.80	4.7 ± 0.56	7.0 ± 1.16	7.4 ± 1.05
Wet control (WC) forest	15	28.5 ± 2.89	5.5 ± 0.84	5.0 ± 1.06	7.8 ± 1.44	8.0 ± 0.90
Wet disturbed (WD) forest	11	30.6 ± 2.31	6.8 ± 0.86	5.0 ± 0.96	9.2 ± 1.76	9.1 ± 1.34

* $P = 0.044$; ** $P = 0.048$

Both the percentage and total grams of crude lipids were significantly influenced by an interaction between forest type and disturbance ($F_{1,10} = 4.97$, $P = 0.044$; $F_{1,10} = 4.65$, $P = 0.048$, respectively; Table 4.3). Both measures were highest in milk from brushtail possums living in DC forest; these values were significantly higher than those for DD and WC forest, but not those from WD sites (Table 4.3). Site DC1 (Table 4.1) contributed to the higher values of grams of lipids obtained for the DC treatment overall (likelihood ratio test of the REML random effect: $\chi^2_1 = 4.20$, $P = 0.040$), but not to the percentage of lipids (likelihood ratio test of the REML random effect: $\chi^2_1 = 2.70$, $P = 0.100$).

Timing of births.—In total, 89.7% (52 of 58) of all births occurred during autumn/winter, with the majority (44.8%) of births in May ($n = 26$). The mean date of birth across all sites during this season was 19 May ± 3.1 days (range: 1 March–5 July, $n = 52$). Timing of births (Table 4.4) did not differ between forest type ($F_{1,6} = 0.03$, $P = 0.875$, $n = 58$) or with habitat disturbance ($F_{1,6} = 0.00$, $P = 0.968$, $n = 58$). Mean date of birth was similar in disturbed (22 May ± 7.9 days; range: 13 April–18 June, $n = 11$) and control (19 May ± 3.3 days; range: 1 March–5 July, $n = 41$) forest. The mean date of birth in the northern wet forest sites (15 May ± 3.1 days; range: 20 April–23 May, $n = 22$) was a week before that in the southern dry forest sites (22 May ± 4.8 days; range: 1 March–5 July, $n = 30$). The remaining 10.3% (6 of 58) of births were 'out of season'; that is, outside the main March–July birthing season. However, 3 of these 6 births were to mothers known to have lost young earlier in the year.

Table 4.4.—Timing of births in brushtail possums ($n = 58$) in Tasmania by treatment (forest type/disturbance). Mean values are presented ± SEM. N/A = not applicable.

Treatment (forest type/disturbance)	Date of birth (autumn/winter)	N	Date of birth (spring/summer)	N
Dry control (DC) forest	24 May ± 5.2 days	24	15 Oct.	1
Dry disturbed (DD) forest	13 May ± 11.7 days	6	13 Oct. ± 25.5 days	2
Wet control (WC) forest	15 May ± 3.6 days	17	17 Oct. ± 8.0 days	3
Wet disturbed (WD) forest	14 May ± 6.5 days	5	N/A	0

Body condition and survival of young.—The mean body mass of young caught in October, at the onset of late lactation and pouch emergence, was 400.5 ± 25.0 g ($n = 10$); the smallest pouch young to emerge from the pouch was 390 g (at 162 days old). Young began to be captured away from (but in close vicinity of) their mothers at a minimum weight of 750 g. The heaviest young caught on its mother's back or in her direct vicinity was 1.05 kg, and the heaviest dependent young was 1.25 kg. Body condition of back young (mean: 1.1 ± 0.08, range: 0.64–2.85, $n = 36$; Table 4.5) did not differ by forest type ($F_{1,6} = 0.59$, $P = 0.479$) or disturbance ($F_{1,6} = 0.43$, $P = 0.542$).

Table 4.5.—Body condition index (as calculated in Lohr et al. 2009) of brushtail possum back young ($n = 36$) in Tasmania by treatment (forest type/disturbance). Mean values are presented $\pm$ SEM.

Treatment (forest type/disturbance)	Body condition index	n
Dry control (DC) forest	1.2 ± 0.19	13
Dry disturbed (DD) forest	1.1 ± 0.09	9
Wet control (WC) forest	0.9 ± 0.11	7
Wet disturbed (WD) forest	1.0 ± 0.11	7

Survival of pouch young born during the study was 86.2% ($n = 58$) across all sites, and we observed no pattern in pouch young survival by forest type ($F_{1,6} = 0.11$, $P = 0.755$) or disturbance ($F_{1,6} = 0.13$, $P = 0.731$; Table 4.6). Of the 8 females whose pouch young died, 1 female lost her young both years and 3 of the 8 females bred again in the year that their pouch young died. Although 2 of these females (body masses of 2.5 and 2.7 kg) had not bred previously, other primiparous mothers (body mass range: 2.4–4.0 kg) raised their young to independence.

Table 4.6.—Survival of brushtail possum pouch young (n = 58) in Tasmania by treatment (forest type/disturbance). Survival across all sites was 86.2%.

Treatment (forest type/disturbance)	Dead pouch young	Live pouch young	Survival (%)
Dry control (DC) forest	3	22	88.0
Dry disturbed (DD) forest	2	7	77.8
Wet control (WC) forest	3	16	84.2
Wet disturbed (WD) forest	0	5	100.0

DISCUSSION

Our results suggest that forest type and habitat disturbance do not affect the reproductive capacity of female brushtail possums. Individual brushtail possums appear to be resilient to disturbance, with little variation in reproductive traits between animals living in the different habitats we examined. Although previous studies have found that forest type and disturbance can influence population-level traits and breeding frequency in this species (Flynn et al. 2011; Hocking 1981) [Chapter 2], it appears that these population responses cannot be explained by variation in the reproductive traits we examined—milk composition and timing of births, survival, and body condition of young.

Differences in breeding success in brushtail possums have been attributed to maternal body mass, with heavier females breeding more often (Ramsey et al. 2002) and producing more young (Cowan 2001; Jolly et al. 1995). However, body mass of females at disturbed or control sites did not differ. Therefore, differences in female body mass do not explain the lower breeding frequency recorded at disturbed sites recorded by Flynn et al. (2011) [Chapter 2].

The timing of births was within the ranges reported for brushtail possum subspecies (Dunnet 1956; Gemmell 1995; Hocking 1981; Kerle 1984; Tyndale-Biscoe 1955). The timing of births to Tasmanian brushtail possums in wet forest varied within this range (up to 37 days) according to the age of the forest regenerating after fire and the resultant nutrient content of the vegetation (Hocking 1981). However, in our study habitat disturbance did not affect the

timing of births; timing did vary slightly (± 1 week) between wet and dry forest, although this could have been an artifact of latitude rather than forest type. This was most likely due to differences in temperature (the north of Tasmania is ~3–6˚C warmer than the south—Australian Bureau of Meteorology 2009b) and the resulting time of flowering of important food plants such as *Eucalyptus* and *Acacia* species (E. M. Flynn, pers. obs.). The mean date of birth (19 May) across all populations in our study (Fig. 4.2) matched that (17 May) recorded by Hocking (1981), suggesting that the timing of births in the brushtail possum in Tasmania appears to have remained consistent over the past 30 years.
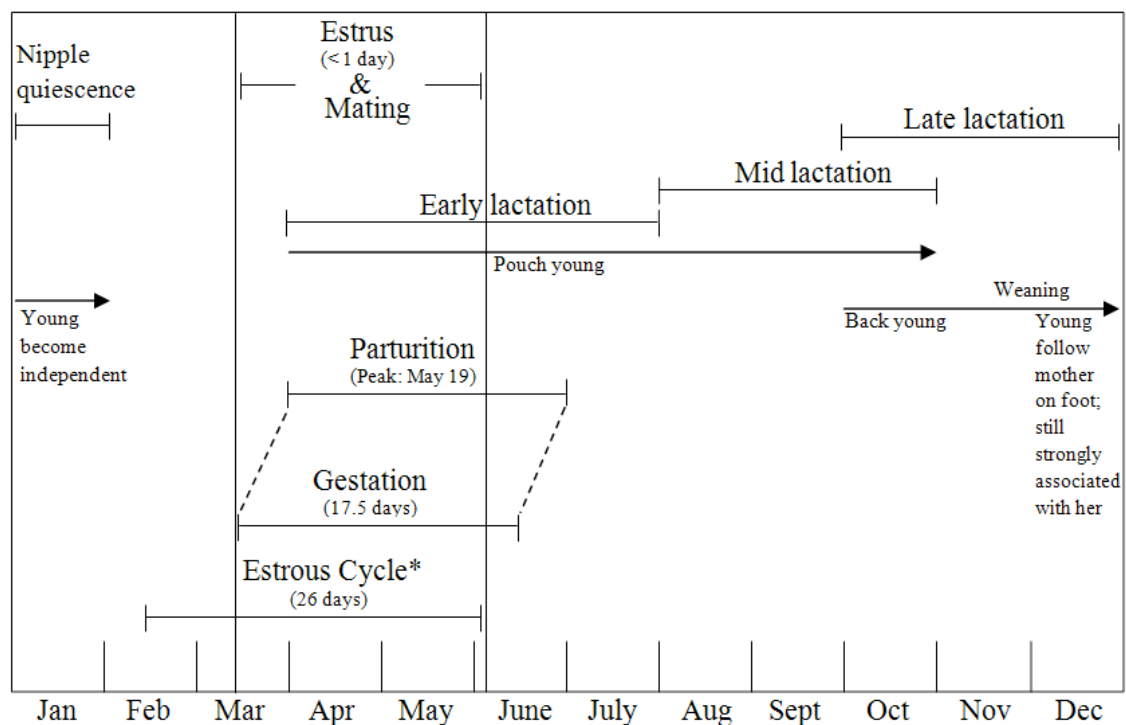
FIG. 4.2. —The reproductive calendar of the female brushtail possum (*Trichosurus vulpecula fuliginosus*) in Tasmania (based on observations from this study and using information from Cowan (1989), Crisp et al. (1989), Hocking (1981), and Pilton and Sharman (1962)). *Note: only 1 breeding season occurs in Tasmania.

In contrast to brushtail possum populations on mainland Australia and New Zealand (Cowan 1990a; Green 1984b; Kerle and Howe 1992), no additional peak of births occurred in late spring/summer in the Tasmanian populations we examined. Although some young were recorded outside the main autumn/winter season, these were probably a consequence of

mortality of offspring earlier in the season rather than a distinct second breeding peak. The lack of a second peak in births in spring/summer could be due to the more seasonal nature of the food supply in Tasmanian forests; this was particularly critical during the period of our study due to drought. In contrast, brushtail possums in urban settings in Tasmania breed throughout the year (E. M. Flynn, pers. obs.; Statham and Statham 1997), presumably reflecting more reliable food sources where suitable refuge sites exist (Green and Coleman 1987; Harper 2005; Statham and Statham 1997).

Survival of pouch young was similar to that reported in other studies (Hocking 1981; Kerle 1984, 1998). However, offspring survival can be highly variable between populations, years, and mothers. Although survival usually ranges between 83–87%, it has been reported as low as 52% (Kerle 1984). Hocking (1981) found that survival of pouch young was higher in populations living in recently burned sites than in mature forest. In contrast, survival of pouch young was similar (range: 80.0–100.0%) across all the populations we examined.

The nutritional composition of milk was within previously reported ranges for late lactation milk for brushtail possums from 3 different populations (Cowan 1989; Crisp et al. 1989; Gross and Bolliger 1959). Milk solids, proteins, and carbohydrates were very similar between sites, but grams of milk lipids were significantly higher at 1 site, DC1 (Table 4.1), than at all others. Milk lipids represent the major source of energy in milk (Jenness 1986) and are important for the condition and survival of young, but body condition of the back young did not differ at site DC1. Similarly, Noblet and Etinenne (1986) found that the growth and body mass at weaning of piglets fed high-fat milk remained similar to those fed milk with normal fat content. However, body composition was different, with a significantly higher proportion of body fat in piglets fed on high-fat milk. Thus, even subtle differences in milk composition can affect the growth and development of mammalian offspring and therefore influence long-term fitness. Milk composition can influence the survival of young mammals, thus linking environment and population dynamics (McMahon and Burton 2005). Such links are complex in marsupials because their milk composition changes markedly from early to late lactation (Green and Merchant 1988). Therefore, although we detected only minor differences in the composition of late lactation milk between sites, it is possible that disturbance or forest type influenced milk composition during earlier stages of lactation.

Differences in milk composition, particularly fat and protein content, can be diet-driven (Chilliard et al. 2001; Del Prado et al. 1997; Griffiths et al. 1988; Jenness 1985; Palmquist et al. 1993). Females at DC1 might have had access to a more varied diet (brushtail possums eat a wide range of foods including *Eucalyptus* leaves, insects, bird eggs, carrion, fungi, and blossoms—Cowan 1990b; Nugent et al. 2000) or to high abundance of a food item, resulting in higher milk fat content. We did not measure milk output. However, indices of food were approximately equivalent between study sites (Flynn et al. 2011) [Chapter 2], so it is unlikely that food supply affected total milk production.

Contrary to our initial hypothesis, this study shows that the reproductive capacity of individual brushtail possums in Tasmania is not affected by habitat type or disturbance history. However, a population-level study at the same sites (Flynn et al. 2011) [Chapter 2] reported a male-biased adult sex ratio and lower abundance and breeding frequency in brushtail possums in disturbed compared to control forest sites, thus fewer young were recruited at disturbed forest sites. It appears that in brushtail possums, population size and breeding frequency are more influenced by the availability of suitable foraging or refuge habitat rather than environmental influences on the physiology of individual animals. For example, the lower density of hollow-bearing trees at disturbed sites (Flynn et al. 2011) [Chapter 2] can limit the number of breeding females that the habitat can support (Koch *et al.* 2008a). Undisturbed, mature forests likely represent higher-quality habitat to possums because tree hollows suitable for breeding can take more than 140 years to develop (Koch et al. 2008b). Hocking (1981) found overall higher breeding success in brushtail possums living in recently burnt versus mature forest, but this could be because the harvest prior to the burn at his sites was not as intensive or extensive as the harvesting at our study sites, so that more tree hollows persisted. After fire, soil fertility (O'Connell et al. 1979) and density and nutritional content of vegetation (Ahlgren and Ahlgren 1960; Attiwill 1980; Cowan et al. 1950) often increase in the short-term. An increase in food quality or quantity following post-harvest burning could have allowed brushtail possums to compensate for reduction in suitable tree hollows. These factors, and the differences in the age of the sites post-harvest and burn, might account for the differences in breeding frequency, pouch young survival, and abundance of young between our study and that of Hocking (1981). Taken with the results of earlier work (Flynn et al. 2011) [Chapter 2], our results underscore the observation

that brushtail possums are physiologically resilient (Barnett et al. 1979) and able to cope with habitat disturbance at the individual level. However, the loss or alteration of habitat features such as tree hollows that are critical for reproduction might reduce the reproductive capacity of a brushtail possum population, resulting in lower population densities.

ACKNOWLEDGMENTS

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Appendices

Appendix A. Use of pouch condition to assess reproductive status of females (synthesised from Hocking (1981), Sharman (1962), and observations from this study)

Pouches of nulliparous females are clean, have very small and inactive nipples, and lack the red/brown waxy residue that denotes the presence of previous young (a), whereas the pouches of females that have previously produced young (but are currently reproductively quiescent) lack an active/enlarged nipple or mammary gland and have dried red waxy residue inside (b). In some cases the last nipple and mammary gland to be active is still distinguishable by shape or length from the unused nipple and mammary gland.

When a female is breeding, her pouch is very waxy without distinction between nipples (c). A pregnant female's pouch is extremely clean (comparable to a nulliparous pouch) with swollen nipples (d).

Pouches of females that are reproductively active (have produced young) are denoted by several factors: presence of a young in the pouch (dependent on the stage of lactation and growth of young), wet red waxy residue, an enlarged nipple and mammary gland (with/without young attached), and/or a clean, moist, warm pouch (e). The pouch grows with the young throughout lactation.

Near the end of lactation, pouches tighten such that the young can no longer enter them ('pouch vacation') (f). Weaning follows shortly after this occurs (denoted by the presence of red/brown waxy residue).

(a) Pouch of nulliparous female

(b) Pouch of female that is currently reproductively quiescent with signs of previous young (note: the nipple and mammary gland on the left can be distinguished the from those on the right as the last to be active)

Photo by Susan M. Jones

(c) Pouch of breeding female

(d) Pouch of pregnant female

Photo by Lisa Cawthen

(e) Pouch of a female in late lactation (reproductively active): note active, enlarged teat and mammary gland on the left and the size and cleanliness of the pouch (pouches of reproductively active females in earlier stages of lactation are smaller and aredenoted by the presence of young)

Photo by Lana Tremayne

(f) Pouch of a female in late lactation (reproductively active) that has undergone pouch vacation (the pouch has tightened around the nipples so that young can no longer enter); the red/brown waxy residue indicates that weaning is occurring (Hocking 1981)

Appendix B. Developmental landmarks of pouch young

The age at appearance of these traits was similar across sites although exact comparisons are difficult because young were not necessarily caught at all sites at appropriate ages.

Table 1.–Timing of developmental landmarks observed in pouch young of brushtail possums in Tasmania across all sites during our study ($n = 58$). Age was estimated using the nomogram of Lyne and Verhagen (1957).

Developmental landmarks	Age of young (days)*
Movement	13**
Vibrissae	19
Claws: white/delicate	19
Claws: yellow/strong	24
Ear and nose pigment	38
Vocalisation	41
Guard hairs	89
Eyes open	100
'Velvet' fur	112
Fully furred	126

*Approximate age only, as observations were not made every day of development. Some landmarks may have been expressed before being first observed.

**Young can be sexed from this early age because the scrotum is discernable at/near birth (Ullmann 1993).

Appendix C. Body condition of back young
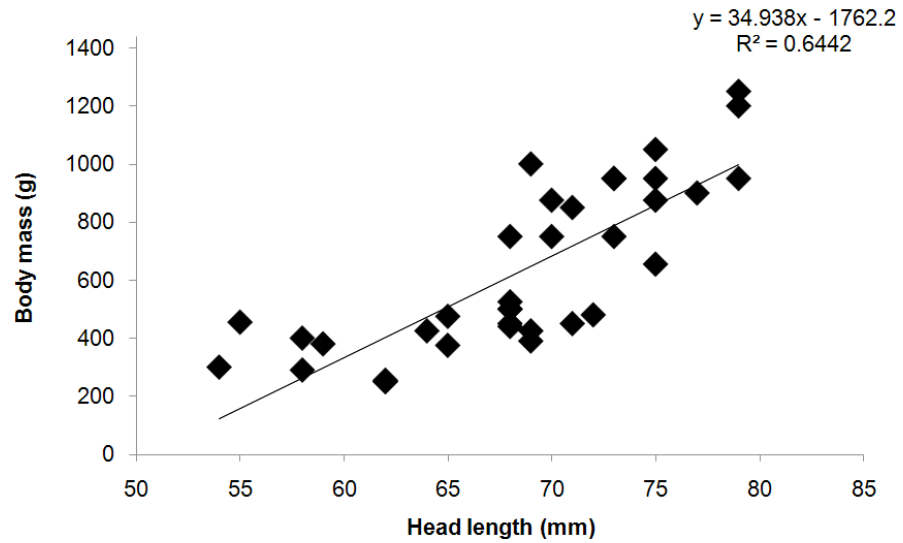
FIG. 1.–The body condition index of back young was calculated as the ratio of observed to expected body mass for each individual ($n = 36$) (Krebs and Singleton 1993; Lohr et al. 2009). Linear regression between head length (mm) and body mass (g) was used to generate the equation for calculating 'expected' values: expected mass (g) = -1762.2 + (34.938 x head length (mm)).

Appendix D. Milk Analysis Protocols

<u>Total solids</u>: Total solids were assessed by measuring out 100 µL of whole milk, transferring it to an Eppendorf tube, and subtracting the mass of the tube to calculate the mass of the milk. Weight was measured to the nearest 0.01 mg with a BP61 Sartorius Analytical Balance (Lower Saxony, Germany). Open tubes of milk were cooked at 100°C for 5 days (120 hr) until weight did not change between subsequent weighings, ensuring that all liquid evaporated. Upon removal from the oven, tubes were immediately capped to prevent moist air from entering the tubes, then cooled for 30 min in a desiccating chamber before final weighing.

<u>Total lipids</u>: Total solids were assessed by the creamatocrit method (Fleet and Linzell 1964). Roughly 60 µL (3/4 tube) of well-mixed whole milk were drawn by capillary action into a standard unheparanised 75 µL microhaematocrit capillary tube. One end of the tube was plugged with plasticine and the tube was run in a Sigma 1–15 haematocrit centrifuge (Lower Saxony, Germany) at 3,000 RPM for 15 min. Tubes were immediately stood upright to prevent the lipid from setting at a slant. Total length of liquid in the capillary tube and total length of lipids (solid and lipid fraction) were measured with vernier callipers to the nearest millimetre.

<u>Calculations were as follows:</u>
Creamatocrit (percentage of fat (solid and liquid fractions)) = (length of lipids/ length of total liquid)*100

The results were then standardised using a micro-analysis modification (K. Newgrain pers. comm.) of the Roese-Gottlieb ether extraction method (Horwitz 1980) to calculate grams of lipid. When working with such small amounts, it is important to avoid hygroscopic plastic (K. Newgrain pers. comm.). The dried, residual solids from the milk solid analysis were manually emulsified. Afterward, they were subjected to alternating washes of petroleum ether and diethyl ether to dissolve and extract the lipids from the solids. The ether from each

wash was saved and evaporated off, leaving behind the lipids. All weights were measured to the nearest 0.01 mg with a BP61 Sartorius Analytical Balance (Lower Saxony, Germany). Results from the creamatocrit and ether extraction methods were compared by regression analysis to derive the equation:

$y = 1.159x - 0.9124$ ($r^2 = 0.929$, $n = 64$) (Fig. 1),

where 'y' is lipid g/100 mL of milk from the ether extraction method and 'x' is percent crude lipids from the creamatocrit method.
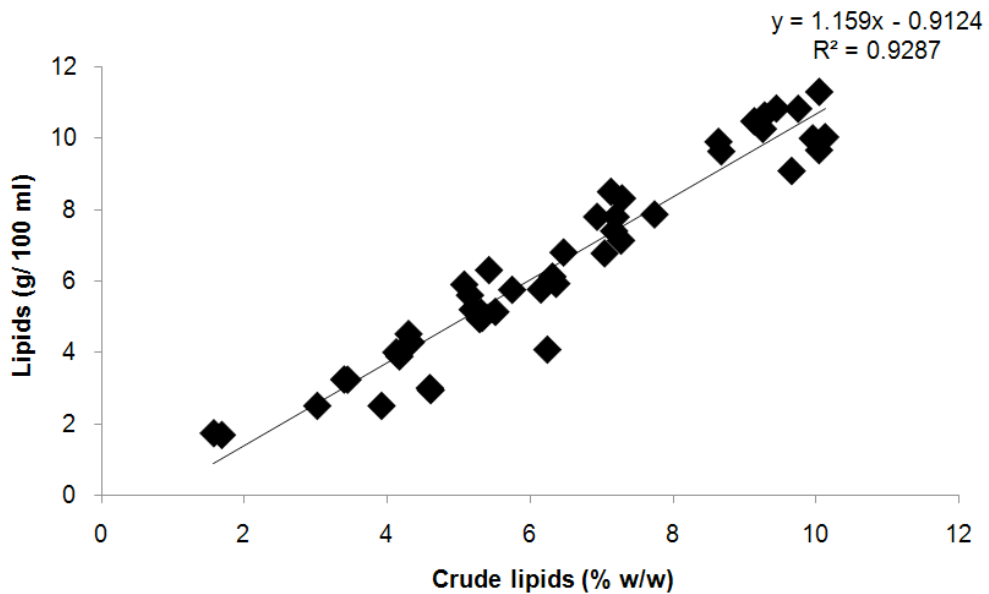
FIG. 1.—Linear regression of the results of both the creamatocrit assay (Lucas et al. 1978) and Roese-Gottlieb ether extraction method (Horwitz 1980; K. Newgrain pers. comm.) used to calculate the standardised value of g/100 mL of lipid in whole milk.

Total carbohydrates: Total carbohydrates were assessed by the phenol-sulfuric method (Dubois et al. 1956) as modified by Messer and Green (1979); this assay's prime range of efficiency is for up to 100 µg of carbohydrate. 10 µL of well mixed whole milk was mixed into 6 mL of distilled water to maximise the efficiency of this assay. In order for this assay to work properly, it must be conducted in a test tube so that the heat dissipates properly. 200 µL of dilute milk was combined with 1 mL of 3.55% phenol solution (17.75 g phenol (Sigma-Aldrich, Missouri, USA), volumed up to 500 mL with distilled water). 3 mL of 100% sulphuric acid was added rapidly, directly to the surface of the liquid (avoiding the

walls of the test tube). The spectrophotometer was first zeroed using distilled water, then again with a reagent blank (200 µL of distilled water in 1 mL phenol solution and 3 mL sulphuric acid). After 30 min, samples were read at 490 nm using a Philips PYE UNICAM SP6-550 UV/VIS spectrophotometer (Amsterdam, Netherlands). A standard was generated by serially diluting a 1g/L (100 µg) β-Lactose (L3750-100G; Sigma-Aldrich, Missouri, USA) and distilled water solution.

6 samples from different sites were randomly selected (from each year) to generate a 'milk turbidity blank' to control for and cancel out the matrix effects of milk turbidy in this spectrophometric assay. To do this, 200 µL of dilute milk was added to 4 mL of distilled water (replacing the volume of the reagent used in the assay), and resultant absorbencies were averaged to create the milk turbidity blank absorbance value, which was subtracted from all samples.

<u>Total proteins:</u> Total proteins were analysed by the Bradford method (Bradford 1976); this assay's prime range of efficiency is 10-100 µg of protein. 50 µL of well mixed whole milk was mixed into 5 mL of distilled water to maximise the efficiency of this assay. 100 µL of dilute milk was combined with 5 mL of Bradford reagent (100 mg of Coomassie Brilliant Blue G (Sigma-Aldrich, Missouri, USA) dissolved in 50 mL 95% ethanol, 100 mL 85% (w/w) phosphoric acid, volumed up to 1 L with distilled water, and filtered with high grade laboratory filter paper). The spectrophotometer was first zeroed using distilled water, then again with a reagent blank (100 µL of distilled water in 5 mL of Bradford reagent). After 1 hr, samples were read at 595 nm by a Philips PYE UNICAM SP6-550 UV/VIS spectrophotometer (Amsterdam, Netherlands). A standard was generated by serially diluting a 1 g/L (100 µg) solution of bovine serum albumin (BSA) (A2153-10g; Sigma-Aldrich, Missouri, USA) dissolved in distilled water (refrigerate overnight to eliminate 'foaming' and increase dissolution of protein).

Milk turbidity blanks were created and used for this assay in the same manner that they were for the protein assay, but for this assay 100 µL of dilute milk was added to 5 mL of distilled water (replacing the volume of the reagent used in this assay).

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Chapter 5

Resource availability drives male-biased sex ratio in disturbed habitats in an arboreal mammal

Brushtail possum (*Trichosurus vulpecula*) trapping, processing, and habitat (tree hollows)

Abstract

The influence of habitat disturbance on genetic variation and persistence of fauna is a critical focus in conservation biology. This paper integrates ecology and genetics to investigate causal mechanisms of a sex ratio bias and uses genetics to investigate the severity of impacts of habitat disturbance on fauna. Genetic analysis suggests that there are two populations of brushtail possums influenced by geographic distance, with near-random mating and only moderate differentiation, across the east coast of Tasmania, the island state of Australia. Moderate genetic diversity suggests that these populations are unlikely to have experienced small population sizes in the past. Whilst habitat disturbance did not affect the mating system or genetic diversity of the brushtail possums in this study, an adult male sex ratio bias indicates that adult female-specific resources have declined to the extent that there has been a response in population dynamics. This suggests that current forestry management practices, while eliciting an intermediate population response, are not causing genetic erosion. However, *Eucalyptus* forests regenerate slowly and populations may suffer genetic erosion if refuge abundance does not increase until mature forest elements are sufficient to provide habitat for full population recovery. This requires further monitoring if long-term ecological impacts (e.g., further forest harvesting or severe storm/wildfire before the sites have regenerated) continue.

Introduction

Integrating ecological and genetic approaches can provide new insights into how the cascading effects of habitat disturbance affect fauna. Such knowledge critically underpins scientifically defensible conservation management actions that will preserve ecosystem integrity. Disturbance results in changes in habitat structure, integrity, connectivity, and the spatial pattern of resource availability at a broad scale; as such, it can impact persistence of individual species as well as altering community structure in both plants and animals (Dodson and Gentry 1991; Krebs 2001). The ultimate impact of forest disturbance on mammal communities depends on a species' degree of forest-dependence (Tyndale-Biscoe and Calaby 1975), as well as the intensity (Kavanagh and Stanton 2005), spatial scale (Dumbrell et al. 2008), and temporal scale of the disturbance (Fisher and Wilkinson 2005). Forest-dwelling species thought to be most at risk from habitat disturbance are those with specialised diets or habitat requirements (e.g., tree hollows), low fecundity, or low dispersal

potential (Lindenmayer et al. 2003; Michaels 2000; Weins 1997). Increasing severity of habitat disturbance causes different levels of faunal responses, beginning with population dynamics and ecological processes, then physiological responses, reproductive ability, and finally genetic influences (Carey 2005; Gaines et al. 1997; John-Alder et al. 2009; Lindenmayer and Franklin 2002).

Genetic responses of species to habitat disturbance are influenced by ecological parameters such as life history, dispersal ability, and sensitivity of demographic parameters to environmental variation (Gibbs 2001). Habitat disturbance can disrupt population gene flow through decreased dispersal, increasing both genetic drift and inbreeding, and altering genetic population structure, particularly if the disturbance results in population isolation (Cushman 2006; Gibbs 2001; Lacy 1997; Moore et al. 2008; Whitlock and Barton 1997). Variance in sex ratio, changes in mating system, and decreased genetic diversity in response to habitat disturbance may alter the fitness, resilience, and long-term adaptability of populations, compromising their ability to respond to environmental challenge (Frankham 1995; Hoffmann and Parsons 1991; Lacy 1997; Pray et al. 1994). However, genetic impacts may not always be detected because habitat loss, replacement by exotic competitors, and the demographic instability that accompanies low abundance may prevent populations from persisting long enough to be impacted by genetic processes (Caughley 1994). Demographic instability can make populations more susceptible to environmental fluctuation and catastrophes, and increase the likelihood of local extinction (Frankham et al. 2002; Lacy 1997). Differences in sex ratios between populations may reflect adaptation to local ecological conditions (Johnson and Ritchie 2002) although little is known about adaptive causes or the mechanisms through which this occurs (Cockburn et al. 2002).

Mating systems, which are influenced by resource abundance (Clutton-Brock 1989), affect the long-term survival of populations through their influence on gene transfer between generations. The influence of habitat disturbance on mating systems is not well studied even though changes in mating system can have serious implications for long-term population persistence. For example, multiple paternity can increase genetic variation in a population (Sugg and Chesser 1994), while high reproductive skew can cause a severe decrease in genetic diversity (Anthony and Bumstein 2000). Loss of genetic diversity, the most severe

impact of habitat disturbance, can compromise the fitness of individuals and the ability of species to respond to environmental challenge and change (Frankham 1996; Frankham et al. 2002).

There are few studies that focus specifically on the genetic implications of habitat disturbance in forest fauna. Ideal focal species for investigating responses to habitat disturbance are those that require slow-forming mature forest elements or trees for foraging, nesting, or movement such as arboreal vertebrate populations. Microbats, marsupials, monkeys, rodents, birds, and reptiles (Carey 1996; Kays and Allison 2001; McIlroy 1978) are dependent on standing trees with mature forest elements, and are strongly affected by structural damage from logging (Laurance 1996), with populations generally recovering slowly after forest harvesting (Alexander et al. 2002; Cork and Catling 1996; Tyndale-Biscoe and Calaby 1975). In Australia, 303 vertebrate species are dependent on tree hollows (10% of reptiles, 13% of amphibians, 15% of birds, and 31% of mammals) (Gibbons and Lindenmayer 2002). Arboreal marsupials are considered to be the faunal group most susceptible to disturbance from forestry and agriculture in Australia because of their specific habitat requirements (Goldingay and Daly 1997; McIlroy 1978; Tyndale-Biscoe and Calaby 1975). Structural attributes of the forest, in particular tree hollows (Gibbons and Lindenmayer 2002; Koch et al. 2008), have been shown to be vital habitat components for arboreal marsupials (Cork and Catling 1996; Pausas et al. 1995).

The brushtail possum (*Trichosurus vulpecula*), a medium-sized, arboreal, omnivorous marsupial that is dependent on tree hollows for refuge and breeding (Fitzgerald 1984; Gibbons and Lindenmayer 2002; Kerle 1984; Nugent et al. 2000; Sadlier 2000), is an ideal focal species for studies of forest habitat disturbance. Studies on mainland Australia indicate that habitat disturbance, through its effect on resource availability and dispersal (e.g., patchiness), can result in a bias in sex ratio of brushtail possum offspring (Isaac et al. 2005; Johnson et al. 2001) and changes in adult sex ratio and mating system in the mountain brushtail possum (*Trichosurus cunninghami*) (Martin and Handasyde 2007; Martin and Martin 2007). In mountain brushtail possums, the mating system reflected patterns of resource distribution: polygyny (the natural mating system) was observed in populations living in unlogged forest where higher levels of resources increased interactions between possums; monogamy was

observed in populations in logged areas, where possums infrequently came into contact because resources were spatially dispersed. In Tasmania, Hocking (1981) found an initial and short-lived male bias in adult sex ratio post-burn that reflected sex-differential survival (favouring males), followed by a reduction in breeding success, survival of pouch young and juveniles, growth rates, and population density, which he attributed to a decrease in quality and abundance of food as the forest regenerated. Flynn et al. (2011a) [Chapter 2] found that while brushtail possum population sizes were similar between wet and dry *Eucalyptus* forest, there were significantly fewer animals in disturbed (logged) than undisturbed habitats, probably in response to habitat 'quality' and resource abundance. As genetic variation is related to population size, such population reduction may compromise the ability of species to adapt genetically to environmental challenge (Frankham 1996). In addition, Flynn et al. (2011a) [Chapter 2] found that adult sex ratios differed between disturbed and control sites: populations living in disturbed sites in both wet and dry forest exhibited male-biased adult sex ratios (66:34 male:female), while populations in undisturbed (control) sites in both forest types displayed adult sex ratios at parity (38:40 male:female), which is typical of the species (Hocking 1981; Hope 1972; Winter 1976).

In this study, we integrate ecology and genetics to investigate the effects of habitat disturbance on sex ratio variance, mating systems, and genetic diversity in the Tasmanian brushtail possum (*T. v. fuliginosus).* Most studies of this nature focus on birds because they are widespread, easily identifiable, and many aspects of their biology which help scientists interpret molecular results (such as ecology, reproductive strategies, physiology, and morphometrics) have been well studied (Mindell 1997). Arboreal mammals have received little attention in this respect. We will address the following questions.

1. What is the mechanism behind the observed sex ratio variance? The following alternative hypotheses were tested:
    a. A bias in male offspring is produced through maternal sex allocation, the ability of the mother to choose the sex of her offspring to the benefit of herself and/or offspring. There are two hypotheses to explain how this may occur. The Trivers-Willard hypothesis proposes that maternal age and body condition

influence the sex of offspring and that maternal investment affect each sex differently (Gaulin and Robbins 1991; Hrdy 1987). The local resource competition hypothesis proposes that sex allocation results from maternal competition with offspring based on dispersal and philopatry in light of restricted resources (Clark 1978; Hewison and Gaillard 1995; Silk 1983). To identify the origin of the adult sex ratio bias, we examined the sex ratio of offspring, maternal body condition, and resource availability.

b. If there is no male bias in dependent young, then reduced dispersal of subadult males may skew subsequent adult sex ratio. To explore this, we examined sex-related recruitment between age cohorts using population age structure and conducted population assignment tests to estimate the proportion of adult males that were immigrants.

c. If there is no sex bias in the younger age cohorts, the adult male sex bias may result from immigration of adult males into the sites. To address this hypothesis, we assessed abundance, relatedness, and the proportion of immigrants of adult males.

d. If there is no significant immigration of adult males, then the number of adult females may be limited at these sites. To determine whether this is the case, we assessed female abundance, breeding success, and resource availability.

2. Is the level of habitat disturbance sufficient to affect the mating system? We assessed dispersal decisions (population assignment tests to identify the origin of individuals and to detect first generation immigrants) and paternity.

3. Is the level of habitat disturbance sufficient to affect genetic diversity? We assessed measures of genetic diversity and population structure within and among sites.

The outcomes of this research will improve knowledge of the effect of habitat disturbance on the population and genetic structure of an arboreal mammal over the period from 4–11 years post harvest. This information will inform forest managers of the outcomes of current management prescriptions in relation to arboreal mammals.

Materials and methods

Study sites and animal capture

This study was carried out at 10 study sites across eastern Tasmania, the island-state of Australia (Flynn and Jones in prep) [Chapter 3] (Figure 5.1, Table 5.1). Six sites were located in dry *Eucalyptus* forest in southeast Tasmania: three were harvested (disturbed) and three were relatively undisturbed and served as control sites. Four sites were located in wet *Eucalyptus* forest sites in northeast Tasmania: two were harvested and two were undisturbed. All disturbed sites were logging coupes in State Forest and were logged 4–11 years ago. Relatively undisturbed (control) sites were dominated by mature forest and subject to storms and wildfire. All study sites were embedded within a surrounding matrix of mature or older-aged regenerating forest. Sites had a mean area of 38.57 ha (range: 21.49–62.30 ha). Dry forest sites received 423.8–651.1 mm of rainfall per year and wet forest sites received 884.4–1190.6 mm of rainfall per year. Dry forest sites were 301–398 m above sea level and wet forest sites were 184–529 m above sea level). The geology of the sites, which influences soil type, is adamellite for the wet forest sites, while in the dry forest sites, DD1 and DD2 grow on dolerite and DD2, DD3, DC2, and DC3 are on sandstone.

Study sites were trapped twice yearly (during spring/summer and autumn/winter) from spring/summer 2007–spring/summer 2008. Hessian-covered 300 x 300 x 660 mm wire Mascot cage traps (Mascot Wire Works, New South Wales, Australia) were deployed in each site for an average spatial trapping effort of 1.77 traps/ha (range: 1.08–2.73 traps/ha) (Flynn et al. 2011a) [Chapter 2]. Traps were deployed in 50 x 20 m spacing in two transects per site over four nights per trip: one night of prebaiting and three nights of active trapping. Traps were baited with ¼ apple and a small ball of peanut butter, vanilla essence, and oats. Checking traps and processing animals commenced at dawn in an effort to minimise stress in captured animals (Johnson and McIlwee 1997).
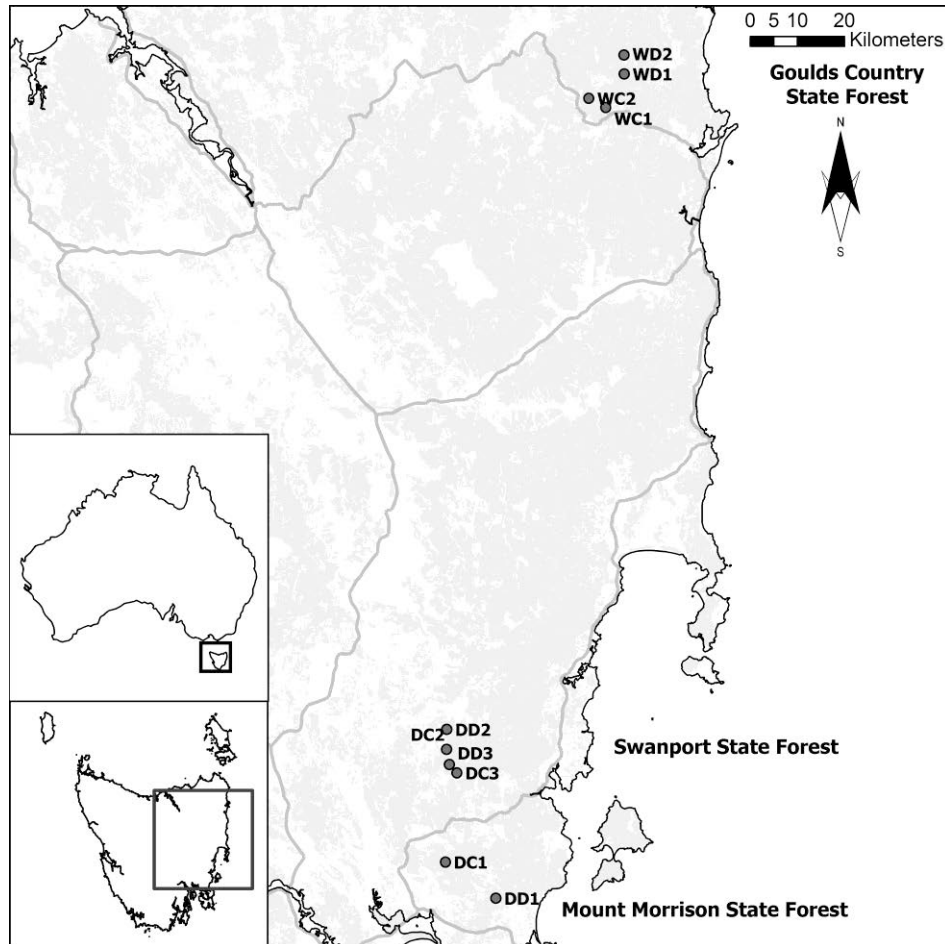
Figure 5.1. Location of study sites across eastern Tasmania, the island state of Australia. Dry *Eucalyptus* forest sites are in the southeast and wet *Eucalyptus* forest sites are in the northeast of the state. Shading indicates forested area while lack of shading denotes non-forest area (agriculture, scrub, lakes, urban areas, etc.). Solid lines denote major roads (DPIW 2001; Harris and Kitchener 2005). (Treatment (forest type/disturbance) is indicated by the first two letters of a site name, followed by the site number: 'DD' = dry disturbed, 'DC' = dry control, 'WD' = wet disturbed, and 'WC' = wet control forest.)

Table 5.1. Description of study sites including forest type classification and both method and date of harvest of disturbed sites

Site name	Broad forest type[1]	Silvicultural method	Harvest date (yr)[7]
DD1	*E. pulchella - E. globulus - E. viminalis* grassy shrubby forest [Dry]	Partial harvest (SED, AGR)[2]	2000/2004
DD2	*E. obliqua* forest [Dry]	Partial harvest (SR, AGR)[3]	1998/1999
DD3	*E. amygdalina* forest on sandstone [Dry]	Partial harvest (SED, SR)[4]	1997
DC1	*E. obliqua* forest [Dry]	N/A	N/A
DC2	*E. obliqua* forest [Dry]	N/A	N/A
DC3	*E. amygdalina* forest on sandstone [Dry]	N/A	N/A
WD1	*E. regnans* forest [Wet]	Commercial thinning[5]	2002
WD2	*E. regnans* forest [Wet]	Clearfell, burn, and sow (CBS)[6]	2002
WC1	*E. regnans* forest [Wet]	N/A	N/A
WC2	*E. regnans* forest [Wet]	N/A	N/A

[1] Broad forest community (DPIW 2001; Harris and Kitchener 2005)

[2] Mosaic of seedtree retention (SED) (resulting in 7–12 trees/ha) and advanced growth retention (AGR) (basal area reduced to < 4 m^2/ha) followed by a low intensity burn for regeneration (Wilkinson 1994)

[3] Mosaic of shelterwood retention (SR) (basal area reduced to12–14 m^2/ha in wet forest and 9–12 m^2/ha in dry forest) and advanced growth retention (AGR) (basal area reduced to < 4 m^2/ha) followed by a low intensity burn for regeneration (Wilkinson 1994)

[4] Mosaic of seedtree retention (SED) (resulting in 7–12 trees/ha) and shelterwood retention (SR) (basal area reduced to 12–14 m^2/ha in wet forest and 9–12 m^2/ha in dry forest) followed by a low intensity burn for regeneration (Wilkinson 1994)

[5] Stocking reduced to at least 200 well-formed trees/ha; no regeneration burn (Wilkinson 1994)

[6] All live trees (> 5 m) are felled, followed by a regeneration burn and seed sowing (Wilkinson 1994)

[7] From Timber Harvesting Plan for the coupe (Forestry Tasmania, unpublished data)

Upon capture, all back young, juvenile, subadult, and adult brushtail possums were processed as in Flynn et al. (2011a; 2011b) [Chapters 2 and 4]. Animals were sexed and tagged with a subcutaneous ISO FDX-B transponder chip (passive integrated transponder (PIT) tag). For animals > 1 kg, body mass was weighed with a Salter 10 kg balance to the nearest 50 g.; back young < 1 kg where weighed with a Salter 1 kg balance to the nearest 5 g. A suite of morphometric measurements were taken with vernier calipers to the nearest millimetre for size, body condition, and ageing purposes: head, leg, pes, and tail length, as well as the left testis length and width on males, excluding the epididymus. Testis volume was calculated from the equation for an oblate sphere: $V = \frac{\pi}{6} W^2 L$, where V = testis volume (mm^3), W = width, and L = length (Abbott and Hearn 1978; Hocking 1981). Relative age (adult, subadult, juvenile, back young, pouch young) was determined based on these parameters (Flynn and Jones in prep) [Chapter 3]. In Flynn and Jones (in prep) [Chapter 3] a small blood sample was taken from the lateral ear vein of brushtail possums and analysed for plasma cortisol concentration (a proxy for adrenal responsiveness) and haematocrit (a proxy for body condition). Results for both these parameters in adult males were reanalysed in the context of the current study. In addition, tissue samples were collected for genetic analysis from each brushtail possum. Pouch young were not sampled for ethical reasons. Duplicate 3 mm biopsies were collected from the base of the ear (so as not to affect ear erection) and stored in 70% ethanol.

Genetic analysis

DNA extracted from 209 individuals using the HotSHOT (salt extraction) method (Truett et al. 2000) was genotyped using six brushtail possum microsatellite loci (Tv16, Tv19, Tv53, Tv54, Tv58, and Tv64) (Taylor and Cooper 1998). Allelic diversity (A), and observed (H_O) and expected (H_E) heterozygosities were calculated using GENALEX Ver. 6.3 (Peakall and Smouse 2006). Genotypic linkage disequilibrium and deviation from Hardy-Weinberg expected genotypic proportions were assessed via exact tests (option 1: 1000 dememorizations, 1000 batches, 1000 iterations) by GENEPOP Ver. 4.1.10 (Raymond and Rousset 1995). GENEPOP adjusts significant test results (p-values) to account for multiple testing using sequential Bonferroni correction (Rice 1989). Inbreeding coefficient (F_{IS}) and

allelic richness (AR) were calculated in FSTAT 2.9.3.2 (Goudet 1995). Frequency of null alleles was calculated in CERVUS Ver. 3.0.3 (Kalinowski et al. 2007).

Three-way analysis of molecular variance (AMOVA) was used to assess the partitioning of genetic variation between regions (northeast and southeast Tasmania), and between and within populations. Isolation-by-distance, as examined by a Mantel test, was used to test for any correlation between genetic and geographic distances. Relatedness by population was assessed using Queller and Goodnight (1989) estimators. We chose relatedness over F_{IS} to infer restricted dispersal as F_{IS} is derived from allele frequencies, which are cumulative over more generations than relatedness, which is derived from genotype and thus reflect short-term ecological timescale and fine-scale populations processes (Garrick et al. 2010). This is a more suitable time scale for the 4–11 year post-disturbance time frame of this study. AMOVA's, isolation-by-distance, and relatedness (Peakall and Smouse 2006) were calculated in GENALEX Ver. 6.3. Pairwise F_{ST} and its significance (after Bonferroni correction) were calculated in FSTAT 2.9.3.2 (Goudet 1995). Population differentiation was assessed via pairwise exact G tests (genotypic) (Goudet et al. 1996) (option 3: 1000 dememorizations, 1000 batches, 1000 iterations) with significance combined across loci by Fisher's method (Fisher 1932; Ryman and Jorde 2001) using GENEPOP Ver. 4.1.10 (Raymond and Rousset 1995). Significant test results (p-values) were adjusted by sequential Bonferroni correction to account for multiple testing (Rice 1989).

Genetic clustering was estimated using STRUCTURE Ver. 2.3.2 (Pritchard et al. 2000), which indicates distinct populations without any *a priori* population description. STRUCTURE was run in the manner described in Evanno et al. (2005): 10 000 burn-in iterations followed by 10 000 Markov chain Monte Carlo iterations, 20 runs for each K value (theoretical number of distinct populations), multiple K's (1–12), and the admixture model with correlated allele frequencies between populations. This model was appropriate to detect subtle population structure (Falush et al. 2003). Both mean likelihood of K and delta (Δ) K were calculated from the output as in Evanno et al. (2005) for analysis purposes. Any locus with potential null alleles was excluded from this analysis to ensure robustness of results.

Population assignment tests and detection of first generation immigrants were performed in GENECLASS 2 (Piry et al. 2004). Assignment tests of individuals were run with the following parameters: frequency-based criteria for computation of Paetkau et al. (1995), missing allele frequency of 0.01, and a threshold of 0.05. Detection of first generation immigrants was run with: L_{home}/L_{max} likelihood ratio, Bayesian criteria for computation of Rannala and Mountain (1997), Monte-Carlo resampling with simulation algorithm of Paetkau et al. (2004), 1000 simulated individuals, and a threshold value of 0.01.

Paternity was assigned with the program CERVUS Ver. 3.0.3 (Kalinowski et al. 2007), using the genotypes of known mother/young pairs against those of adult males in the area ('candidate males'). Simulation of paternity analysis was done with 10 000 iterations with the following settings: 100 000 offspring, 66 candidate fathers, 0.84 proportion of males sampled, 0.84 proportion of loci typed (as calculated by the program), one minimum typed locus, and the standard mis-scoring error rate of 0.1 (Marshall et al. 1998). Confidence levels were 80% (relaxed) and 95% (strict). All adult males trapped during this study were considered to be candidate fathers in the site where they were trapped (range 4–27 males per site). The proportion of fathers sampled was derived from field observations in the concurrent capture-mark-recapture study and the average estimation of the captured proportion of the 'total population' across all sites (Flynn and Jones in prep) [Chapter 3]. The percentage of the population sampled for genetic analysis ranged from 63.2–99.7%.

Highest logarithm of the ratio of likelihood ratios scores (Δ, equal to the difference in likelihood ratio (LOD)), the likelihood of a particular male versus a randomly chosen one being the father, were used to identify the most likely genetically compatible father (Marshall et al. 1998). Zero, negative, and positive LOD scores imply that a candidate male is equally, less, or more likely the father than an arbitrary male (Glen et al. 2009). Paternity assignment was accepted at 80% (Slate et al. 2000).

Considering the capacity of CERVUS to detect and handle null alleles, paternity analysis was run twice to determine which was more accurate: once each with genotypes either including or excluding the locus with the null allele (Tv54). CERVUS deals with null alleles through locus specific error rates and exclusion probabilities. Furthermore, any miscalculations due to null alleles are unlikely to change the assignment result as LOD scores are unambiguous (Slate et al. 2000; Wagner et al. 2006).

Measures of individual genetic diversity were calculated for adult males to explore male reproductive success: internal relatedness (IR) which estimates the relatedness of an individual's parents (Amos et al. 2001); heterozygosity weighted by locus (HL) (Aparicio et al. 2006); and both standardised expected and observed heterozygosity (SH_{EXP} and SH_{OBS} respectively) which measure individual heterozygosity (Coltman et al. 1999). Calculations were made using default settings in IRmacroN3 (Amos 2005), available from http://www.zoo.cam.ac.uk/zoostaff/amos/#ComputerPrograms.

Statistical analysis

All statistical analyses were carried out using SAS 9.1 (SAS Institute Inc. 2003) and all results are reported ± standard error (SEM). Two-way analysis of variance (ANOVA) with factors disturbance and forest type were performed on site means to assess difference in genetic diversity parameters and on overall site scores to assess differences in both the percentage of subadults in the population and correct population assignments. Differences in the percentage of correct population assignment between different age classes (adult and subadult) and by sex within those age classes were analysed by one-way ANOVA with factor age or sex, respectively. Differences in male attributes between males known to have bred and all other adult males were analysed using one-way ANOVA with factor paternity (successful versus unsuccessful), performed on site means of physiological (analysed in Flynn and Jones in prep [Chapter 3]), morphometric, and molecular attributes. Chi square analysis was used to compare differences in counts. When no differences were detected between forest type or disturbance history, data were grouped from sites by treatment (forest type/disturbance): dry disturbed ('DD'), dry control ('DC'), wet disturbed ('WD'), and wet control ('WC') forest.

Results

Relevant field observations

The percentage of subadults in the population did not vary significantly by forest type (male: $F_{1,6} = 2.45$, p = 0.168; female: $F_{1,6} = 3.10$, p = 0.129) or disturbance (male: $F_{1,6} = 4.05$, p = 0.091; female: $F_{1,6} = 2.35$, p = 0.176). The absence of subadults in wet disturbed forest was of note as females were breeding there and young were surviving until at least near weaning (E. Flynn pers. obs.). Subadult males comprised an average of $10.5 \pm 3.9\%$ of all males (range 0–19.75%) and subadult females comprised an average of $12.2 \pm 4.4\%$ of all females (range 0–20.0%).

Field observations indicate that adult male abundance was equivalent between disturbance classes: 53 males were found in disturbed forest versus 50 males in control forest ($\chi^2_1 = 0.04$, p = 0.979). In adult females, abundance was influenced by disturbance class within forest type (27 in disturbed forest and 48 in control forest); this relationship was significant in wet forest (7 females in disturbed sites versus 23 in control sites ($\chi^2_1 = 4.27$, p = 0.039)), but not in dry forest (20 females in disturbed sites versus 25 in control sites) ($\chi^2_1 = 0.28$, p = 0.870).

Paternity assignment and male attributes

Increased success in paternity assignment occurred when the locus with the null allele (Tv54) was included in analyses, presumably due to higher resolution with an additional locus. The analyses excluding Tv54 failed to identify four fathers that were assigned paternity when Tv54 was included in the analysis, and assigned paternity to two males that were not identified when Tv54 was considered. All other assignments between analyses were the same. The results from analyses including the locus with the null allele were accepted. Sixteen of 26 young were assigned fathers. Paternity assignment was accepted with 80% confidence (12 of 16 were identified with 95% confidence). The observed percentage of assignment resembled the expected percentage calculated by CERVUS (62% versus 66%, respectively). There was one mismatched locus (Tv54, the locus with the null allele) between a known mother and offspring pair; the assigned father was not a mismatch. Twelve 'fathers' exhibited no locus mismatch between known mother/offspring/candidate male genotypes, three 'fathers' had one mismatched locus, and one 'father' had two mismatched loci. Exclusion probability was 0.999 with known maternity.

There were no significant differences between physical attributes (body mass, head, leg, or tail length, or testis width, length, or volume) of males known to be successful breeders and all other males based on one-way ANOVAs (p = 0.523–0.923, n = 103) (Table 5.2). No significant differences were detected in plasma cortisol concentration (examined in an attempt to identify dominant males (Jolly et al. 1999; Miller et al. 2010)) (p = 0.633, n = 103) and individual measures of genetic diversity (p = 0.674–0.830, n = 103) between males known to be successful and all other males. Furthermore, no differences were detected in haematocrit (examined as a proxy for body condition (Sanchez-Guzman et al. 2004)) between successful breeders and other males (p = 0.723, n = 96). While home ranges were not measured as part of this study, fathers were trapped in the close vicinity of relevant mothers: 12 of 16 fathers were trapped 50–100 m from where mothers were trapped and four were caught 250 m away. This suggests that successful males may have territories that overlap or adjoin those of the mothers. All known fathers were resident animals, trapped in their respective site for at least one year. Of the males whose reproductive success was unknown, 38/87 (43.7%) were captured only once: these are most likely transient animals. The fathers of the unassigned progeny were most likely peripheral males, adjoining the mother's territory so that they were not trapped/sampled. Paternity of young revealed that all three females that breed in subsequent years chose to breed with different males each year. Only one male was known to father more than one young (different females in the same year at site WD1). It is worth noting that this male was trapped with a back young on his back, which was highly unusual as parental behaviour is exclusively female in this species (Callister 1991; Russell 1982).

Table 5.2. Physical, physiological (from Flynn and Jones in prep [Chapter 3]), and molecular traits of known brushtail possum fathers (n = 16) compared to all other adult males (n = 87) in Tasmania. Data were analysed by two-way analysis of variance (factors forest type and disturbance) on site means.

Characteristics	Known fathers			All other males		
	Mean	SE	Range	Mean	SE	Range
PHYSICAL						
Body mass (kg)	3.31	0.11	2.9–3.8	3.4	0.04	2.7–4.8
Head length (mm)	105.0	2.20	94.0–126.0	101.0	0.60	90.0–112.0
Leg length (mm)	119.0	2.10	119.0–134.0	120.0	1.50	99.0–158.0
Pes length (mm)	66.0	4.70	56.0–74.0	69.0	1.20	59.0–75.0
Tail length (mm)	359.0	7.90	299.0–403.0	359.0	3.70	294.0–424.0
Testis length (mm)	23.0	1.00	21.0–28.0	24.0	0.30	18.0–32.0
Testis width (mm)	19.0	0.90	16.0–23.0	19.0	0.30	10.0–26.0
Testis volume (mm^3)	45.0	4.10	33.0–71.0	46.0	1.90	19.0–89.0
PHYSIOLOGICAL						
Plasma cortisol concentration (ng/mL)	14.5	2.1	3.9–35.7	15.1	1.1	0.61–48.8
Haematocrit (%)	43.9	1.2	38.2–46.3	43.1	0.5	32.2–49.2
MOLECULAR						
Internal relatedness (IR)	0.11	0.07	-0.06–0.72	0.12	0.03	-0.21–0.71
Standardised observed heterozygosity (SH_{OBS})	0.61	0.05	0.33–0.83	0.59	0.02	0.33–0.83
Standardised expected heterozygosity (SH_{EXP})	0.89	0.07	0.24–1.22	0.88	0.03	0.23–1.24
Heterozygosity weighted by locus (HL)	0.30	0.05	0.08–0.60	0.32	0.02	0.09–0.80

Genetic diversity

Genetic analyses using six microsatellite loci detected a moderate level of genetic diversity across all measures (mean: number of alleles (A) = 6.0 ± 0.32, allelic richness (AR) = 4.4 ± 0.11, observed heterozygosity (H_O) = 0.62 ± 0.02, expected heterozygosity (H_E) = 0.63 ± 0.02, and inbreeding coefficient (F_{IS}) = 0.09 ± 0.02) that did not differ between populations by disturbance ($F_{1,6}$ = 0.08–1.27, p = 0.354–0.769) or forest type ($F_{1,6}$ = 0.31–2.89, p = 0.091–0.576) (detailed in Table 5.3). F_{IS} did not deviate significantly from zero in any population, indicating that there was no heterozygote deficiency in these populations.

Table 5.3. Diversity diagnostics (averaged across sites) derived from analysis of six microsatellite loci of brushtail possums in Tasmania (N = number of animals sampled, A = number of alleles/locus, AR = allelic richness/locus, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient)

Site	Forest type	Disturbance class	N	A	AR	H_O	H_E	F_{IS}
DD1	Dry	Disturbed	33	7.0	4.2	0.59	0.58	0.14
DD2	Dry	Disturbed	6	5.0	5.0	0.58	0.60	0.14
DD3	Dry	Disturbed	19	6.8	4.7	0.68	0.71	0.09
DC1	Dry	Control	24	6.7	4.3	0.63	0.60	0.05
DC2	Dry	Control	15	6.7	4.8	0.73	0.69	0.03
DC3	Dry	Control	15	5.5	4.4	0.62	0.63	0.04
WD1	Wet	Disturbed	10	5.2	4.1	0.62	0.57	-0.02
WD2	Wet	Disturbed	6	4.0	3.8	0.56	0.61	0.15
WC1	Wet	Control	24	6.8	4.3	0.60	0.64	0.09
WC2	Wet	Control	24	6.8	4.5	0.61	0.70	0.19

Tv54 was the only locus not in Hardy-Weinberg Equilibrium after sequential Bonferroni correction, suggesting the possible presence of a null allele. No other loci or populations differed from Hardy-Weinberg Equilibrium (Table 5.4). Tv54 had a high frequency of null alleles (frequency ≥ 0.20), as indicated when using CERVUS (Chapuis and Estoup 2007) (Table 5.4). A moderate null allele frequency was also suggested at Tv58 (0.05 ≤ frequency < 0.20), but there was no indication of null alleles at this locus in any other analysis. There were no deviations from Hardy-Weinberg equilibrium at Tv58 and none of the parent/offspring genotypes displayed a mismatch at this locus. There was no evidence of linkage disequilibrium; all loci/population combinations were independent.

Table 5.4. Brushtail possum deviation from Hardy-Weinberg expected genotypic proportions (via exact tests) by site/locus and mean null allele frequency by locus. Significant p-values (p < 0.05) in bold; stars indicate significance (p < 0.05) after sequential Bonferroni correction (by locus).

Locus	Deviation from Hardy-Weinberg expected genotypic proportions by site										Mean null Allele Frequency
	DD1	DD2	DD3	DC1	DC2	DC3	WD1	WD2	WC1	WC2	
Tv16	0.17	0.02	0.10	0.17	0.59	0.92	0.54	1.00	0.13	0.02	0.03
Tv19	0.86	0.77	0.08	0.07	0.84	0.71	0.65	0.13	0.01	0.30	0.01
Tv53	0.92	0.89	0.80	0.22	0.91	0.23	0.41	0.08	0.05	0.28	0.02
Tv54	0.00*	0.09	0.00*	0.00*	0.01	1.00	0.48	0.15	0.43	0.00*	0.40
Tv58	0.09	0.82	0.58	1.00	0.19	0.35	1.00	1.00	0.56	0.03	0.06
Tv64	0.02	0.94	0.55	0.25	0.96	0.56	0.67	0.78	0.66	0.32	-0.03

Population structure and gene flow

A Mantel test revealed a significant correlation between geographic distance and genetic relatedness (R = 0.09, p = 0.01). Thus isolation-by-distance is in effect and geographic distance weakly, but significantly influences genetic distance. Three-way AMOVA showed that a significant proportion of variation occurs among regions (3%) and populations (5%) (p = 0.010), with slightly more variation resulting among populations. Within-population genetic variation is large (92%) and reflects the resultant variation of sexual reproduction (Excoffier et al. 1992).

The results from the STRUCTURE analysis indicate that there are either one or two populations of brushtail possums living on the east coast of Tasmania. The natural log of K (ln(K)), which suggests the actual K value (number of populations), was highest for K = 1, though values were very similar for both K = 1 and K = 2 (Figure 5.2). ΔK is often a more reliable measure but cannot be calculated for K = 1. The modal value represents 'true K' (Evanno et al. 2005): in this case, ΔK is ambiguous, suggesting that there are one or two populations.
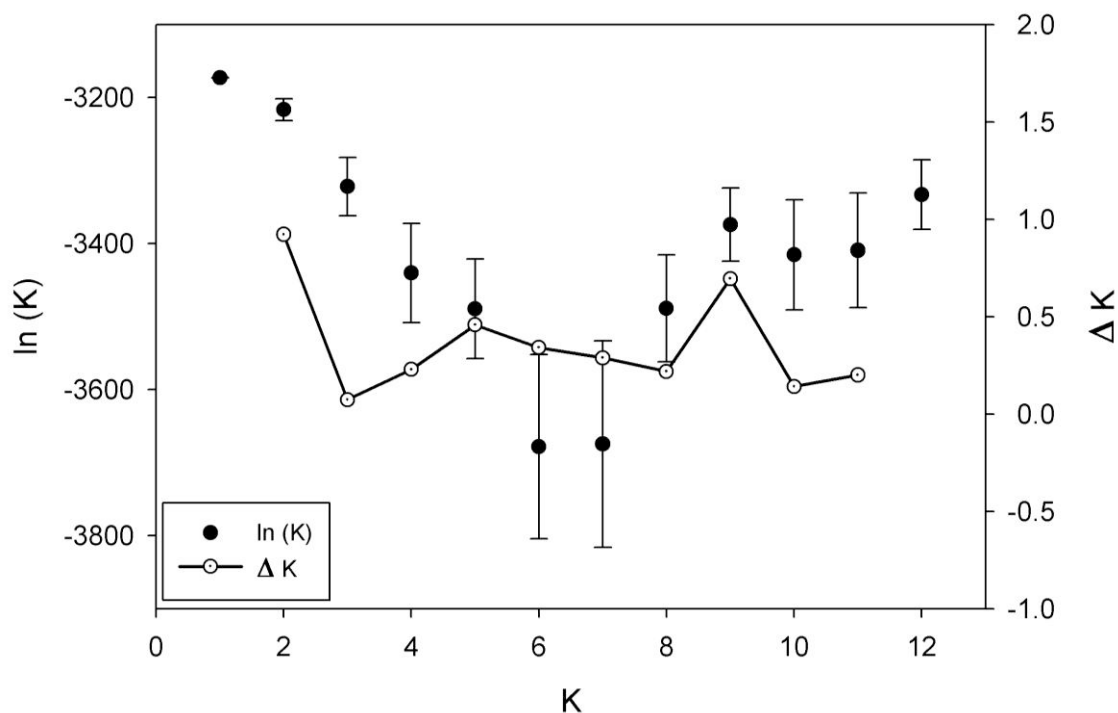
Figure 5.2. STRUCTURE results indicating the number of populations of brushtail **possums living on the east coast of Tasmania as indicated by this study: ΔK and ln(K) for** K = 1–12. Values for ln(K) (indicating the number of populations) of both K = 1 and K = 2 are very similar. ΔK, the more reliable measure (Evanno et al. 2005), is ambiguous in this case as K = 1 cannot be calculated. These results suggest that there are one or two populations.

Overall F_{ST} for the populations in this study was 0.07, indicating moderate differentiation between populations (F_{ST} values of 0.05–0.15) (Kraaijeveld-Smit et al. 2007). Most of the significant variation in F_{ST} values were between northern and southern sites (wet and dry forest) (76%) (Table 5.5). The majority of these (56%) were moderate ($F_{ST} \geq 0.05$) and the remaining proportion was low ($F_{ST} < 0.05$) (Kraaijeveld-Smit et al. 2007). There were no significant F_{ST} values within the wet forest sites and few within dry forest sites (33% (5/15)). Of the dry sites, DD1 and DC1 are spatially closer to each other than to the other sites; the same is true of DD2, DD3, DC2, and DC3 (Figure 5.1). Between these two spatial subgroups of sites, three pairs of sites show variation in F_{ST} indicating moderate differentiation ($F_{ST} \geq 0.05$). The remaining significant variation was weak ($F_{ST} < 0.05$). In only one case is there a significant F_{ST} that is not between these two subgroups of dry forest sites (DC2 and DC3, $F_{ST} < 0.05$).

Table 5.5. Pairwise F_{ST} across microsatellite loci by site (overall F_{ST} = 0.07) in brushtail possums in Tasmania. Negative estimates indicate values of zero with estimation error. The solid boxed area indicates the northern (wet forest) sites only and the dashed boxed area indicates the southern (dry forest) sites only. Significant p-values (p < 0.05) in bold; stars indicate significance (p < 0.05) after Bonferroni correction.

Population	DD1	DD2	DD3	DC1	DC2	DC3	WD1	WD2	WC1	WC2
DD1	0.00									
DD2	0.05	0.00								
DD3	0.06*	0.02	0.00							
DC1	0.01	0.02	0.04*	0.00						
DC2	0.05*	0.04	0.02	0.05*	0.00					
DC3	0.02	0.04	0.03	0.03	0.03*	0.00				
WD1	0.04	0.06	0.05	0.06*	0.06	0.04*	0.00			
WD2	0.06*	0.04	-0.01	0.06*	0.05	0.04	0.01	0.00		
WC1	0.05*	0.06*	0.02*	0.06*	0.04*	0.02*	-0.00	0.01	0.00	
WC2	0.06*	0.06*	0.01*	0.06*	0.03*	0.03*	0.03	0.00	0.02	0.00

Most of the significant population differentiation results from the exact G tests were between northern and southern sites (wet and dry forest sites, respectively) (59%) (Table 5.6). The majority of results between the northern (wet) and southern (dry) sites (79% (19/24)) were significant after Bonferroni correction. Within the dry forest sites, 80% (12/15) of results were significant after Bonferroni correction. There was one significant p-value after Bonferroni correction (17% (1/6)) within the wet forest sites (between WC1 and WC2).

Table 5.6. Results of pairwise exact G tests, indicating population differentiation, across microsatellite loci by site in brushtail possums in Tasmania. The solid boxed area indicates the northern (wet forest) sites only and the dashed boxed area indicates the southern (dry forest) sites only. Significant p-values ($p < 0.05$) in bold; stars indicate significance ($p < 0.05$) after Bonferroni correction.

Population	DD1	DD2	DD3	DC1	DC2	DC3	WD1	WD2	WC1	WC2
DD1	0.0000									
DD2	0.0004*	0.0000								
DD3	0.0000*	0.0883	0.0000							
DC1	0.0005*	0.0625	0.0000*	0.0000						
DC2	0.0000*	0.0253	0.0006*	0.0000*	0.0000					
DC3	0.0005*	0.0021*	0.0031*	0.0000*	0.0000*	0.0000				
WD1	0.0001*	0.0055	0.0024*	0.0000*	0.0001*	0.0001*	0.0000			
WD2	0.0000*	0.1629	0.4954	0.0001*	0.0146	0.0079	0.3490	0.0000		
WC1	0.0000*	0.0000*	0.0004*	0.0000*	0.0000*	0.0000*	0.6195	0.1763	0.0000	
WC2	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0447	0.0728	0.0020*	0.0000

Relatedness (r) varied by population with only DD1 and DD2 being more related than expected on average (r = 0.159, p = 0.001 and r = 0.125, p = 0.002, respectively). In both of these sites, this was driven by relatedness of the females (with DD1 being the only significantly highly related group of females (r = 0.213, p = 0.001)). While there was variation between populations (more so between both males and whole populations than between females), no other significant variation in relatedness or lack of relatedness was detected in either sex at any site.

Population assignment tests indicate whether an individual's genotype reflects that of the population in which it was trapped. It can be extrapolated that correctly assigned animals are from the local area where they were trapped. Those incorrectly assigned may have immigrated from nearby, unsampled areas. $75.0 \pm 3.33\%$ (range: 52.6–90.0%) of all animals were correctly assigned to the site in which they were trapped. There was no significant difference in the percentage of correct assignment by disturbance ($F_{1,6} = 0.01$, p = 1.000) or forest type ($F_{1,6} = 0.49$, p = 0.511). Several patterns were apparent from these results based on one-way ANOVAs: that a significantly greater percentage of adults were correctly assigned than subadults (p < 0.001, n = 178) and that within both adults and subadults, significantly more females were correctly assigned than males (p = 0.004, n = 153; p < 0.001, n = 25, respectively) (Table 5.7).

Table 5.7. Population assignment and detection of first generation immigrants by sex and age class of brushtail possums in Tasmania

Sex and age class	N	% correctly assigned	Number of first generation immigrants
Adults (male and female)	153	77	10
Adult males only	85	82	8
Adult females only	68	97	4
Subadult (male and female)	25	32	7
Subadult male only	14	7	7
Subadult female only	11	46	0

Discussion

Sex ratio

Our results indicated that the mechanism behind the male-biased adult sex ratio (66:34 male:female) in brushtails possums living in disturbed sites was resource-limited female abundance. We came to this conclusion through the sequential testing of four alternative hypotheses.

a. Maternal sex allocation: Our data suggest that maternal sex allocation is not occurring in disturbed sites. The sex ratio of dependent young (pouch young and back young) did not vary from parity across forest type or treatment (Flynn et al. 2011b) [Chapter 4], even though fewer hollow-bearing trees, considered to be a critical resource for breeding for female brushtail possums (Gibbons and Lindenmayer 2002; Koch et al. 2008), were found in both disturbed than in control sites and in wet than in dry forest (Flynn et al. 2011a) [Chapter 2]. There were also no differences in maternal body condition (as indicated by haematocrit values) between forest type or disturbance (Flynn and Jones in prep) [Chapter 3]. Though refuge availability differed between forest type and disturbance, maternal body condition and sex ratio of offspring did not.

b. Reduced dispersal in subadult males: Recruitment of subadults into the adult population was similar among all forest types and disturbance histories. Population assignment tests also reveal that subadults, particularly males, were dispersing. Thus, failure to disperse was not the cause of the sex ratio bias in adult males.

c. Influx of adult males: Disturbed sites may be attractive to dispersing males as food availability is high (Driessen et al. 1991; Hocking 1981; Vernes and Haydon 2001). If this were the case, we would expect lower relatedness among males living in populations in disturbed sites than in the control sites. No such pattern was observed, lessening support for this explanation. In addition, similar abundance of adult males between control and disturbed sites provides further evidence against a male movement-driven bias in adult sex ratio.

d. Limited numbers of females: This was the most parsimonious explanation for the sex ratio bias as there were fewer females in disturbed sites than in control sites, the effect being observed in wet forest but not dry forest. Breeding frequency across the study was also significantly lower in disturbed than in control sites (Flynn et al. 2011a) [Chapter 2],

most likely in response to decreased availability of suitable quality refuges for breeding. Resource abundance influences the ecology and life history of a species (Clutton-Brock 1989). Significantly fewer hollow-bearing trees were detected in wet versus dry and in disturbed versus control forests (Flynn et al. 2011a) [Chapter 2]. Cawthen (2007; unpublished data) found that although hollow-bearing trees may remain in the landscape after harvest, brushtail possums do not use them until forest structure regenerates around them. Therefore, we suggest that the male-biased sex ratio observed in disturbed sites was a result of a resource-driven decrease in adult females, with the limited resource being tree hollows in mature, structured forest.

In support of this conclusion, it is worth noting that subadults were not caught in the wet disturbed sites, although young were produced in these sites throughout the study and were known to survive until close to weaning (E. Flynn pers. obs). Female abundance in mammals is commonly resource-limited, while males are female-limited (Clutton-Brock and Harvey 1978). Females require high quality tree hollows to reproduce (Gibbons and Lindenmayer 2002). In contrast, males are able to utilise more marginal den sites (Cawthen 2007). These results suggest a shortage of the tree hollow resource in which adult females defend the high quality hollows, adult males are able to take advantage of sub-optimal refuge, and there is a shortage of suitable refuges for juveniles which are under duress to disperse to find refuge. Logged sites may represent sub-optimal habitat for brushtail possums because the refuge resource abundance is limited.

Mating system

The mating system of the mountain brushtail possum can reflect patterns of resource distribution (Martin and Martin 2007); the brushtail possum is likely to exhibit the same range of possibilities in the plasticity of its mating system. Habitat disturbance, as examined in this study, did not appear to influence the mating system of the populations of Tasmanian brushtail possums. While our results were inconclusive on this point due to low numbers of maternal/offspring pairs sampled because of the difficulty in trapping back young as late lactation progresses, the relevant field observations support findings from other brushtail possum studies. The results of population assignment and detection of first generation immigrants support what we know is typical of the species: female offspring are philopatric

and male offspring disperse (Clout and Efford 1984; Kerle 1984). The high proportion of reproductively unsuccessful males in the populations supports the supposition that the mating system is polygynous (Clout and Efford 1984; McKay and Winter 1989). In addition, female mate fidelity appears to be low, as demonstrated in Taylor et al. (2000). It is commonly reported that male brushtail possums that breed successfully are significantly larger (e.g., body size and weight) than those that do not breed (Clinchy et al. 2004; Isaac et al. 2005; Winter 1976). Our results showed no difference between male body mass and either forest type or disturbance (Flynn and Jones in prep) [Chapter 3], suggesting that there was no clear physical, physiological, or molecular basis for female mate selection in these populations. However, proximity to the female did seem to play a role in mate selection. As in Taylor et al. (2000) and Clinchy et al. (2004), fathers tended to be resident males that lived near or immediately adjacent to the females. It is likely that females choose their mates on a criterion that was not detected in this study such as testosterone concentration, pheromones, degree of sternal gland activity, or timing (who was available at the right time could be crucial as oestrous lasts for less than one day (Pilton and Sharman 1962)). A male-driven mechanism, such as male-male scramble competition, could also be occurring (Schwagmeyer 1988).

Genetic diversity and population genetic structure

In this first study of population genetic structure in Tasmanian brushtail possums, our results are parsimonious with two populations (in northern wet forest and southern dry forest), influenced by distance, living on the east coast of Tasmania. While STRUCTURE results were ambiguous towards one or two populations, pairwise F_{ST} results suggested structuring between the northern and southern sites (wet and dry forest). Results from exact G tests (a more powerful test that F_{ST} (Goudet et al. 1996)) indicate a strong population differentiation between northern and southern sites. This is most likely a result of distance rather than a reflection of habitat on population genetics. Population differentiation results further indicate homogeneous sites in the north (wet forest) and distinct sites in the south (dry forest). Greater structuring among southern sites than northern sites is most likely primarily an artefact of their greater geographic separation; however, we cannot exclude the possibility that brushtail possum dispersal rates vary with forest type. While there is distinct

population differentiation indicated across the northern and southern sites in this study, intermediate sites across this distance may also contain genetically distinct populations as might geographically broader sampling within the state-wide distribution of the brushtail possum.

Moderate differentiation across the geographic scale of this study, suggests that brushtail possum populations should be managed as independent units (Palsbøll et al. 2007). Habitat disturbance did not influence population genetic structure. In contrast, habitat disturbance, through decreased immigration, resulted in genetically distinct populations that need to be managed independently to ensure long-term persistence in other forest-dependent species: koala (*Phascolarctos cinereus*) (Lee et al. 2010), grizzly bear (*Ursus arctos*) (Proctor et al. 2005), and orangutan (*Pongo pygmaeus*) (Goossens et al. 2005). Populations of the greater glider (*Petauroides volans*) (Taylor et al. 2007) and the Eurasian red squirrel (*Sciurus vulgaris*) (Hale et al. 2001; Wauters et al. 1994) living in disturbed forest habitat had significantly less genetic diversity than populations from undisturbed, continuous forest. Populations may become isolated because of habitat loss or anthropogenic barriers to gene flow (i.e., road, paddock, or savannah) (Balkenhol and Waits 2009; Radespiel et al. 2008). Habitat availability influences the ability of individuals to disperse, thus secondarily influencing genetic divergence among populations (Gibbs 2001). Population isolation and subsequent genetic erosion are the most common genetic impacts of habitat disturbance in the literature.

The moderate genetic diversity that we report suggests that the eastern Tasmania population is unlikely to have experienced small population size in the past despite glaciation, disease, hunting pressure from the fur industry, and population control measures. Diversity parameters indicated lower genetic variability in Tasmanian brushtail possums than is reported in studies of brushtail possums on mainland Australia (Clinchy et al. 2004; Stow et al. 2006; Taylor and Cooper 1998; Taylor et al. 2004) (Table 5.8). However, the Tasmanian values were closer to those of brushtail possum populations in New Zealand, which have founder effects (founded using a small number of individuals from both Tasmania and mainland Australia) (Taylor et al. 2000; Taylor et al. 2004; Taylor and Cooper 1998) (Table 5.7). These results must be interpreted with caution as different microsatellite loci were used in each study. Tasmanian and offshore island populations in these studies show the same

pattern of decreased diversity (Table 5.8). Low genetic variability is typical of island populations (Bidlack and Cook 2001; Frankham 1997; Stow et al. 2006), including marsupial species (Eldridge et al. 1999; Mills et al. 2004; Sinclair 2001).

Table 5.8. Summary of the range of genetic diversity measures of brushtail possum populations living in mainland Australia, New Zealand, and Tasmania
(A = number of alleles/locus, AR = allelic richness/locus, H_O = observed heterozygosity, H_E = expected heterozygosity)

Geographic location	A	AR	H_O	H_E	Studies
Mainland Australia [offshore islands in brackets]	8.9-16.6 [8.9]	6.1-6.9 [5.1]	0.76-0.88 [0.79]	0.79-0.90 [0.81]	Clinchy et al. 2004; Stow et al. 2006*; Taylor and Cooper 1998; Taylor et al. 2004;
New Zealand [offshore islands in brackets]	7.5-11.8 [3.5]	5.1 [2.9]	0.63 [0.43]	0.67-0.83 [0.41]	Taylor et al. 2000; Taylor et al. 2004*; Taylor and Cooper 1998
Tasmania	6.0-9.2	4.4-6.1	0.62-0.77	0.63-0.80	Taylor et al. 2004; this study

*Indicates studies that report values for populations on both the mainland and offshore islands

Global F_{ST} (0.07) was lower than that seen among brushtail possum populations in southeastern mainland Australia (New South Wales, Victoria, and South Australia) (F_{ST} = 0.145) (Truett et al. 2000) but was typical of a non-social mammal with restricted dispersal (Barrowclough 1983). Within mammals, F_{ST} values decrease with non-sociality and increasing dispersal ability (Barrowclough 1983): ranging from an F_{ST} of 0.283 in highly structured populations (Comstock et al. 2002), to a F_{ST} of 0.005 with species that disperse over long distances (Rueness et al. 2003). While most of the variation in population differentiation and pairwise F_{ST} seemed to reflect geographic distance (northern wet forests versus southern dry forests), the greater structuring and significant pairwise F_{ST} variation indicated between dry forest populations may reflect both the geographic separation of these sites and restricted dispersal among sampled sites with immigrant animals likely to come from unsampled sources, resulting in weak to moderate differentiation between some of these populations.

Conclusions

Integration of genetic data, ecological parameters, and habitat variables provides thorough, detailed analyses that are useful for conservation purposes (Lindenmayer et al. 1999). Habitat alteration through logging appears to have decreased female-specific resources to the extent that there has been response in population dynamics. There were no implications of the level of habitat disturbance in this study on the mating system or genetic diversity of the brushtail possum. According to 'disturbance theory' and the potential cascading impacts of habitat disturbance on fauna (Lindenmayer and Franklin 2002), the intermediate population responses (e.g., sex ratio bias and abundance) observed in brushtail possums in this study could be precursors to the loss of genetic diversity. However, inbreeding and long-term reduction in the potential of a species to cope with environmental challenge (Frankham 2005) could occur if habitat disturbance becomes more severe (e.g., increasingly restricted availability of hollow bearing trees). The population responses of the brushtail possums we observed indicate that current forestry methods and prescriptions for retention of mature forest as experienced in this study may not fulfil all the requirements of an arboreal mammal population for long-term sustainability. If insufficient suitable habitat is retained, populations may not persist during the time taken for mature forest elements to regenerate and once again provide habitat for full population recovery. While brushtail possums are documented as resilient to periods of unfavourable habitat conditions, under longer term unfavourable conditions they may form smaller populations with greatly increased susceptibility to local extinction than the population pre-disturbance (Kerle 1984). Our results, however, suggest that habitat connectivity was preserved using current harvest methods; thus, brushtail possums were able to disperse throughout the landscape and no populations became genetically isolated because of disturbance. This highlights the importance of maintaining areas of mature forest habitat throughout the landscape, particularly corridors and links between areas subject to production forestry activities and intact forest that allow wildlife to disperse and maintain genetic diversity (Bennett 1990; Tewksbury et al. 2002). Due to the rate of genetic drift it takes several generations for genetic isolation to manifest, during which time populations may be demographically isolated, impacting their ability to persist (Gaggiotti 2003; Lande 2002). While this is possible, it is unlikely as population assignment tests suggest that a mean of $25.0 \pm 3.33\%$ of animals at each site have immigrated (or are related to immigrants) into the site.

With the slow regeneration of *Eucalyptus* forest (regrowth of the surrounding forest was sufficient for brushtail possums to once again use tree hollows between 10–17 years post harvest in dry forest (Cawthen 2007; unpublished data)), this shortage of refuge for brushtail possums and other arboreal/tree hollow-dependent vertebrates is unlikely to be resolved in the medium-term. Without an increase in adequate den sites, populations are not likely to increase greatly in size (Ginsberg and Milner-Gulland 1994). As brushtail possums are relatively long-lived (their lifespan is an estimated 8–12 years in the wild) (Clinchy et al. 2004; Crawley 1970), it is possible that the local populations at disturbed sites may decline and experience genetic erosion unless there is sufficient adult female mortality and immigration to maintain genetic variability within the population. Neither high mortality (Flynn and Jones in prep) [Chapter 3] nor immigration of adult females was observed in populations living in habitat 4–11 years post harvest. This again stresses the vital importance of retaining mature habitat in the landscape to facilitate persistence and recolonisation of harvested areas by breeding populations of animals into the future.

Studies of genetic diversity in populations living in disturbed versus intact forest are necessary for understanding the response of species to habitat modification (Sumner et al. 2004). Specifically, investigation into dispersal, relatedness, and population structure can inform conservation management by elucidating the potential impacts of disturbance (Moore et al. 2008). The results of this study suggest that mature forest elements retained in current forest practices can help to maintain habitat connectivity, but whether or not they are sufficient to sustain current brushtail possum populations in disturbed sites in the long-term, particularly if the ecological impacts continue (e.g., further forest harvesting or severe storm/wildfire before the sites have regenerated) requires further investigation. To conserve genetic variation in wild populations, both habitat preservation and population management are vital (Gibbs 2001). It is critical to incorporate the preservation of genetic diversity into management objectives (Frankham et al. 2002).

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Chapter 6

General discussion

Brushtail possum (*Trichosurus vulpecula*) trapping, processing, and release
Far right: A brushtail possum (post-release) with a tree hollow (deep fissure)

Arboreal marsupials are considered to be the group of Australian mammals that is most sensitive to land use practices that result in loss of refuge sites, such as agriculture and forestry (Tyndale-Biscoe and Calaby 1975; McIlroy 1978; Goldingay and Daly 1997). An exception is the common brushtail possum (*Trichosurus vulpecula*), which because of its presence in plantations (Bulinski and McArthur 1999) and urban areas (Statham and Statham 1997), is widely thought to be resilient to such anthropogenic disturbance. Brushtail possums have a highly adaptable life history (e.g., breeding success, behaviour, and diet) that varies amongst the subspecies and between habitats (Smith *et al.* 1969; Hocking 1981; Kerle 1984; Cowan 1990; Kerle and Howe 1992). However, the degree to which the brushtail possum is resilient to habitat disturbance at both the individual and population level has received limited attention. Studies on mainland Australia indicate that habitat disturbance, through its effects on individual dispersal and both resource availability and distribution, can result in a bias in offspring sex ratio of brushtail possums (Johnson *et al.* 2001; Isaac *et al.* 2005) and a change in the mating system of the closely related mountain brushtail possum (*Trichosurus cunninghami*) (Martin and Handasyde 2007). In Tasmania, Hocking (1981) found an initial and short-lived male bias in the adult brushtail possum sex ratio immediately post-fire which he explained by sex-differential survival. Furthermore, breeding success, survival of pouch young and juveniles, individual rates of growth, and population density all decreased as the forest regenerated. He attributed these changes to differences in quality and abundance of food as a result of fire.

The current study used a novel multi-dimensional approach, integrating genetic, physiological and ecological approaches, to investigate impacts of habitat disturbance in wet and dry *Eucalyptus* forest, with a focus on the individual brushtail possum. This kind of multi-disciplinary approach is key to "holistic", and thus more efficient, problem solving in conservation (Wildt *et al.* 2003). The principal assumption of this study was that forest type and habitat disturbance influence brushtail possums at both the individual and population levels. The results, however, indicated that brushtail possums were resilient at the individual-level, but were more sensitive at the population level. The responses of individuals to disturbance indicated that brushtail possums were robust to disturbance, particularly in their physiological ability to adapt to changes to their habitat and to continue to breed. Their physiological status (e.g., adrenal status, generalized immune response, body

condition, and survival) and reproductive capacity were not compromised by either disturbance or forest type. Diet is known to alter milk composition between wild and captive populations of marsupials (Munks *et al.* 1991; Rose and Flowers 2005). Thus floristic differences between forest types, and their intrinsically different nutrition levels and anti-predatory defence mechanisms (e.g., plant secondary metabolites and allelochemicals (Lawler *et al.* 2000; Foley and Moore 2005)), may influence milk composition, which may have implications for growth, body condition, and subsequent survival of dependent young. However, no influence of forest type was found on milk composition of brushtail possums in this study. While habitat influenced milk composition at one site, this was most likely a result of site-specific plant physiology or other dietary components (e.g., carrion, insects, bird eggs, fungi, and blossoms (Fitzgerald 1984; Cowan and Moeed 1987; Nugent *et al.* 2000; Sadlier 2000)) that females may have complemented their variable diet with. This demonstrates the adaptability of brushtail possums to a range of different environments without impacts on reproductive success.

Despite resilience at an individual level, there was some evidence of a population level response to habitat disturbance of the brushtail possums in this study. Significantly lower brushtail possum abundance and breeding frequency were found in populations living in disturbed forest as compared to those living in control forest, in both wet and dry *Eucalyptus* forest. Although there were fewer offspring produced in disturbed forest, birth dates were similar, and young had comparable rates of development (based on size and developmental landmarks) with equivalent body condition to offspring at the control forest sites. A strong male bias was observed in the adult sex ratio of brushtail possum populations living in disturbed sites in both forest types. Despite this, there were no disruptions to the brushtail possum mating system and populations showed no signs of genetic erosion across the study.

As female mammal abundance is resource-dependent (Clutton-Brock and Harvey 1978), the male-biased sex ratios and lower abundance and breeding frequency in brushtail possum populations living in disturbed forest were likely due to limited resource abundance in these sites. This may have implications for population growth and long-term viability at these sites. As foraging habitat within each site and in the surrounding landscape were equivalent,

the limited resource was likely the availability of refuges. Cork and Catling (1996) and Pausas *et al.* (1995) assert that when food supply is adequate, structural components of the habitat interact to determine habitat quality for arboreal marsupials. This is parsimonious with what we know about the habitat requirements of brushtail possums and tree hollow abundance; availability of nest sites is a crucial habitat component as brushtail possums require tree hollows for breeding (Gibbons and Lindenmayer 2002; Koch *et al.* 2008a). In a concurrent study conducted at two of the sites used in this study, brushtail possums living in disturbed forest did not use the tree hollows that were retained in the harvested area ('core' site) during logging, while those living in intact control forest used tree hollows that were available throughout the site (Cawthen 2007). However, brushtail possums began to use tree hollows retained within harvested areas in dry *Eucalyptus* forest 10–17 years post harvest, when forest structure regenerated around them (Cawthen 2007; Cawthen *et al.* unpublished data). Refuge availability impacts brushtail possum population demographics on mainland Australia. Johnson *et al.* (2001) attributed a male-biased sex ratio in brushtail possum offspring in areas of low tree hollow availability to maternal sex allocation, the ability of a female to determine the sex of her offspring to benefit herself and/or her offspring. Male-biased sex ratio decreased competition between mothers and their philopatric daughters in areas with restricted tree hollow availability. This evidence suggests that hollow-bearing trees, and thus refuge abundance, play an important role in the influence of habitat on brushtail possum population size and demographics. This supports the theory of the long-term value of hollow-bearing trees as 'legacies' for future generations in areas subject to habitat disturbance (Fisher and Wilkinson 2005; Lindenmayer and Franklin 2002). Significantly more hollow-bearing trees were found in control as compared to disturbed sites in this study. The population level responses in brushtail possums living in disturbed forest in this study demonstrated the importance of maintaining the availability of ecologically-valuable mature forest elements, particularly hollow-bearing trees, in forests subject to production forestry. Retention of mature forest elements is particularly crucial in the areas surrounding logging coupes (the 'matrix') as the loss of forest structure around hollow-bearing trees retained within harvested areas may mean that such trees do not provide suitable refuge sites for some fauna in the short-term (Cawthen 2007; Cawthen *et al.* unpublished data).

While brushtail possum populations are able to recover from periods of unfavourable habitat conditions, if unsuitable conditions become permanent they may form smaller populations with greatly increased susceptibility to local extinction (Kerle 1984). According to 'disturbance theory' and the potential for cascading impacts of habitat disturbance on fauna (Lindenmayer and Franklin 2002) (Figure 6.1), the differences observed between control and disturbed sites in the abundance and sex ratio bias of the brushtail possum could be precursors to a loss of genetic diversity. This may occur if populations are unable to persist as mature forest elements regenerate, providing habitat for them to fully recover. Genetic erosion, which increases local extinction risk through inbreeding and reducing a population's ability to respond to environmental challenge, can occur when populations become isolated (Frankham 2005). This highlights the importance of maintaining areas of mature forest habitat, particularly corridors and links between areas subject to production forestry activities and intact forest that allow wildlife to disperse and maintain genetic diversity (Bennett 1990; Tewksbury *et al.* 2002).
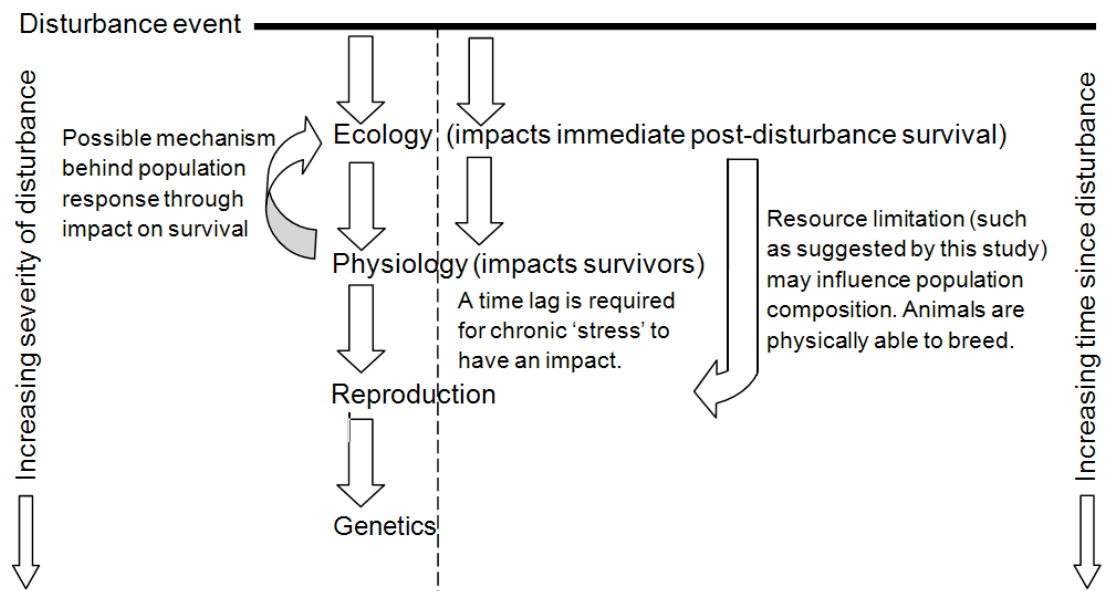
Figure 6.1. Potential cascading impacts of habitat disturbance on ecology, physiology, reproduction, and genetics, considering both increasing severity of disturbance and time from a disturbance event (as synthesised from Gaines *et al.* (1997), Lindenmayer and Franklin (2002), Carey (2005), and John-Alder *et al.* (2009)). The vertical dashed line separates impacts of increasing severity from increasing time. The arrows represent the progression along the cascade (from most susceptible (i.e., population responses and ecology) to most severe impacts (i.e., genetics)) between different levels of impact.

The results of this study, one of few on the influences of habitat disturbance on mammals, particularly medium-sized species, contributes to current disturbance theory by providing insight into the medium-term recovery of forest-dwelling small-medium mammal communities following harvest. Disturbance influenced abundance rather than species composition. A common consequence of habitat disturbance is the displacement of more ecologically sensitive native species that do not tolerate disturbance well by ecologically opportunistic and tolerant invasive species, altering community composition (McKinney and Lockwood 1999). This did not occur in the communities in this study. This suggests that the habitat remaining 4–11 years post harvest was sufficient that native species were able to persist and prevent invasive species from establishing in high numbers in these sites. Furthermore, the stability demonstrated in the composition of mammal communities between disturbance histories in this study support the contention that the matrix surrounding disturbed sites makes an important contribution to medium-term population recovery of small-medium mammals. When habitat is not retained around an area of disturbance, the ability of animals to recover from the disturbance event may be limited, resulting in a greater impact (Dunn 2004). The difference between the responses of brushtail possum populations and the ground mammal communities to disturbance also reaffirm that mammals dependent on mature forest elements are more ecologically 'sensitive' to habitat disturbance than those which do not require mature forest elements (Cork and Catling 1996; Alexander *et al.* 2002). Moreover, the equivalent genetic diversity between brushtail possum populations among treatments demonstrates that disturbance is more likely to affect demography and ecological processes than genetic structure (Gaines *et al.* 1997). Finally, this study also addresses a gap in the literature concerning habitat disturbance and its impacts on milk composition and breeding parameters in mammals, a little-studied aspect, particularly in free-living animals.

This study demonstrates how effectively a multi-disciplinary approach can be used to assess influences of habitat disturbance and forest type on an arboreal mammal, enabling exploration of the mechanism behind observed anomalies in population dynamics. Determining the cause(s) of population decline is important for designing and adapting management strategies and to act to prevent local extinction. In most cases of population decline, identification of a causal mechanism is very difficult due to the number of

environmental factors that may have synergistic effects (Carey 2005). Integration of different types of information from diverse disciplines provides more insights than a purely ecological, physiological, or genetic study (Wildt *et al.* 2003). This premise is exemplified in Chapter 5 where ecology and genetics are integrated to test and eliminate competing hypotheses, allowing elucidation of the mechanism behind the sex ratio bias of the populations of brushtail possums living in disturbed habitat.

The limitations of this study lay primarily in the restricted availability of suitable study sites. Site selection was constrained by the need to choose sites with similar broad forest type, geology, and elevation. Thus, the disturbed sites chosen varied in age since disturbance and silvicultural method employed. This study did not aim to discern between the impacts of different silvicultural methods. The short time frame of this study and the focus on medium-term (4–11 years post harvest) recovery meant that it was impossible to conduct a study that assessed the mammal community at the sites before and after harvest. A longitudinal study before and after a disturbance event provides the greatest power to make inferences about ecological recovery (Stewart-Oaten and Bence 2001). When this is not possible, a 'space-for-time' survey of disturbed and control sites provides an alternative method (Pickett 1989). This space-for-time (or retrospective) study has provided some valuable insights into small-medium mammal recovery after current forest harvest practices used in two broad forest types throughout Tasmania.

The approach taken in this study may be useful in examining the effectiveness of forestry management prescriptions and the status of faunal communities in managed landscapes. The occurrence of mammal species, thus community composition, was dependent on forest type, while habitat disturbance directly influenced abundance (Grinnell 1917; MacArthur 1972; Dueser and Shugart Jr. 1978; Mares *et al.* 1986). Of the small-medium mammals investigated in this study, only the brushtail possum showed a response to site-level habitat disturbance 4–11 years post harvest. No ground-dwelling mammal species responded to forestry disturbance at this temporal scale. Cork and Catling (1996) argued that both ground-dwelling and arboreal mammals are dependent on forest structure. The difference in response in this study is likely due to differences in ecological requirements among species.

Ground-dwelling mammals are dependent on characteristics of the undergrowth (Taylor 1991), much of which remained or increased in the areas harvested in this study. These elements, with the exception of ground logs (abundance of which, as logging debris, is high immediately post harvest but low in the long-term (Scotts 1991; Grove and Meggs 2003)), regenerate relatively quickly following harvesting. Arboreal mammals, on the other hand, depend upon mature forest elements that are slow to regenerate; for example, tree hollows take more than 140 years to develop (Koch *et al.* 2008b).

A multi-disciplinary approach to monitoring both individual and population level responses is crucial to a comprehensive understanding of the mechanisms underlying species decline and persistence. While brushtail possums are considered resilient to anthropogenic influence, in this study they exhibited a response to habitat change that may be detrimental to the long-term viability of populations. A valuable future direction would be ongoing longitudinal monitoring of these study sites to record the recovery of the mammal fauna as the harvested sites regenerate to mature forest and as the surrounding matrix continues to be utilised for wood production. It would also be constructive to conduct a similar study to this over a continuum of matrices varying from purely intact forest to a purely agricultural landscape. Together, this information would allow determination of the critical threshold of mature elements in the matrix necessary to mitigate effects of harvest for guilds/ecological groups of animals and/or individual species. Such knowledge would enable management of production forests to mitigate short to medium-term impacts of harvest on fauna and maximise long-term ecological recovery of both habitat and the mammal community.

An important aspect of studies of habitat disturbance is consideration of recovery time and recovery potential. Populations may recover, even though there is often an immediate response to forest harvest and loss of habitat (Hocking 1981; Lambin 1994; Lopez and Ferrari 2000; Atwood 2006). Recovery potential and time varies with fauna type. Ground-dwelling mammals appear to recolonise relatively quickly (Cork and Catling 1996; Ferron *et al.* 1998; Moses and Boutin 2001), while arboreal mammals recover more slowly due to their reliance on habitat provided by standing trees (Tyndale-Biscoe and Smith 1969; Tyndale-Biscoe and Calaby 1975; Cork and Catling 1996; Laurance 1996; Alexander *et al.* 2002). The results of this study reduce uncertainty about how forest fauna respond to and

recover from habitat disturbance, which is imperative for the development of effective forest management strategies. All species in this study showed resilience and persisted in sites in the medium-term after harvest, probably due to the availability of mature forest and habitat elements in the surrounding landscape. Overall, brushtail possums appear tolerant of forestry disturbance at the site scale examined in this study; this is probably because sufficient habitat resources are retained in the surrounding landscape. The availability of resources in areas surrounding disturbed sites can mitigate the impacts of disturbance and allow animals to recolonise sites after harvest (Brockerhoff *et al.* 2008). The population responses (e.g., abundance, breeding frequency, and the adult male bias observed in the sex ratio) of the brushtail possums, however, suggest that current retention of mature forest within harvest areas may not fulfil all the requirements to sustain an arboreal mammal population in the long-term and may need to be reviewed depending on the management goal.

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Appendices

Eucalyptus trees and brushtail possums (*Trichosurus vulpecula*)

Appendix A. Field data sheets and data collection

(a) Brushtail possum data sheet

BRUSHTAIL POSSUM DATA SHEET:

Date:	Time:	Site:	Transect:
Recorder:	Trap No:		GPS coords of trap:

ADULT DATA:			
Transponder Number:	982 009 10		
Sex:		Age:	Colour:
Wt(kg):		Head length:	
Pes length:		Leg length:	
Tail length:		Left Testis (lxw):	
Pouch condition (active, non-active/prev. young, virgin):			
Stage of Lactation (early, mid, late):		Active nipple (R/L):	

SAMPLE COLLECTION:			
BLOOD			
Blood collected (amt):		Slide ID:	
Hematocrit 1:	Tube (l) 1:	Hemcrit 2:	Tube (l) 2:
Hematocrit 3:	Tube (l) 3:	Hemcrit 4:	Tube (l) 4:
GENETICS:		Biopsy (Y/N):	
MILK			
Oxytocin inj amt:		Milk collected (amt):	
Mammary (lxw):		Nipple (l):	
Length of time under:		Start time:	Stop time:
Recovery time:		Notes:	

POUCH YOUNG DATA: DEVELOPMENTAL LANDMARKS			
Ear/nose pigment (Y/N):		Noise (Y/N):	
Eyelashes (Y/N):		Vibrissae (Y/N):	
Testis (Y/N):		Shivering (Y/N):	
Underhair (Y/N):		Movement (Y/N):	
Claws (Y/N):		Claw colour/integrity:	
Head length:		Body length:	
BACKYOUNG DATA:			
Transponder Number:	982 009 10		
Sex		Colour:	
Head length:		Pes length:	
Leg Length		Tail length:	
Wt(g):		Notes:	

Distinguishing Markings: Dorsal: Ventral:
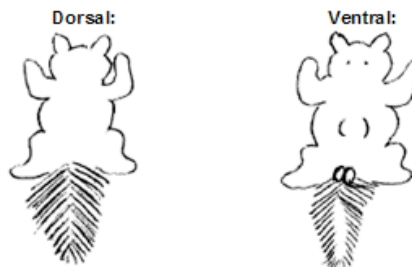
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(June 08 Rev.)
EMF-BTP

(b) Bycatch (non-target species) data sheet

BYCATCH DATA SHEET:			
Date:	Time:	Site:	Transect:
Recorder:		Trap No:	GPS coords of trap:
Species:			
Transponder Number:			
Sex:			
Coat Colour:			
Wt(kg):			
Notes/Distinguishing marks:			

BYCATCH DATA SHEET:			
Date:	Time:	Site:	Transect:
Recorder:		Trap No:	GPS coords of trap:
Species:			
Transponder Number:			
Sex:			
Coat Colour:			
Wt(kg):			
Notes/Distinguishing marks:			

BYCATCH DATA SHEET:			
Date:	Time:	Site:	Transect:
Recorder:		Trap No:	GPS coords of trap:
Species:			
Transponder Number:			
Sex:			
Coat Colour:			
Wt(kg):			
Notes/Distinguishing marks:			

BYCATCH DATA SHEET:			
Date:	Time:	Site:	Transect:
Recorder:		Trap No:	GPS coords of trap:
Species:			
Transponder Number:			
Sex:			
Coat Colour:			
Wt(kg):			
Notes/Distinguishing marks:			

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(06/07 Revise)

(c) Tree hollow survey rules

Method:

In order to assess tree hollow availability and trees most likely to be used by brushtail possums throughout the site, conduct surveys 10 times per site (~200 m intervals)

Survey all *Eucalyptus* trees in 0.25 ha circular (28.2 m radius) plot. Use rangefinder to establish the plot area. Only consider trees with ≥ 500 mm diameter at breast height (DBH) (1.3 m off the ground as measured from uphill side of the tree). Count number of hollows suitable for brushtail possum use (> 50 mm diameter entrance) that are VISIBLE from the ground and look deep enough for entry; these must be > 2 m off the ground. Make note of tree shape (from Koch *et al.* 2008), DBH, and a senescence assessment (rank volume of dead wood in the canopy). Make note of presence/absence of: dead top, basal hollow, possum 'runways' and other signs of use, and fire scarring.

Senescence assessment:

Rank volume of dead wood in the canopy:
Classify tree by the diameter of the largest dead branch in the canopy

| None	Small	Med	Large
(No dead branches < 50 mm)	(50–150 mm)	(150–400 mm)	(> 400 mm)

Tree hollow definition (in context of brushtail possums):
• > 50 mm diameter entrance
• Visible from the ground (be conservative: if you cannot tell that it is a hollow, then
  do not call it a hollow)
• Looks deep enough for entry (you need to able to see that the hollow goes somewhere)
• Must be > 2 m off the ground

Hollow Rules:
• Deep fissures count (assume useable cavity behind it)
• In case of broken off but upturned branch or fractured tree top: if I cannot see the
  hollow, I cannot know that it is there

(d) Tree shape classification for tree hollow surveys (Koch *et al.* 2008) (as modified from Smith and Lindenmayer (1988)):
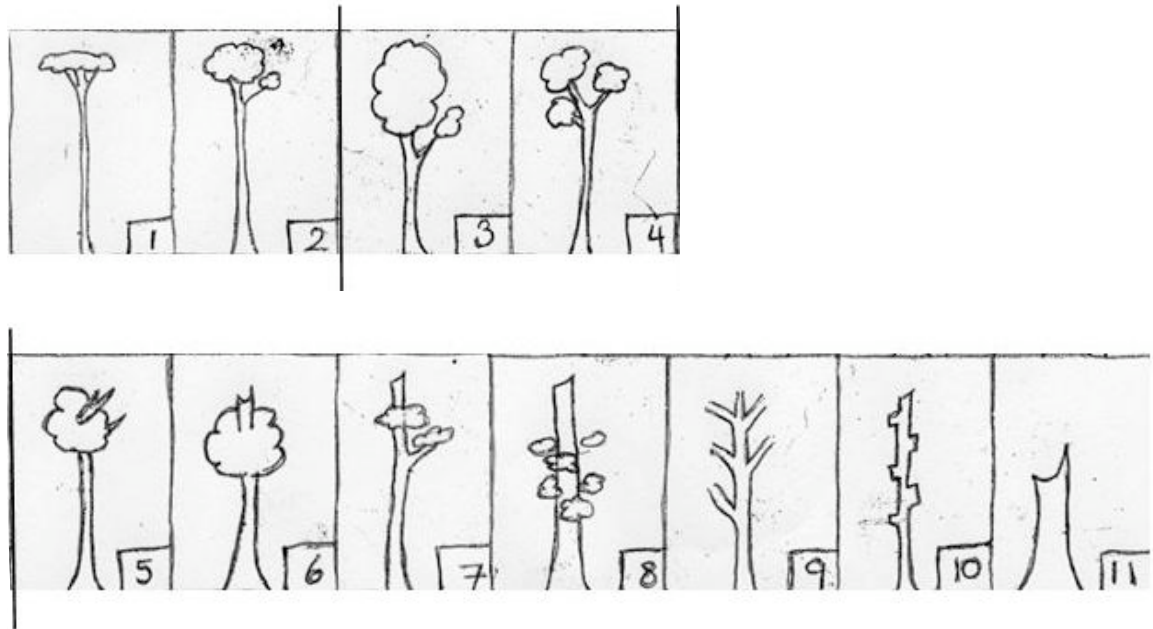
 [Won't be old or big enough to have tree hollows (A. Koch pers. comm.)]

1. Regrowth or medium-growth tree with no major branches off trunk

2. Regrowth or medium-growth tree with major branches off trunk

3. Large tree with no major branches off of trunk

4. Large tree with major branches off of trunk

5. Mature tree with dead branches (live top)

6. Mature tree with dead top but big crown [lots in wet forest (A. Koch pers. comm.)]

7. Mature tree with dead top and small crown

8. Burnt tree with only epicormic growth [FIRE]

9. Dead tree retaining branches (E. Flynn interpretation)

10. Dead tree with nubs left (E. Flynn interpretation)

11. Hollow stump [stump must be > 2 m to be 'useful' to brushtail possums (E. Flynn interpretation)]

(e) Tree hollow survey data sheet

Hollow Assessment

Site	Plot #	Trap # based at	Recorder	Date

Number of trees containing hollows >5 cm diameter entrance in 0.25 ha area (radius 28.2 m) [Spp and # of holes]

Tree #	Spp	DBH	# hollows (>5 cm)	Tree shape (1-11)	Dead top (y/n)	Dead wood (O,S,M,L)	Fire scarring (y/n)	Basal hollow (y/n)	Poss marks (y/n)
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									

(f) Vegetation survey data sheet (designed and issued by Forest Practices Authority, Hobart, TAS)

CUST SITE ID			SITE CODE
LOCATION/RESERVE		SAMPLE NO.	
RECORDER	DATE	MAP	
EASTING	NORTHING	ALTITUDE	
SLOPE	ASPECT	LANDFORM	
GEOLOGY	ROCK COVER	DRAINAGE	
LITTER/BARE G	NON VASC		
COMMUNITY			

R= regrowth _____ = dominant ( )= occasional D = Dead

Strat	Height	Cover	Species

Additional species outside plot but in community and within 500 m of plot	

Species recorded elsewhere but within 1 km of plot	

DISTURB. HIST./ MANGT ISSUES	FAUNA/ HABITAT	OTHER NOTES
log/firewood		
fire		
grazing		
human/mech		
weeds		
disease/other		

(g) Calculating total basal area (an estimate of stand density)

The 'wedge' (Figure 1) was used to calculate total basal area (an estimate of stand density) using the Angle Count Sampling ('sweep') method (Goodwin 1995) in a full circle with a trap as the centre point once in each vegetation survey plot. The wedge is held by the appropriate knot with the knot held close to the observer and the 'body' of the wedge held extended the length of the string at eye level. All 'wooded' stems (> 10 mm diameter at breast height) that were wider than the width of the notch on top of the body of the wedge were counted as a value of one and stems that were an equal width to the notch were counted as a value of 0.5. Stems smaller than the width of the notch were not counted. The values for each survey plot were added together for a total number of stems at the completion of the circle. In wet *Eucalyptus* forest (which is more dense), the first knot is used and the number of stems observed is multiplied by a factor of four to generate total basal area. (Example: If 20 stems are counted, total basal area is 20 x 4 = 80 m^2/ha). In dry *Eucalyptus* forest (which is less dense), the second knot is used and the number of stems observed is multiplied by a factor of two to generate total basal area. (Example: If 20 stems are counted, total basal area is 20 x 2 = 40 m^2/ha).
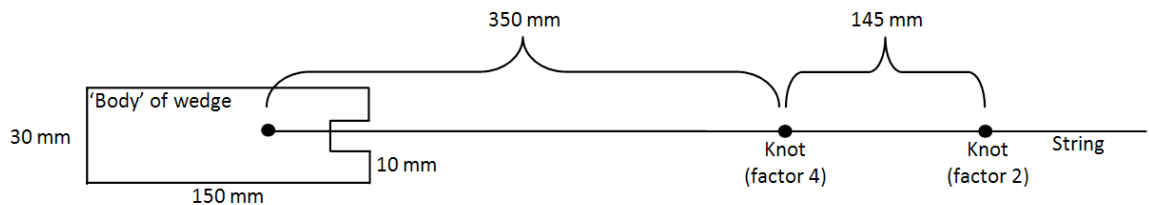
**Figure 1. The 'wedge' used to estimate total basal area**

Appendix B. **Composition of the study sites ('core' and 'matrix' of each site) in the context of the surrounding landscape.** Location of each trap and boundaries of both the core and matrix sites are indicated amongst vegetation type (TASVEG) (DPIW 2001) and the dominant age structure of the vegetation (Stone 1998; Forestry Tasmania 2007a; Forestry Tasmania 2007b; Forestry Tasmania 2007c).
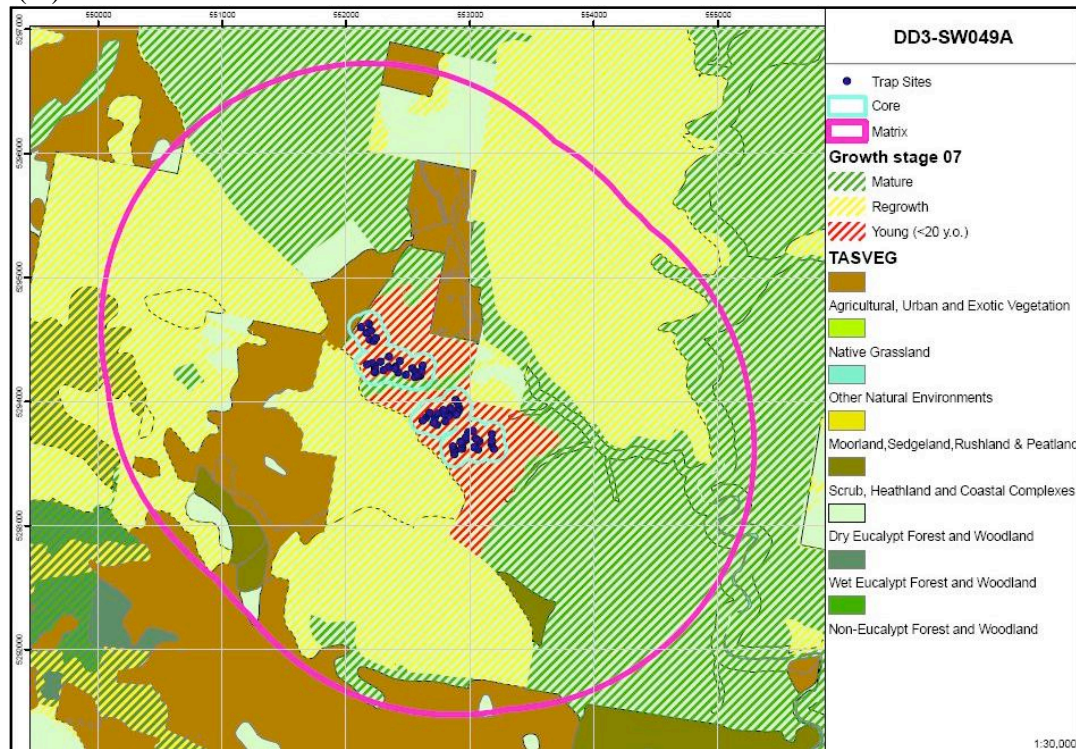
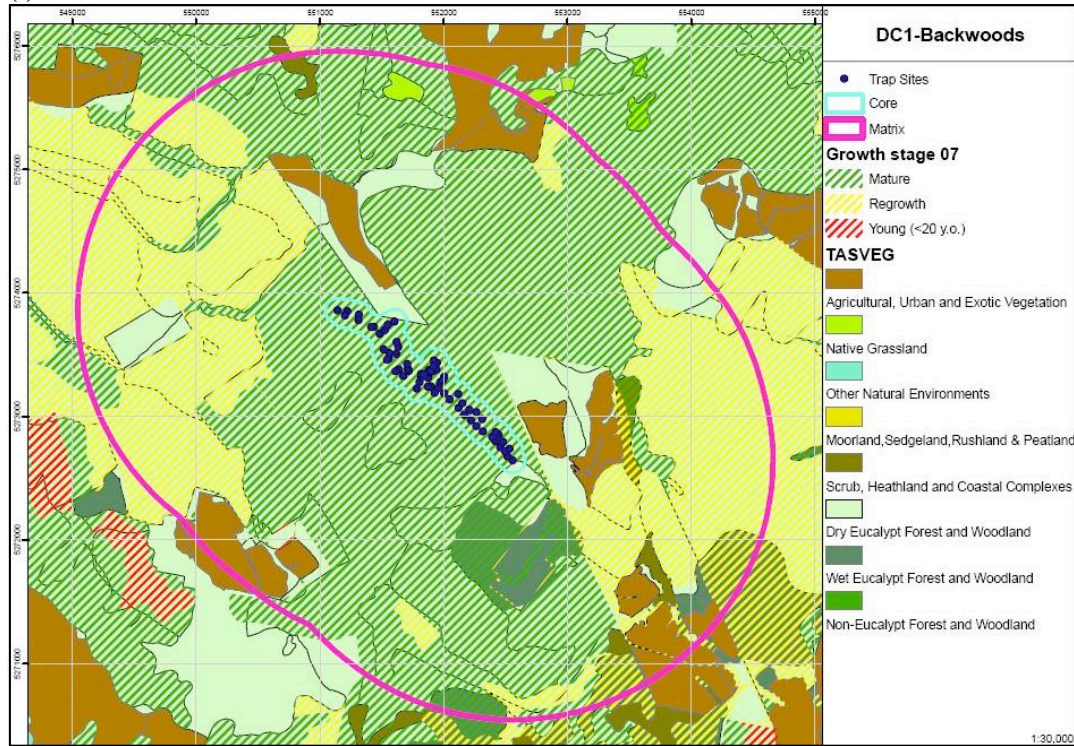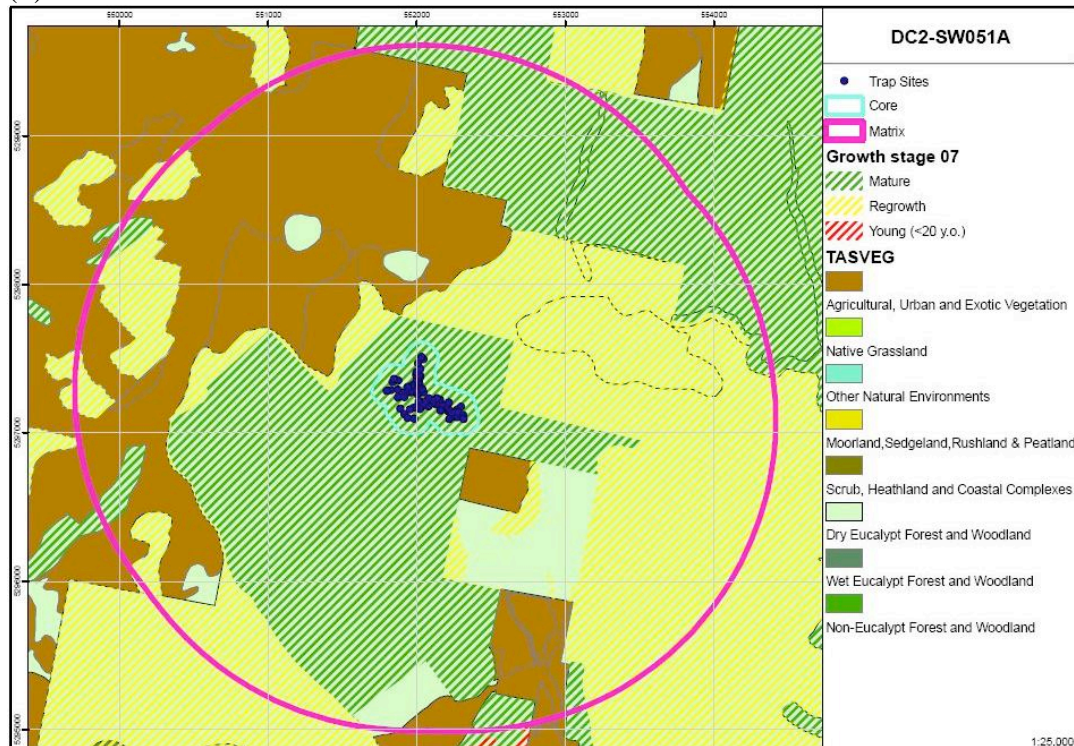(a) Dry *Eucalyptus* forest disturbed sites
(i) DD1
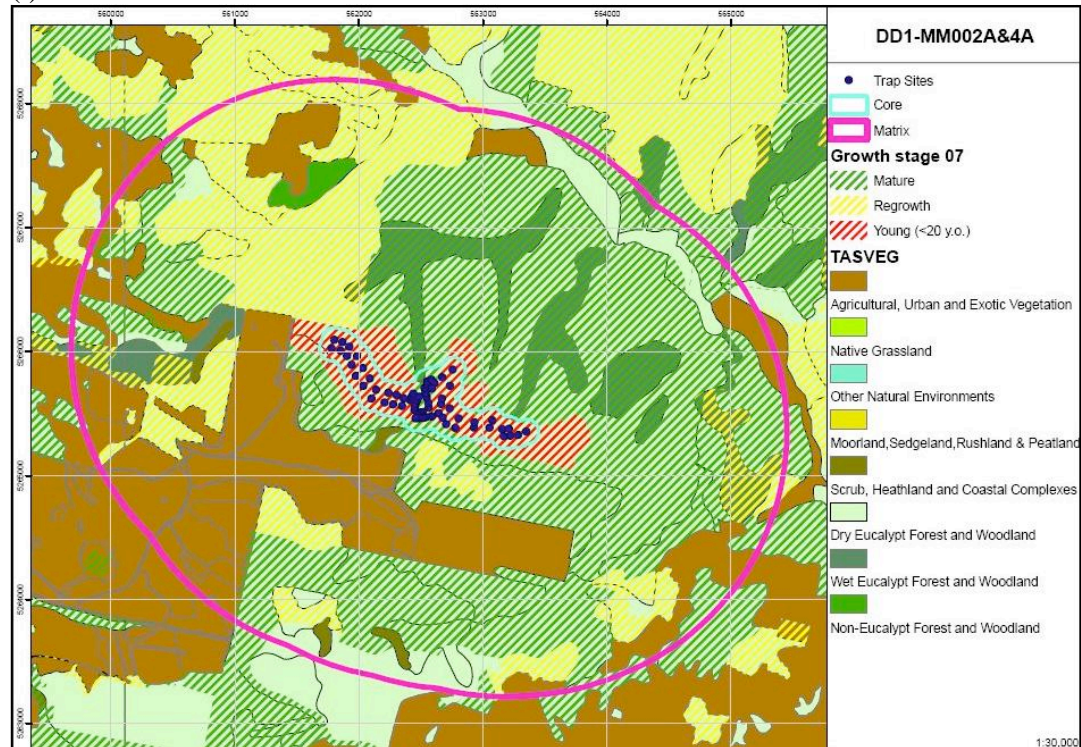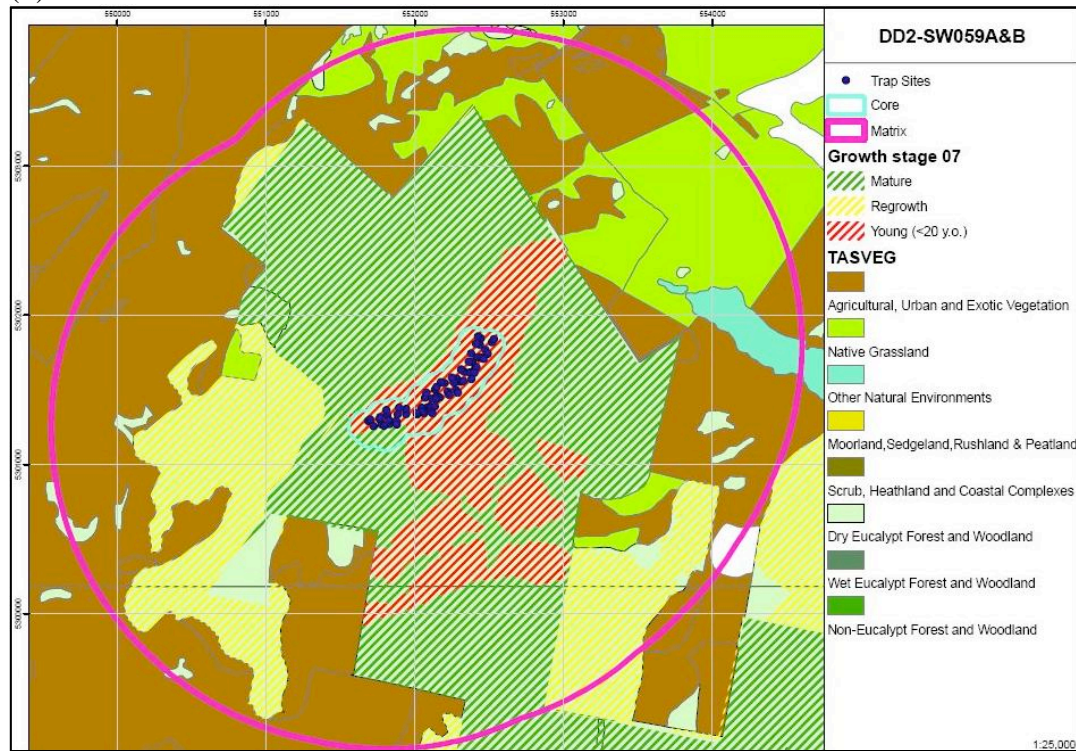
(ii) DD2

(iii) DD3

(b) Dry Eucalyptus forest control sites
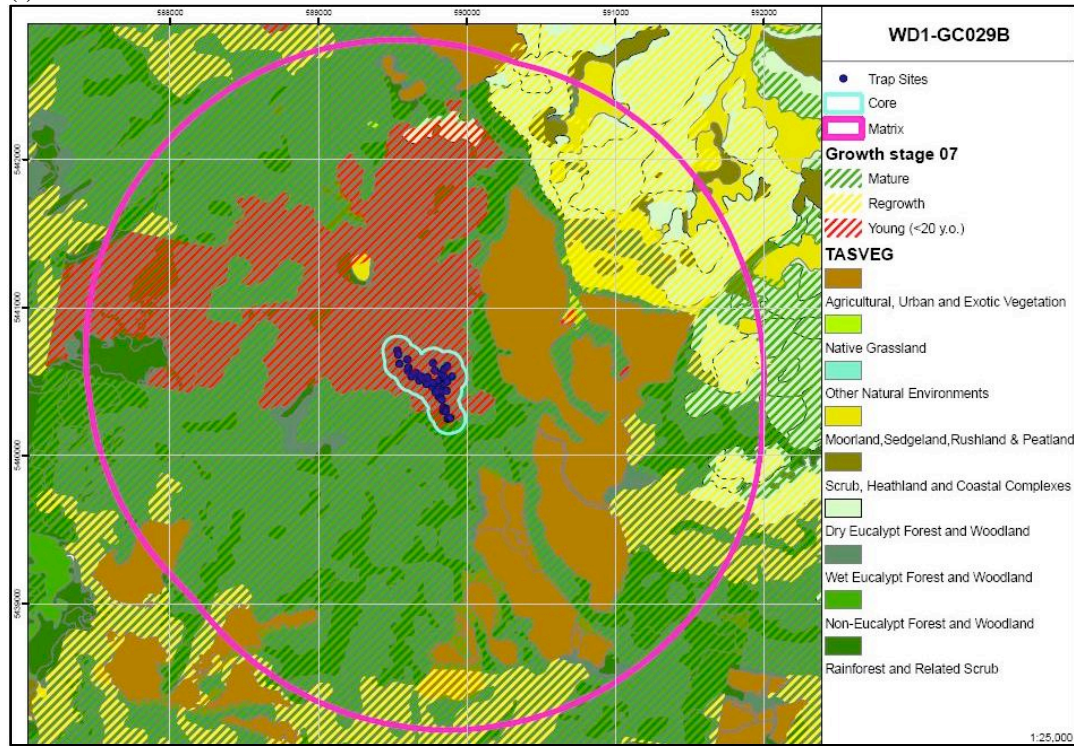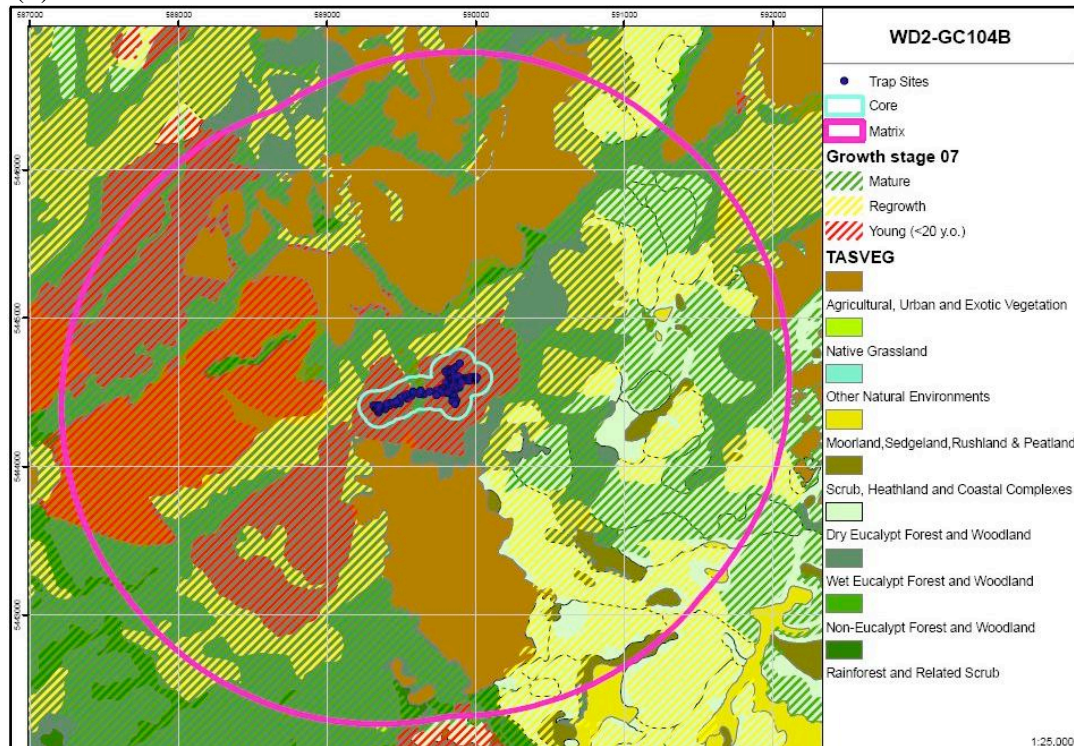(i) DC1

(ii) DC2

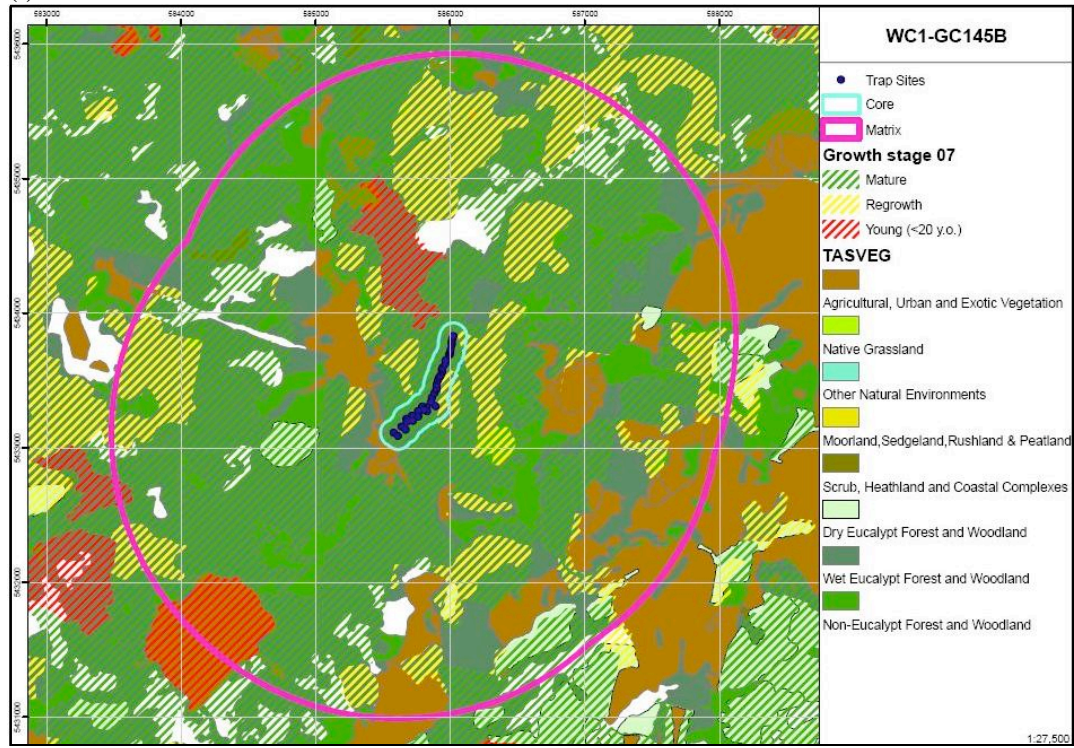(iii) DC3
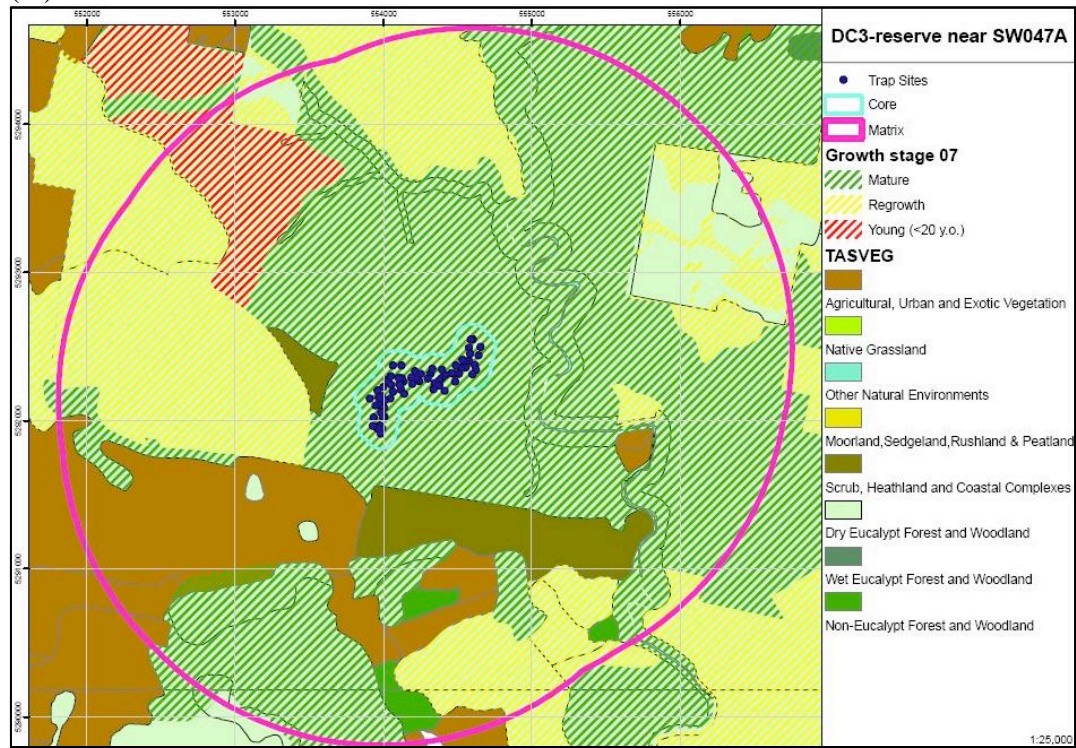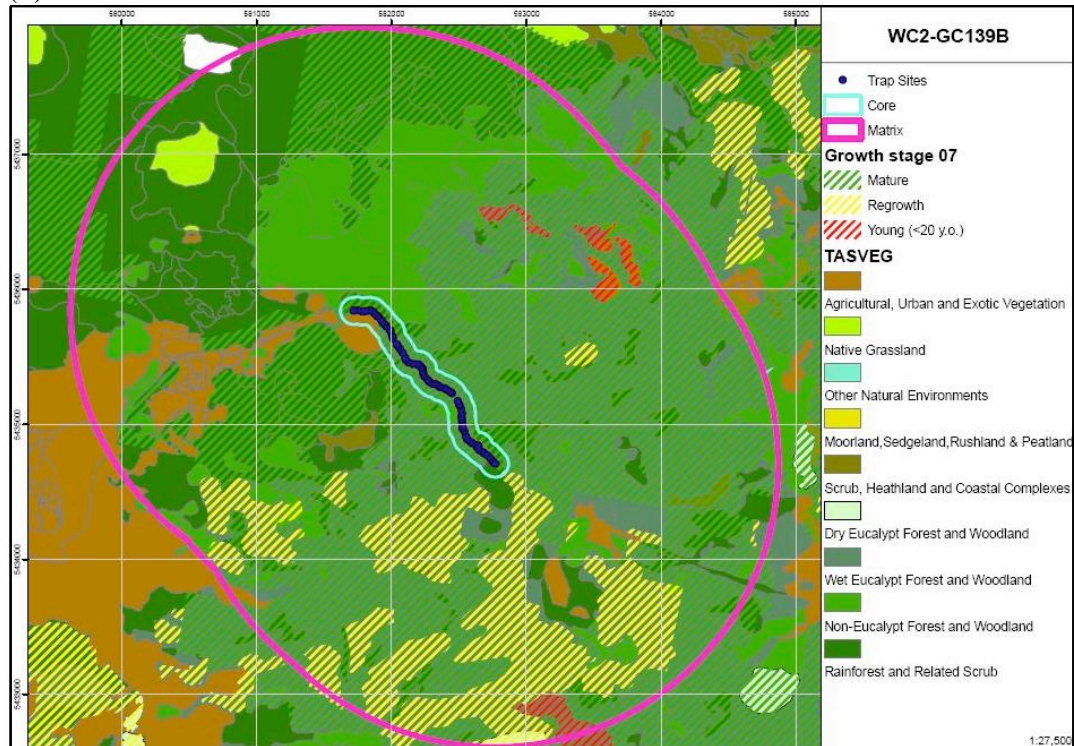
(c) Wet Eucalyptus forest disturbed sites
(i) WD1

(ii) WD2

(d) Wet Eucalyptus forest control sites
(i) WC1

(ii) WC2

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