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Physiological and Behavioural Responses of Australian and Exotic Prey to the Scent of Native and Introduced Predators

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DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: ………………………………………….

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General abstract

This study examined the physiological and behavioural effects of a stress-inducing stimulus (predator odour) on potential prey species (Australian native and exotic). The aim was to determine if differences in the response of prey were related to the scent of evolutionary known predators compared to unfamiliar or short-term introduced ones. In laboratory experiments, responses were always restricted to changes in respiratory variables, with brushtail possums (*Trichosurus vulpecula*), rabbits (*Oryctolagus cuniculus*) and tammar wallabies (*Macropus eugenii*) showing no variation in metabolic rate after exposure to predator odours.

Brushtail possums showed strong changes in ventilatory rate only when faced with the scent of an historical predator, increasing respiratory frequency (ratio after/before exposure = 4.55 ± 1.007) and decreasing tidal volume (ratio after/before exposure = 0.38 ± 0.113) in response to stale dingo urine. The changes were short-lived, lasting for only one minute of exposure. For this reason, it is unclear if the response observed could be considered as a fear reaction. However, there was no habituation after three exposures and this may indicate that possums were initially displaying an investigative approach to a predator scent and then relaxing once assessment was completed.

The effect of predator and novel odours on the ventilation of rabbits appeared to be in general mediated by anxiety, as shown by the responses elicited by both predator and control scents. However, stronger reactions were observed in response to feral cat and quoll odours for both respiratory frequency (ratio after/before exposure to feral cat = 4.39 ± 0.721 and to quoll = 3.75 ± 0.486) and tidal volume (ratio after/before exposure to feral cat = 0.47 ± 0.065 and to quoll = 0.64 ± 0.129). This could be due to different intensity of the olfactory stimuli. Nevertheless, the effects of noxious odours on rabbits clearly demonstrate that they become highly vigilant at any sudden change in their environment.

Tammar wallabies appeared to possess a mechanism for the recognition of predator odours as ventilatory responses were restricted to particular predator scents. After

investigation, fox and cat odour provoked a stronger and more prolonged change in respiratory frequency (ratio after/before exposure to fox = 3.58 ± 0.918 and to feral cat = 2.44 ± 0.272) and tidal volume (ratio after/before exposure to fox = 0.84 ± 0.110 and to feral cat = 0.98 ± 0.155) compared to the other scents, suggesting that wallabies may have perceived these species as more immediate threats.

For wild, free-living brushtail possums and southern brown bandicoots (*Isodoon obesulus*), there was no pattern of avoidance of historical or introduced predator odours, with no difference in number of animals captured in predator and control scented traps. This may indicate that predator odour avoidance has not evolved in these species and that they have poor possibilities of escaping potential predators. However it may also be explained by the long-term predator-free environment in which the study was conducted, and suggests loss of anti-predator behaviour in populations without predation risk.

Wild, free-ranging western grey kangaroos (*Macropus fuliginosus*) reacted to the odour of both historical (dingo) and novel (fox) predators by reducing number of feeding events (5.2±2.08 for fox and 5.9±1.33 for dingo) and time spent foraging (17.7±7.2 sec for fox and 22.2±4.6 sec for dingo) when predator scents were present and by escaping areas tainted with predator odours (41.4±17.5 sec for fox and 33.8±13 sec for dingo). Clearly these results suggest that kangaroos are scared of predator odours. However, a close investigation of predator scents was necessary before a response could be elicited and feeding areas were not completely abandoned.

It is still unclear if small Australian prey, such as southern brown bandicoots and brushtail possums, respond to olfactory cue of predation in the wild, as different results were obtained in the studies conducted in the laboratory and in free populations. However, this research showed that medium and large-sized macropods respond to both native, long-term and introduced predator odours. This indicates that at least some Australian prey species can recognise the odour of potential predators, although responses usually occur after a period of investigation, and do not always result in avoidance behaviour. Differential responses appear to be based on the perceived risk. Use of predator odour is unlikely to be an effective mechanism of deterring herbivores from preferred feeding locations.

Thesis structure

This thesis consists of a series of stand-alone papers intended for publication, therefore each chapter has its own abstract, methods, results, discussion acknowledgements and references section. This causes a small degree of repetition in the method sections and in the citations when the same experiment procedures were used. Chapter 1 gives a general overview on the research undertaken, on the aims and objectives of the thesis and briefly describes the prey and predator species studied. Chapter 2, 3 and 4 describe the physiological experiments carried out on brushtail possums, rabbits and tammar wallabies respectively to investigate their responses to predator odours. Chapter 5 examines the trapping success of brushtail possums and southern brown bandicoots in the wild in two experiments using traps tainted with different predator odours and control traps. Chapter 6 explores the foraging and anti-predator behaviour of western grey kangaroos under increased risk of predation in the wild and investigates the changes in occurrence of behaviour and time allocated to different activities. Chapter 7 is a general discussion, where all the results are reviewed, specific findings are summarised and final conclusions drawn. Consequences and implications for animal conservation and management are discussed.

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Chapter 1: General introduction

Prey populations are often limited and controlled by predators, not simply through direct predation, but because predators influence the behaviour of prey (Paine 1969; Soule *et al.* 1988; Henke & Bryant 1999). In fact, predation can have a number of non-fatal effects, eliciting anti-predatory responses in prey, which result in behavioural modification (Glen *et al.* 2007). Predation pressure has been shown to have a large impact on prey space use (Lima 1990), habitat preference (Jordan *et al.* 1997), feeding rate (Brown *et al.* 1999), activity pattern (Fenn & Macdonald 1995; Boonstra *et al.* 1998; Perrot-Sinal *et al.* 1999) and breeding (Fuelling & Halle 2004). Studies have indicated that predators have an important role in maintaining ecosystem function and population dynamics. For instance, it has been shown that complete removal of predators from a system not only produces an overabundant number of herbivores but as a consequence affects plant communities (McLaren & Peterson 1994). An example is the excessive grazing by herbivores which causes destruction of vegetative cover and of habitat for other species (Burbidge & McKenzie 1989; Terborgh *et al.* 2001). In Australia, the introduction of exotic herbivores has reduced vegetation and caused an increase in aridity (Stanley 1983; Burbidge & McKenzie 1989). Although non-lethal predator-prey interactions have been investigated, extended effects on populations of vertebrate terrestrial animals are still mostly unknown (Lima 1998a).

Odour plays a significant role in predator-prey interactions (Kats & Dill 1998). Predators normally produce pungent scented wastes and scent marking behaviour is widespread in all carnivore families (Macdonald 1980; Gorman & Trowbridge 1989; Gese & Ruff 1997); this is important for both territorial marking and individual recognition (Jorgenson *et al.* 1978; Bartos & Rodl 1990). However, odours left by predators may also be perceived by prey species as a warning signal of predation risk and may be exploited to reduce the danger of encountering a predator by avoiding scent marked areas (Kleiman 1966; Gorman 1980; Macdonald 1980; Gorman & Trowbridge 1989). Prey species have evolved mechanisms for recognition, and avoidance of predators (Endler 1991), which may include sensitivity towards particular predator-derived scents (Kats & Dill 1998; Lima 1998b), so that prey become genetically inclined to avoid predator odours (Stoddart 1982; Weldon *et al.* 1993; Nolte *et al.* 1994).

This thesis explores an olfactory sensory fear pathway to investigate the responses of prey to predator scents. A number of studies have shown that predator odours can elicit fear responses in prey, however, predator odour recognition is not always associated with a clear anti-predator behavioural response (see review in Apfelbach *et al.* 2005). As fear is an emotional state provoked by an animal's perception of a possible danger (Boissy 1995), the examination of physiological parameters of animals under the stress of predation risk may help to better interpret responses to predator scents. There has been only limited research on anti-predator responses of prey in the natural environment combined with physiological laboratory experiments. Prey species in this study were subjected to various olfactory stimuli from predators in both laboratory and field environment to enable a better understanding of behavioural responses observed in reaction to predator odours. In addition, most of the former studies conducted on predator odour recognition used only one particular predator or synthetic odour against an unscented control (Fendt 2006). In this research responses to different predator odours and sources were compared with the reactions to specific control scents, such as biological non-predator odours, as well as an unscented treatment.

This study also investigates if there is any pattern of response with respect to the history of predator-prey relationships, as different reactions to different predators are expected to be selected for animals which have coevolved compared to ones that have been coexisting for only a short time (Russell *et al.* 2003; Russell 2005). Australia has a unique mammalian fauna, which evolved in biogeographical isolation for around 35 million years (Van Dyck & Strahan 2008), therefore since European settlement, the rapid introduction of exotic predators has caused naïve prey to face evolutionary unfamiliar predators (Burbidge & McKenzie 1989). Contrary to Australian native fauna, which has only had limited contact with introduced predators, the exotic prey brought to Australia have had long evolutionary association with these specialised predators (Pongracz & Altbacker 2000; Short *et al.* 2002; Malo *et al.* 2004; Delibes-Mateos *et al.* 2007) and various studies have already shown that scent has an important function in their predator avoidance (Dickman

1992; Monclús *et al.* 2005; Monclús *et al.* 2006a; Monclús *et al.* 2006b). Australian fauna may be more responsive to native or long-term introduced predators, as insufficient time has elapsed to evolve avoidance to short-term introduced ones. It is still unclear how Australian prey animals respond to odours of native and introduced predators, since some studies showed that native prey species avoid predator scents (Montague *et al.* 1990; Woolhouse & Morgan 1995; Morgan & Woolhouse 1997), while others did not (Banks 1998; Banks *et al.* 2003). Comparative laboratory and field analyses of the similarities and differences in the responses to potential predator odours of Australian and exotic prey may complement other results and help to clarify the physiological and behavioural consequences of predation risk on prey species.

In particular this research had the following objectives:

- To determine if two different Australian prey species (arboreal and terrestrial) showed any physiological response to potential predator odours in the laboratory, and if these responses differed for historical and short-term introduced predators.
- To determine if an exotic terrestrial herbivore showed a physiological response to the odour of potential predators in the laboratory and if responses differed for Australian and introduced predators.
- To determine if three Australian prey species (arboreal and terrestrial) in the wild avoid the faecal scent of historical and short-term introduced predators and if there was any change in their use of space or in time allocated to anti-predator activities.
- To determine if certain predator scents were more effective in provoking a response in Australian native prey and if so, to determine if odours of historical predators would be more efficient than those from recently introduced ones.

Study species: predators

Predator-based odours used in this study were derived from different scent sources, such as predator skin, fur, urine and faeces, all of which have shown to be able to elicit responses in prey species in previous studies (Apfelbach *et al.* 2005). Predators used as scent donors were both Australian native, long-term introduced and short-term introduced. These categories were chosen to allow comparisons between prey reactions to different kinds of predators, such as to mammalian and non-mammalian or to native and introduced ones. All the mammalian predators studied use wastes for scent-marking behaviour (Macdonald 1979; Kruuk & Jarman 1995; Henry 1996), therefore their odour has the potential to represent a cue of predation risk for prey species.

Native predators

Reptilian predator

The black-headed python (*Aspidites melanocephalus*) was chosen to represent the non-mammalian predator category. This snake is locally common across northern Australia (Hoser 1989; Cogger 1996). It is mainly nocturnal, terrestrial (Torr 2000) and it feeds on a range of reptiles as well as birds and mammals (Pearson 2005). Various prey species have been observed to respond to the odour of snakes both in laboratory studies (Weldon *et al.* 1987; Miller & Gutzke 1999; Stapley 2003) and in field observations (Gutzke 2001). However, in my knowledge no study has previously assessed the use of black-headed python odour to deter potential prey species.

Marsupial predators

Australian (Hayes *et al.* 2005; Russell & Banks 2007) and exotic mammals (Dickman 1992) have been shown to avoid marsupial predator scents, such as quolls (*Dasyurus* species). Quolls are mainly nocturnal opportunistic predators with both arboreal and terrestrial habits and with a diet that includes brushtail possums (*Trichosurus vulpecula*), bandicoots, rabbits (*Oryctolagus cuniculus*), small wallabies, birds, reptiles, amphibians and invertebrates (Belcher *et al.* 2008; Oakwood 2008; Serena & Soderquist 2008). Quolls commonly scent mark, use latrines (Kruuk & Jarman 1995; Belcher *et al.* 2008) and have strongly scented body and faeces (Braithwaite & Begg 1995). Therefore their scent could represent a reliable cue of predator presence to prey. In this thesis, two species of quoll, the spotted-tailed (*Dasyurus maculatus*) and the northern quoll (*Dasyurus hallucatus*), were used to represent the marsupial predators category.

Long-term introduced predator

This category includes a predator which was introduced in Australia over 3000 years ago (Corbett 1995, 2008) and has today an important stable role in Australian ecosystems. The dingo (*Canis lupus dingo*) represents the only long-term established top order predator on the mainland (Johnson *et al.* 2006). In fact, since the disappearance of the thylacine (*Thylacinus cynocephalus*), the dingo has become the main predator for medium-sized and large native herbivores such as wallabies and kangaroos (Robertshaw & Harden 1986; Thomson 1992; Corbett 1995). Populations of red (*Macropus rufus*), eastern grey (*Macropus giganteus*), western grey kangaroos (*Macropus fuliginosus*) and euros (*Macropus robustus*) all appeared to be less abundant where dingo was present (Caughley *et al.* 1980; Newsome 1990; Pople *et al.* 2000). In recent studies dingo odour seemed to represent a reliable predator cue, able to induce avoidance responses in prey (Hayes *et al.* 2005; Parsons *et al.* 2007).

Short-term introduced predators

Predators which arrived in Australia less than 150 years ago (Van Dyck & Strahan 2008) were used to represent the short-term introduced predator category. This included the dog (*Canis lupus familiaris*), the red fox (*Vulpes vulpes*) and the feral cat (*Felis catus*). The dog is closely related to the dingo and in Australia they are known to produce hybrids (Glen & Dickman 2003). Australian mammals seem to often fall prey to dogs (Seebeck 1979; Meek 1999; Isaac 2005), therefore dogs can have serious impacts on native fauna (Fleming *et al.* 2001).

The cat was introduced in Australia in the early nineteenth century and now occurs throughout the whole mainland (Denny 2008). It is thought to be associated with the decline of several species of native animals (Dickman *et al.* 1993; Short & Smith 1994; Smith & Quin 1996). In fact, although the rabbit represents its main prey (Molsher *et al.* 1999), the cat exploits a wide range of native fauna (Newsome 1990), including invertebrates, reptiles, birds, both terrestrial and arboreal mammals like small macropods, bandicoots and possums (Paltridge *et al.* 1997; Molsher *et al.* 1999; Denny 2008).

The red fox was introduced in Victoria in the 1860s (Coman 1983) and it rapidly became widespread in Australia, except in the northern tropics (Johnson *et al.* 2006). The fox is an opportunistic predator and although it mainly predates on rabbits (Newsome *et al.* 1997), it replaces them with several other foods, such as small mammals and birds, when these are scarce (Amores 1975; Brunner *et al.* 1975; Green & Osborne 1981; Paltridge 2002; Delibes-Mateos *et al.* 2007). The fox is the main cause of the continuing decline, local loss and extinction of populations of small and medium-sized Australian native mammals (Kinnear *et al.* 1988; Burbidge & McKenzie 1989; Saunders *et al.* 1995; Short 1998). Most attempts to reintroduce in Australia locally extinct species have failed because of fox predation (Short *et al.* 1992).

Scent marking with urine and faeces is used as territorial signal by all these introduced predators (Macdonald 1979, 1980; Kruuk & Jarman 1995; Henry 1996). Studies have shown that odours derived from dog were effectively used to suppress feeding in prey species (Montague *et al.* 1990; Arnould & Signoret 1993; Epple *et al.* 1993; Mason *et al.* 1994; Englehart & Muller-Schwarze 1995; Arnould *et al.* 1998); cat scent elicited anti-predatory responses in its prey (Blanchard *et al.* 1990; Blanchard *et al.* 1993; Dielenberg *et al.* 1999; Blanchard *et al.* 2001) and fox odour has been regularly avoided by historical prey (Dickman & Doncaster 1984; Sullivan *et al.* 1985; Sullivan & Crump 1986; Rosell 2001) and also by some Australian native rodents (Hayes *et al.* 2005).

Study species: prey

Prey species used as study subjects were mainly Australian marsupials, with the only exception of an introduced herbivore. Olfaction is highly sensitive in the prey studied in this research (Bell 1980; Salamon 1996) and may therefore be used for predator detection. The species investigated provided a range of taxonomic affiliation, size, lifestyle (arboreal and terrestrial) and distributional history (native and introduced) to allow examination of the pattern of response to different potential predators. All the prey species used can be considered pests in some circumstances and require population control at some level. At present, control methods include shooting, poisoning, release of disease and controlled sterility (Robinson & Wheeler 1983; Burbidge & McKenzie 1989; Twigg *et al.* 2000), however a good comprehension of the behaviour of these animals is essential to maximise any control strategy.

Native prey

Arboreal prey

The common brushtail possum is an endemic arboreal Australian marsupial (Kerle 2001) with well developed olfaction, as individual communication is mainly by scent and sound (Green 1984; Kerle & How 2008). Although the brushtail possum is still the most widely distributed marsupial in Australia (Kerle 1984), populations have significantly declined in the last two centuries (Kerle *et al.* 1992; Kerle 2001; Gilna *et al.* 2005) with severe impacts on density and distribution of the species also in Western Australia (How & Hillcox 2000). Population decline seems to be coincident with the arrival in Australia of the fox (Burbidge *et al.* 1988; Isaac 2005). Attempted reintroductions in Australia have failed as populations disappeared within short time, with mortality mainly owing to exotic predators (Pietsch 1995). The ability of this species to survive in urban areas has obscured the significant reduction in its distribution and abundance across Australia, though on the other hand, has created problems for their noisy and destructive activities (Kerle & How 2008).

Terrestrial prey

The southern brown bandicoot (*Isoodon obesulus*) is a small nocturnal terrestrial marsupial (Buchmann & Grecian 1974). It was very abundant before the European settlement but has now only a fragmented distribution; its reduction is mainly due to introduction of exotic carnivores and habitat modification (Paull 2008). Bandicoots are know to possess well developed olfactory sensitivity (Stoddart 1980) and use olfaction for orientation and food detection (Buchmann & Grecian 1974; Quin 1992).

The tammar wallaby (*Macropus eugenii*) is the smallest member of the *Macropus* genus and it was once widespread in many zones of mainland Australia, while today is isolated in remnant populations inhabiting coastal and insular areas of Western and South Australia (Poole *et al.* 1991; Smith & Hinds 1995; Hinds 2008). Some tammar wallaby populations are still culled under destruction permits to control damage to crops and pasture (Wright & Stott 1999). Feral cats and foxes are considered to have a significant impact on the decline of the tammar wallaby (Short *et al.* 2002; Hinds 2008).

The western grey kangaroo is a large macropod with abundant distribution, occupying a broad geographic climatic range (Coulson 2008). Western grey kangaroo populations can achieve very high local densities, with a negative effect on natural habitat and pastures (Coulson 2008). Culling is currently the most widely used technique to reduce western grey kangaroos' damage to crops and fields (Poole 1995).

Some studies have been conducted on the sensitivity of these prey to predator stimuli, and predator odour recognition has been suggested for some of them (Morgan & Woolhouse 1997; Blumstein *et al.* 2002; Russell & Banks 2005; Parsons *et al.* 2007), however there is still no clear evidence of these animals ability to distinguish predator odours.

Exotic prey

The European rabbit was introduced in Australia in 1858 and quickly spread across the continent, creating serious environmental disturbance as it influences habitat composition through consumption of vegetation and its burrowing habits (Williams & Myers 2008). The rabbit not only causes damage to the environment and but also loss to Australian agriculture (McNeeley *et al.* 2003; Williams & Myers 2008). Predation by foxes, cats and dingoes has been recently examined as a possible biological control of rabbit populations, integrating other pest management techniques (Newsome 1990; Banks *et al.* 1998; Banks 2000). Rabbits have shown to respond to various predator odours with a decrease in feeding and trapping rate (Robinson 1990; Boag & Mlotkiewicz 1994; Morgan & Woolhouse 1997); in particular they have shown physiological and behavioural responses when faced with fox scent (Monclús *et al.* 2005; Monclús *et al.* 2006a).

Research outcomes

This research has two important potential applications. The first is to understand the effects of predators on prey in order to improve the conservation of Australian native animals. Biodiversity loss in Australia in general and locally in Western Australia can be largely explained by the vulnerability of Australian fauna to introduced predators and reduction in vegetative cover owing to exotic herbivores (Burbidge & McKenzie 1989). It is important to understand if and how marsupial prey can detect and respond fearfully to the odour of potential predators, as this may help to explain predator-prey relationships and to predict the impact of predators on potential prey populations.

The second application is the possibility of using predator odours to control prey species which are considered as vertebrate pests. The prospect of using the odour of predators to influence the spatial distribution of mammalian herbivores in certain areas of Australia is an interesting potential outcome of this research. Predator odours could be used in the wild to help deterrence of animals from specific areas, since they may represent effective stimuli to develop an association with the fear of encountering a predator (McLean 1995; McLean *et al.* 2000). The interdependence of prey and predators and the trade off between foraging and predation risk (McNamara & Houston 1987; Abrams 1991, 1993; McNamara & Houston 1994) may be exploited to help to change habitat use by herbivores (Gilliam & Fraser 1987; Abrahams & Dill 1989). Because of their indirect effects on their prey, predators can facilitate plant recruitment (Pace *et al.* 1999; Polis *et al.* 2000; Schmitz *et al.* 2000) by excluding herbivores from certain areas (Glen *et al.* 2007). In Western Australia, control of herbivores is important to increase plant productivity in zones undergoing rehabilitation (Jones *et al.* 2003). Furthermore, deterring animals from unsafe areas such as road verges is particularly important as collisions between vehicles and animals not only involve substantial costs (Klocker *et al.* 2006), but often result in death of native species and injury to people (Rowden *et al.* 2008).

New approaches need to be explored to minimise environmental impact in Australia and to reduce the decline of native mammals. A good understanding of animal behaviour and predator-prey strategies may help to create new plans for the conservation of Australian marsupials and may be applied to control pest species and to improve animal management.

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Chapter 3: Effects of potential predator and non-predator odours on the physiology of the wild rabbit, *Oryctolagus cuniculus*

Abstract

This study examines the extent of the physiological response of wild rabbits, (*Oryctolagus cuniculus*) to various potential predator and control scents as shown by changes in metabolic rate and ventilatory variables. The intensity of the rabbits' reaction to potential native (quoll, *Dasyurus hallucatus* and snake, *Aspidites melanocephalus*) and introduced (fox, *Vulpes vulpes* and feral cat, *Felis catus*) predator odours was compared to that to non-threatening scents (distilled water and horse, *Equus caballus* urine). No changes in metabolic rate were observed. In contrast, both control and predator scents elicited a response in ventilatory variables, with the rabbits showing long-term higher respiratory frequencies (mean before exposure = 60.87 ± 4.46 breath min^{-1} and mean after the first minute of exposure = 181.03 ± 22.74 breath min^{-1}) and lower tidal volumes (mean before exposure = 5.8 ± 0.478 mL and mean after the first minute of exposure = 1.05 ± 0.069 mL) after introduction of all the scents, except distilled water. However, a stronger reaction was observed in response to feral cat and quoll scents for both respiratory frequency (ratio after/before exposure to feral cat = 4.39 ± 0.721 and ratio after/before exposure to quoll = 3.75 ± 0.486) and tidal volume (ratio after/before exposure to feral cat = 0.47 ± 0.065 and ratio after/before exposure to quoll = 0.64 ± 0.129). The change in ventilatory rate as a reaction to the introduction of the odours can be interpreted as an increase in alertness.

Introduction

Olfactory cues suggesting the presence of a predator are known to affect the behaviour of many animals (Kats & Dill 1998). The deterrence effect of predator odours on herbivores has been successfully examined as a method of protecting against browsing damage. For instance, in a study by Swihart (1991), bobcat (*Lynx rufus*) urine reduced tree damage by woodchucks (*Marmota monax*) and in a study by Rosell (2001), fox (*Vulpes vulpes*) and raccoon (*Procion lotor*) urine prevented foraging damage by gray squirrels (*Sciurus carolinensis*). Snowshoe hares (*Lepus americanus*) were observed to suppress feeding after exposure to mustelid scent (Sullivan & Crump 1984; Sullivan 1986), fox urine (Sullivan & Crump 1986) and also to lynx (*Lynx canadensis*), bobcat (*Lynx rufus*), wolf (*Canis lupus*) and coyote (*Canis latrans*) odours (Sullivan *et al.* 1985a). Most of these studies concluded that the effects observed were due to fear responses. However, it is not easy to discriminate between a fear response and avoidance of an unpleasant odour during feeding trials. For example, rabbits (*Oryctolagus cuniculus*) showed repellence not only to real predator odours but also to a commercial product based on putrescent whole egg solids (Mason *et al.* 1999).

Physiological responses to predator scents have been used to quantify animals' fear of predation in various studies (Monclús *et al.* 2005; Feoktistova *et al.* 2007). Alertness in response to the odour of a predator had energy costs measurable as an increase in metabolic rate in a study by Ward *et al.* (1996) on hedgehogs (*Erinaceus europaeus*). Fox scent induced hormonal stress in rats (*Rattus norvegicus*) by increasing corticosterone levels (Vernet-Maury *et al.* 1984). In addition, studies have shown that high predation risk may result in animals being extremely stressed and individual growth and reproduction were affected as a consequence (Magnhagen 1991; Boonstra & Singleton 1993). For example, female grey-sided voles (*Clethrionomys rufocanus*) have been observed to suppress reproduction under the influence of predator scents (Fuelling & Halle 2004). Therefore, non-lethal effects of predation can significantly affect the fitness of prey and population dynamics (He & Kitchell 1990; Houston *et al.* 1993; Van Buskirk & Arioli 2002).

The introduction of the European rabbit and its spread throughout Australia has had negative environmental and economic consequences (Pech *et al.* 1992). Rabbits are not only responsible for the degradation of indigenous vegetation but for promoting soil erosion as they prevent the regeneration of plants by grazing (Williams *et al.* 1995; Williams & Myers 2008). In addition, rabbits have been shown to sustain populations of feral cats (*Felis catus*) and foxes. For example, Delibes-Mateos *et al.* (2007) demonstrated how fox predation in Australia is centred on rabbits when these are abundant and Molsher *et al.* (1999) illustrated how rabbits are usually the main prey of feral cats in many regions. Although density temporarily declines after high predation (Parer 1977; Newsome 1989, 1990), elevated reproductive rate and colonisation obstruct a real long-term suppression (Banks 2000).

Rabbits have reacted aversively to mink (*Mustela vison*) odour (Robinson 1990) and stopped feeding when exposed to a repellent derived from lion (*Panthera leo*) faeces (Boag & Mlotkiewicz 1994). They increased vigilance (Monclús *et al.* 2006) and corticosterone levels, and experienced an intense weight loss (Monclús *et al.* 2005) when presented with fox scent. As rabbits' habitat use is reduced under predation risk and because their breeding patterns appear to be food dependent (Cooke 1974; King & Wheeler 1985; Wheeler & King 1985), predator presence could have considerable indirect impact on their populations. A decrease in food intake would cause a decline in body condition (Banks *et al.* 1999), which may trigger reduced fecundity (Parer 1977). For example, snowshoe hares experienced a deterioration in body condition as a result of shifting from high predation risk zones to low-quality food areas with lower risk and were then observed to have limited reproductive success (Boonstra *et al.* 1998). Therefore, the possibility of using predator scents to increase the perception of predator presence in areas populated by rabbits, may play a major role in the management of their populations.

This study investigates the physiological response of wild rabbits to the scent of mammalian, reptilian and marsupial predators. The majority of the physiological experiments previously carried out on prey reactions to predator odours used only one particular predator scent, and synthetic or non-odourant controls (Fendt 2006). In the present study the effects of exposure to different predator scents were instead compared with the responses to a biological non-predator odour and a non-scented control. By examining the physiological responses of rabbits to these odours I aimed to determine if they can respond to olfactory cues of predation and to better understand their reactions to potentially dangerous stimuli.

Methods

Study animals

The six rabbits used in this study were provided by the Western Australian Department of Agriculture and Food and were captured using wire mesh cage traps, baited with diced carrots following the procedure used by Twigg *et al.* (1996). Each rabbit was identified by a unique ear-notch. The rabbits were then maintained in the animal house at Curtin University of Technology in indoor enclosures in couples of the same sex. They were maintained on a 12/12 light/dark photoperiod at 21°C. Rabbits were provided with food (rabbit pellets and fresh vegetables), water *ad libitum*, branches to gnaw and carton boxes to use as refuges. The rabbits had been living in the laboratory for a couple of weeks prior to the beginning of the tests to ensure they were acclimatised to the new environment.

Experimental procedures

Measurements were made during late February to early May 2008 at day time, during the rabbits' inactive phase. Rabbits were removed from their enclosures in the morning, weighed to ±1g and then placed into a 10 L metabolic chamber that consisted of a Perspex box set in a temperature-controlled room. Air passed through the chamber at 2.5 L min^{-1}. After the rabbits had attained a quiet resting state, 2 mL of liquid or 2 cm x 6 cm of solid scent source were introduced in the inlet airline to the chamber for 5 min. The order of odour introduction was selected randomly. Only one scent was used at a time and at least one hour passed between presentation of successive odours to ensure that the previous scent had flushed out of the chamber; washout was calculated to be 18.4 min after Lasiewski *et al.* (1966). Metabolic rate (MR) and ventilation were monitored during this time to verify that rabbits had re-attained a resting state indicating a stable resting metabolic rate (RMR) before introduction of a new scent. At the end of each experiment, the rabbit was removed from the chamber and its body temperature measured with an Omron MC-510 ear thermometer. The animal was then weighed to ±1g, before being returned to its enclosure. Body mass of a rabbit on a specific day was calculated to be the mean of masses obtained before and after the experiment.

Metabolic measurements

Open-flow respirometry was used to measure the rates of oxygen consumption (VO_2, mL O_2 g^{-1} h^{-1}) and carbon dioxide production (VCO_2, mL CO_2 g^{-1} h^{-1}) in the zone of thermolneutrality at a temperature of 30°C (Lee 1939). Flow rate was controlled by an Aalborg GFC 171 mass flowmeter at 2.5 L min^{-1}. A sub-sample of excurrent air passed through a column of drierite to remove water vapour, then carbon dioxide (CO_2) was measured by a CA-2A Sable System analyser and finally oxygen (O_2) was measured by a Servomex 572 analyser. Throughout the experimental period analog voltage outputs were recorded every 20 seconds for CO_2 and O_2, converted to a digital signal with a Pico Technology ADC 11 data logger and saved on a computer using a custom-written Visual Basic (V6) data aquisition program (Withers, P.). Baselines of background O_2 and CO_2 levels were established for at least 20 min before and after each experiment and MR was averaged for 20 min both before and after the introduction of the odours. Calculations were completed using a custom-written Visual Basic (V6; Withers, P.) program for VO_2 and VCO_2 after Withers (2001). The O_2 analyser was calibrated using compressed nitrogen (0% O_2) and room air (20.95% O2) and the CO_2 analyser with compressed nitrogen (0% CO_2) and a 0.53% CO_2 calibration gas (BOCS, Perth, Western Australia).

Ventilatory measurements

Rabbits' respiratory frequency (f_R, breaths min^{-1}) and tidal volume (body temperature and pressure saturated, BTPS, V_T, mL) were calculated at 1 min intervals, using the metabolic chamber as a whole body plethysmograph (Malan 1973; Withers 1977; Dawson *et al.* 2000; Larcombe 2002; Cooper & Withers 2004), calibrated after Szewczak and Powell (2003). Minute volume was calculated as mean f_R * mean V_T. Warm and humid air inspired by the rabbits caused pressure changes in the chamber; these were detected by a custom-made pressure transducer with a Motorola MPX2010 sensor, whose analog voltage outputs were converted to a digital signal using a Pico Technology ADC 11 data logger, and were recorded on a personal computer every 2 msec for approximately 20 sec using PicoScope. Respiratory variables were measured before and after introduction of an odour at 1 min intervals for 5 min. Calculations were made using a custom-written Visual Basic (V6) program (Withers, P.) after Malan (1973) and Cooper & Withers (2004).

Scent sources

The experiment included fox and feral cat as introduced predators, northern quoll (*Dasyurus hallucatus*) and black-headed python (*Aspidites melanocephalus*) as Australian native predators. I used predator waste (feral cat urine and quoll scats), skin (python) and fur (fox) as cues to suggest the presence of different predator types. In addition, horse (*Equus caballus*) urine was used as non-predator control, while distilled water was used as an unscented control. Urine samples of feral cat was obtained from Department of Environment and Conservation (DEC) and collected as a by-product of their cat research program. Quoll scats were obtained from a captive individual maintained at University of Western Australia. Horse urine and python skin was donated by private owners. Fox skin was obtained from fresh road kill victims, found by driving on country roads in the early mornings. In order to avoid differences in the rabbit's responses due to the various sources of predator scent used, it would be ideal to use the same source (fur, skin, urine or faeces) for all the predators considered in the experiment. Unfortunately this was not possible for this study. However, urine, faeces and scent glands of canids and felids contain a number of common sulphur compounds (Epple *et al.* 1995) and in most of the studies previously conducted, prey avoided predator odours regardless of the source (Stoddart 1976, 1982; Dickman & Doncaster 1984; Gorman 1984; Sullivan *et al.* 1985b; Dickman 1992; Blanchard *et al.* 2003b). Therefore, scents in this experiment were obtained from different sources but only one source was used to represent each predator.

Data Analysis

All statistical analyses were performed in StatistiXL for Microsoft Excel Version 1.7 (Nedlands, Western Australia). All values are presented as mean ± standard error (SE; N=6), unless stated otherwise, where N is the number of animals and n is the number of measurements.

Before and after exposure differences

Each scent used in the experiment was tested for its effect on metabolism and respiratory values. Two-tailed paired *t*-tests were used to determine if MR, f_R and V_T of rabbits after exposure to each scent were significantly different than before the introduction of each odour. Any increase in MR and in f_R or any decrease in V_T was considered as a response to the scent introduced in the chamber. Since the differences within the before and after values of each scent were tested individually and results were not combined to find a general difference in the response to the odours, the use of P value correction for multiple comparisons tests was considered inappropriate in this case (Aickin & Gensler 1996; Perneger 1998; Bender & Lange 2001).

Odour differences and time effect

One-way analysis of variance (ANOVA) with Student-Newman-Keul (SNK) *post hoc* tests were used to compare the effects of different scents on the rabbits' MR. The effect of odours and time on the ventilatory variables was detected with two-way ANOVA with SNK *post hoc* tests values for before exposure and after exposure. The ratio of after/before was also analysed with two-way ANOVA with SNK *post hoc* tests, as analyses based only on absolute differences are greatly affected by individual variability (Martin & Bateson 2007). Repeated measure ANOVA was used on before, after and ratio values for MR, f_R and V_T to control for between subjects effects.

Results

The mean body mass of the rabbits before and after the experiments was 1.113 ± 0.028 kg (N=6; n=50). When asleep, rabbits maintained a stable RMR and showed a very regular breathing pattern, with f_R and V_T indicating slow and deep breaths (Fig.3.1A). Every time they were exposed to a scent, rabbits would wake from their resting state showing an increase in f_R and a decrease in V_T (Fig.3.1B).

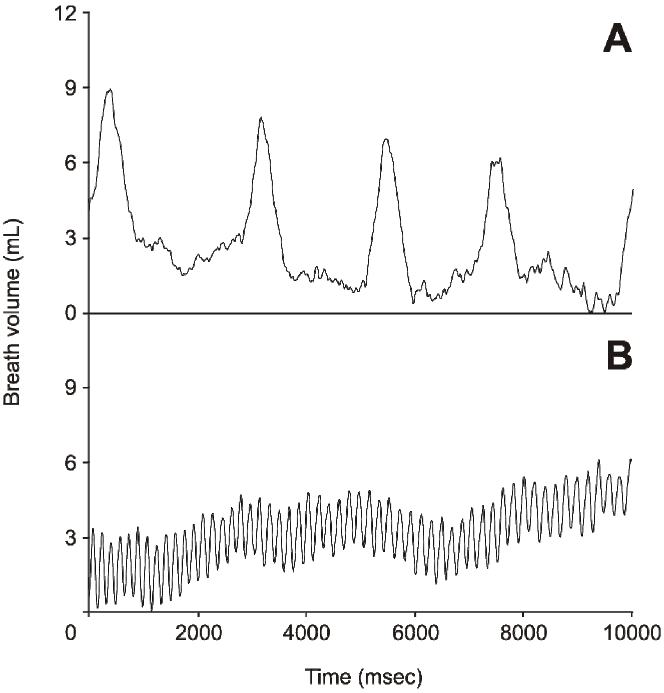

Figure 3.1: Example of breathing traces for rabbits. A) Before exposure to the odours and B) after introduction of the scents.

Metabolic Response

Before and after exposure differences

Rabbits' RMR was calculated to be 523.5 mL O_2 h^{-1}. Mean VO_2 before the introduction of the scents was 0.47 ± 0.017 mL O_2 g^{-1} h^{-1} and showed little variation after exposure (0.49 ± 0.020 mL O_2 g^{-1} h^{-1}). Mean VCO_2 was 0.57 ± 0.02 mL CO_2 g^{-1} h^{-1} before introduction and 0.59 ± 0.02 mL CO_2 g^{-1} h^{-1} after. *T*-tests performed on each scent showed VO_2 values not to be significantly different after exposure to any treatment ($P \geq 0.184$; Fig.3.2A). Similar results were found for VCO_2, with values before the introduction of all the odours not statistically different from the ones after exposure ($P \geq 0.181$; Fig.3.2B).

Odour differences

There was no difference in MR in response to predator or control odours (Tab.3.1). ANOVA indicated that values of both VO_2 ($F_{5,30}=0.465$; P=0.799) and VCO_2 ($F_{5,30}=0.647$; P=0.666) did not differ between scents.

Individual differences

There was a very high individual variability in before and after values for both VO_2 (before $F_{5,30}=11.37$; P<0.001 and after $F_{5,30}=7.95$; P<0.001) and VCO_2 (before $F_{5,30}=8.108$; P<0.001 and after $F_{5,30}=6.893$; P<0.001). However, these differences were not detected in the ratio after/before for either VO_2 ($F_{5,30}=2.323$; P=0.068) or VCO_2 ($F_{5,30}=1.815$; P=0.140).

Breathing Response

Before and after exposure differences

Mean resting f_R was 60.9 ± 7.14 breath min^{-1} and mean f_R after the first minute of exposure was 181.03 ± 15.22 breath min^{-1} over all the measurements (N=6; n=50). Minute volume was calculated to be 319.02 mL min^{-1} over all the experiments. *T*-tests showed that f_R in the first minute after introduction of each odour was significantly higher compared to prior to exposure ($P \leq 0.036$) except for distilled water ($T_5=1.642$; P=0.161; Fig.3.2C).

Mean V_T was 5.8 ± 0.44 mL before introduction of the scents, while mean V_T after the first minute of exposure was 3.04 ± 0.20 mL. Similar to that observed for f_R, *T*-tests analyses performed on each scent confirmed that V_T was significantly lower after the first minute of exposure to each odour ($P \leq 0.047$), compared to before, except for water ($T_5=1.525$; $P=0.188$) and fox ($T_5=2.026$; $P=0.099$; Fig.3.2D).

Odour differences

There was no significant difference in f_R recorded before exposure to any of the scents (ANOVA $F_{29,150}=1.773$; $P=0.122$). There was an over all difference in f_R after exposure (ANOVA $F_{29,150}=3.27$; $P<0.001$), with odour having a significant effect (ANOVA $F_{5,150}=17.392$; $P<0.001$). *Post hoc* tests revealed that feral cat and quoll odours elicited a higher f_R compared to all the other treatments (SNK $P \leq 0.01$). Statistical analysis of the ratio of after/before values (Tab.3.1) showed similar results, with a significant effect ($F_{5,150}=3.593$; $P=0.004$) of feral cat and quoll odours on f_R (SNK $P \leq 0.023$).

ANOVA indicated that V_T before exposure to horse odour was significantly different from those before introduction of the other scents ($F_{5,150}=6.032$; $P<0.001$). However, this difference was owing to the large individual variability in V_T levels amongst rabbits during different experiments and was not reflected in the V_T after exposure. Therefore, the difference detected in the before values was not considered to have affected V_T after introduction of horse urine and was ignored. In general, there was a significant difference in V_T after exposure to the odours ($F_{29,150}=1.618$; $P=0.034$). Scent had a significant effect on V_T ($F_{5,150}=7.064$; $P<0.001$) and *post hoc* tests revealed that feral cat and quoll scents elicited significantly lower V_T values compared to every other treatment (SNK$\leq$0.01), including horse. Analysis of after/before ratios (Tab.3.1) confirmed a similar odour effect on V_T ($F_{5,150}=5.6$; $P<0.001$).

Individual differences

Even under uniform experimental conditions, there was a significant individual variability ($F_{5,150}=3.42$; $P=0.014$) in the f_R of the rabbits before introduction of the scents. This was reflected also in the ratio after/before ($F_{5,30}=8.57$; $P<0.001$), but not in the after values ($F_{5,30}=0.996$; $P=0.437$). The same pattern was observed for V_T, with significant variability in before ($F_{5,30}=3.808$; $P=0.009$), ratio after/before ($F_{5,30}=4.620$; $P=0.003$) but not in after values ($F_{5,30}=0.987$; $P=0.442$).

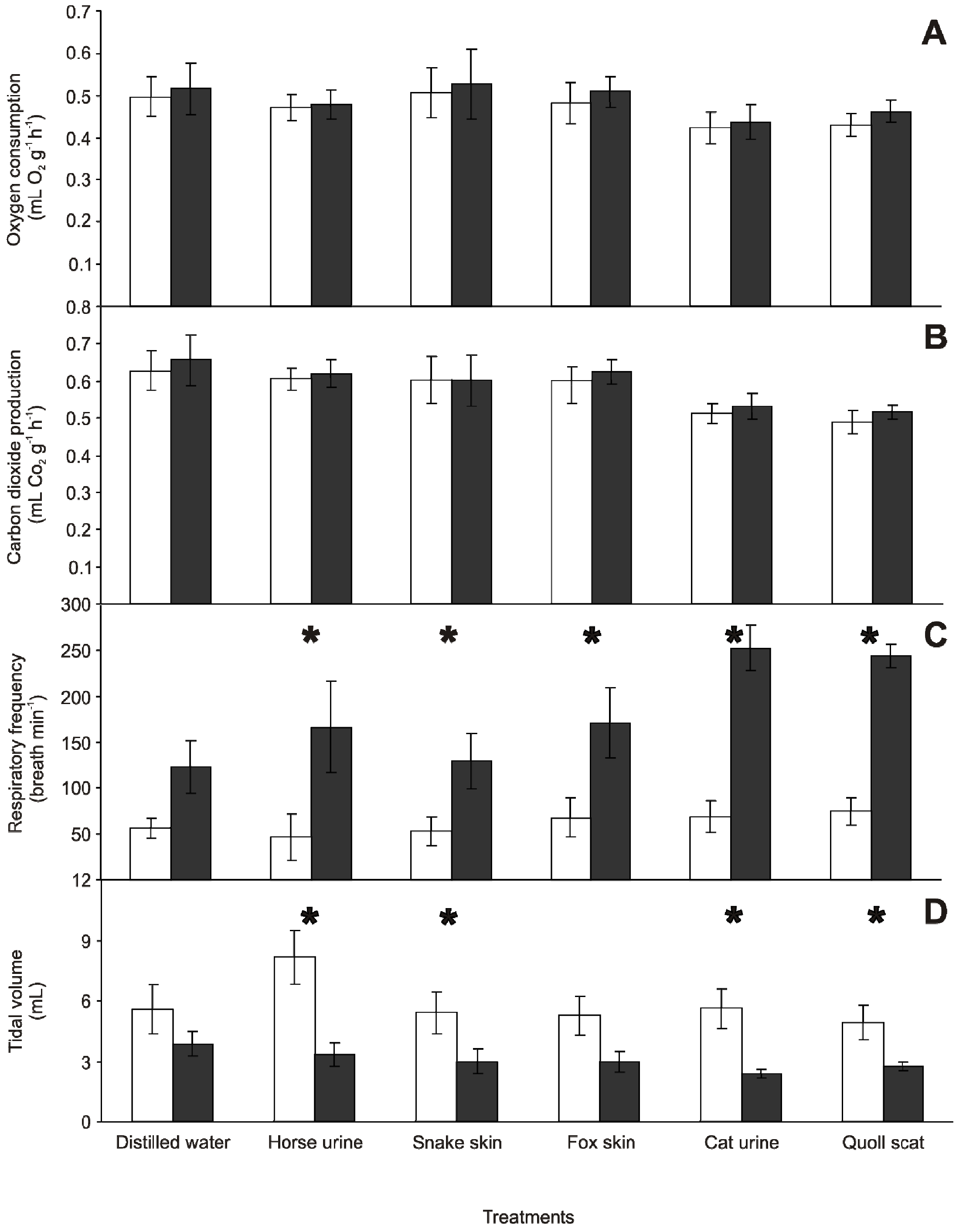

Figure 3.2: The effect of various odours on A) oxygen consumption, B) carbon dioxide production, C) respiratory frequency and D) tidal volume for rabbits during the first minute of exposure. White bars represent values before and black bars represent values after exposure. Asterisk indicates significant difference between before and after values at $P<0.05$. Values are mean ± SE (N=6).

Table 3.1: After/before mean ratio ± SE (N=6) of oxygen consumption, carbon dioxide production, respiratory frequency and tidal volume for rabbits in the first minute of exposure to different odours. Asterisks indicate significant difference from other scents at P<0.01.

	Oxygen consumption	Carbon dioxide production	Respiratory frequency	Tidal volume
distilled water	1.03 ± 0.030	1.04 ± 0.032	3.08 ± 1.648	0.80 ± 0.140
horse urine	1.01 ± 0.008	1.02 ± 0.015	4.11 ± 0.928	0.47 ± 0.113
snake skin	1.02 ± 0.048	0.99 ± 0.024	3.08 ± 0.907	0.59 ± 0.103
fox skin	1.07± 0.044	1.03 ± 0.022	3.36 ± 1.122	0.65 ± 0.129
cat urine	1.04 ± 0.040	1.04 ± 0.039	4.39 ± 0.721**	0.47 ± 0.065**
quoll scat	1.09 ± 0.063	1.07 ± 0.044	3.75 ± 0.486**	0.64 ± 0.129**

Time effect

There was no significant effect of time on f_R after introduction of any scents ($F_{4,150}$=1.401; P=0.236) nor on the after/before ratio ($F_{4,150}$=1.976; P=0.101). The increases observed in the rabbits' f_R after the introduction of the odours did not diminish over time, being maintained for the whole duration of the exposure to the various scents (Fig.3.3A). Time did also not significantly affect V_T after exposure to the odours ($F_{4,150}$=1.094; P=0.362) or the after/before ratio ($F_{4,150}$=0.528; P=0.715). The decrease of V_T in response to the introduction of the scents did not return to initial values during the 5 min of exposure to all the treatments (Fig.3.3B).

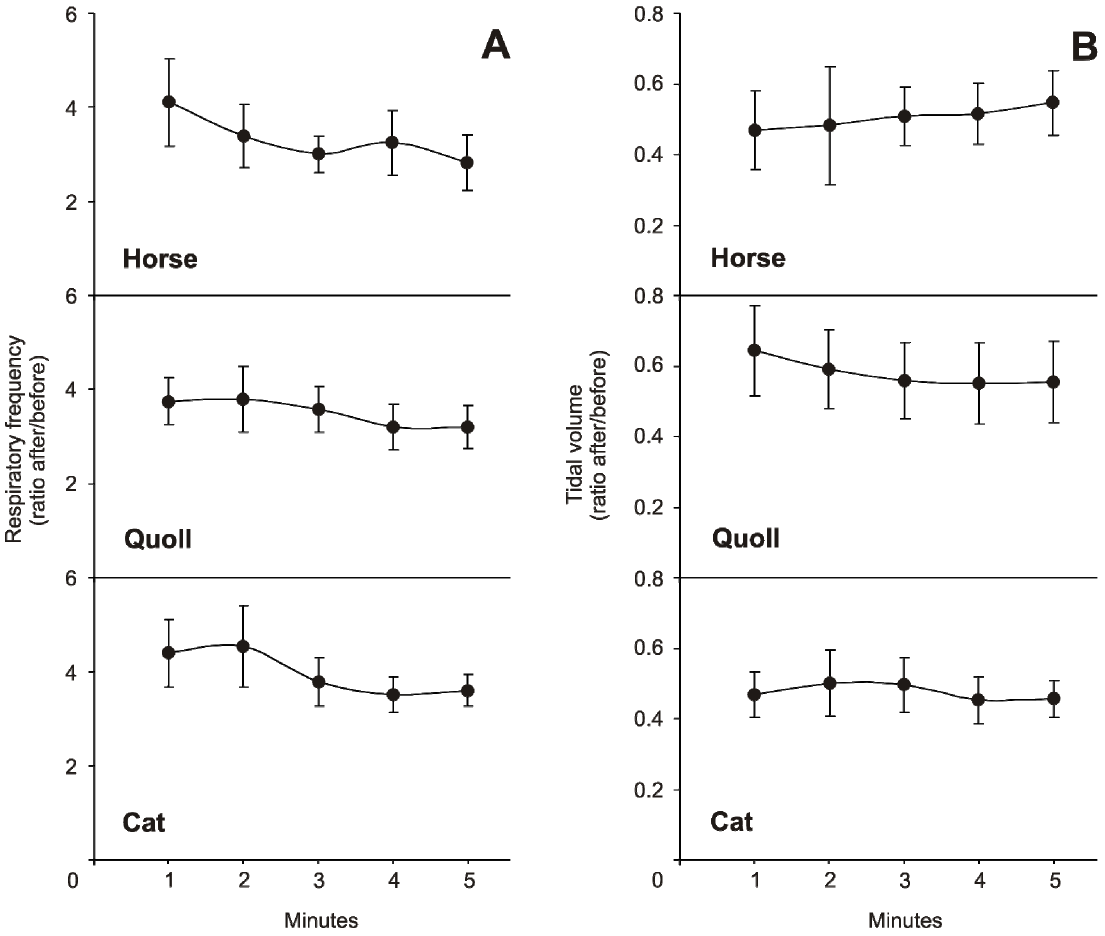

Figure 3.3: A) respiratory frequency and B) tidal volume responses to different odours for rabbits did not change over the five minutes exposure time. Examples of response to a control (horse), to a native (quoll) and to an introduced predator (cat). Values are mean ratio ± SE (N=6).

Discussion

During the experiments, rabbits' RMR was calculated to be 523.5 mL O_2 h^{-1}. For a placental mammal of comparable size BMR was calculated to be 559.49 mL O_2 h^{-1} (McNab 1988) and 614.1 mL O_2 h^{-1} (Hayssen & Lacy 1985). While resting, the rabbits' mean f_R was 60.9 ± 7.14 breath min^{-1} and minute volume was 319.02 mL min^{-1} over all the experiments. Previous studies of small mammals showed the resting respiratory rate to be between 60 and 70 breath min^{-1} for a rabbit-sized mammal (Kleinman & Radford 1964) and minute volume to be around 419.85 mL min^{-1} (Stahl 1966) under basal conditions. Values in this study were slightly lower than but similar to the predicted ones. Small differences are presumably due to the fact that in the majority of previous studies, data were collected on restrained animals, while rabbits used in this experiments were allowed to rest comfortably for a long time in the metabolic chamber before starting the measurements. As restraint and measurement duration are known to affect ventilation (Chappell 1992; Dawson *et al.* 2000; Cooper & Withers 2009), this probably explains the slightly lower values in this study and indicates that rabbits were calm and resting before introduction of the odours, allowing scope for an appropriate response in case of alertness after exposure to the odours.

Metabolic Response

No metabolic response was observed in rabbits after exposure to any of the odours. VO_2 and VCO_2 did not show any significant variation despite the strong responses recorded in the ventilatory variables. As MR was averaged for 20 min and the scents introduced in the chamber for only 5 min, it was unlikely to observe a response in the metabolic variables in this time frame. On the other hand, shorter measurements of MR could result in over or underestimation, since it is necessary to allow enough time for the air to mix and reach equilibrium with the rest of the chamber before measuring VO_2 and VCO_2 levels.

MR did not differ in response to either exposure to control scents or to potential predator odours. It has been previously argued that measurements of the MR may not be ideal when used to study the immediate physiological consequences of predation risk as MR is less sensitive to stressors compared to other physiological parameters, such as ventilatory or heart rate (Allen *et al.* 1986; Chabot *et al.* 1996; Schapker *et* *al.* 2002). However, some studies have shown MR to be successful in detecting a response indicating perception of predation risk. For example, bees and wasps exposed to alarm pheromones increased their VCO_2 (Moritz & Burgin 1987) and hedgehogs presented a significant increase in VO_2 without any observable increase in activity after exposure to badger (*Meles meles*) scent (Ward *et al.* 1996). In this last study measurement of MR response was shorter (10 min averages) than the one in my experiments (20 min averages) and this may explain why sudden changes could be more easily detected. An increase in MR could be expected under a stressful situation, such as perceiving a predator odour, which may indicate the presence of a potential danger, however long-averaged measurement of response may conceal sudden and short-term changes.

Ventilatory Response

A ventilatory response was observed for all odours, with significant increases in f_R and decreases in V_T, except for distilled water, which was used as an unscented control to test for disturbance. In addition, elevated f_R and low V_T were maintained for the whole duration of the exposure to all the scents, suggesting that the reactions to the odours were alarm responses, characterized by long-lasting fast breathing patterns. Rapid responses in respiratory rate have been associated with fear in previous studies on crustaceans (McMahon 1995; Schapker *et al.* 2002). In general, rabbits seemed unable to discriminate among the predator and the herbivorous control odours used in the experiment, since all scented treatments elicited physiological responses. However, alteration of ventilatory rate has been reported to be an index of response to sudden environmental changes, representing animals' readiness for a consequent behavioural reaction (Wilkens 1976; McMahon & Wilkens 1983; Burmistrov & Shuranova 1996). For example, crayfish (*Procambarus clarkii*) responded to environmental disturbances with an increase in heart and ventilatory rate before physical movement occurred (Schapker *et al.* 2002). Therefore, significant responsiveness to stimuli may indicate high sensitiveness to alterations in the environment. This may conform to the resilient nature of rabbits and may explain their flexibility to adapt promptly to changes, as also suggested for rats (Burwash *et al.* 1998).

The intensity of the rabbits' response was not equally strong for all the odours considered. Changes in the ventilatory variables were greater with cat and quoll odours than with all the other scented treatments. Cats share an evolutionary history with European rabbits (Malo *et al.* 2004) and are considered major predators for rabbits living in Australia (Molsher *et al.* 1999). Therefore, odour recognition was expected to some extent. On the other hand, rabbits did not evolve with quolls but they showed an equally strong reaction to their scent. Rabbits have been previously observed to show responses to unknown predator odours, such as lion faeces (Boag & Mlotkiewicz 1994). As highly predated animals, facing a variety of different threats from various types of predators, rabbits may be naturally wary and simply cautious in the presence of strong olfactory signals, since also unfamiliar scents could represent the risk of encountering unknown predators. However, cat and quoll odours were easily perceived by the experimenter and smelt unpleasant. Therefore, the pungency of these scents may have contributed to the results. It has been questioned before whether the responses to predator waste by prey species do characterise a specific anti-predator strategy, or can rather be interpreted as a general effect induced by a strong olfactory signal (Kemble & Bolwahnn 1997; Fuelling & Halle 2004). Responses may have been greater with cat urine and quoll scat because they were perceived with stronger intensity and not because they represented a cue of predation risk.

Python and fox scent elicited a smaller response in the rabbits, consistent with the one observed to the unfamiliar non-predator scented control (horse urine). This may be due to a low effectiveness of skin derived odours. However, in previous studies, olfactory information released by skin chemicals facilitated detection of snake by California ground squirrels, (*Spermophilus beecheyi*; Hennessy & Owings 1978), wood rats (*Neotoma albigula*; Richardson 1942), kangaroo rats (*Dipodomys merriami*; Webster 1973), two species of gerbils (Kotler *et al.* 1993) and other ophidian prey (Weldon & Burghardt 1979; Weldon & Schell 1984; Burger 1990). In other studies, hair scent successfully served as a danger cue for white-tailed deer (*Odocoileus virginianus*; Seamans *et al.* 2002) and cat fur odour elicited a defensive behaviour in rats (Blanchard *et al.* 2003a). Furthermore, coat odours have been considered to be more effective than those of faeces as predator related stimuli, since they may indicate a higher risk of encountering an actual predator compared to other scents (Blanchard *et al.* 2003a). Therefore, the presence of fur/skin odour should provide a warning sign that a predator is nearby, while scents derived from organic waste should only indicate that a predator has been previously around but may not be anymore. However, fur/skin derived stimuli are difficult to test efficiently, as components responsible of eliciting responses are hard to control (Apfelbach *et al.* 2005). Fox fur was removed from dead animals, while python skin was obtained from molting individuals. Therefore, it may be possible that the intensity level of the fur and skin sources used in the experiment did not resemble those normally encountered by prey in the presence of a real predator.

There was a significant individual variability in MR, f_R and V_T values amongst rabbits throughout the experiment. High variability may be due to different maturation and experience of the various rabbits. Rabbits used in this study were wild caught, of unknown age and history. It is known that animals usually limit responsiveness and differently select stimuli to respond to when at different development stages (Inglis 1979; Fishman 1999). For example, Boyce (1983) found that juvenile rabbits were more alert than adults and Vitale (1989) observed diversities in anti-predator behaviour in rabbits of different ages. Based on body masses, it is likely that rabbits used in this study were of different group ages. Other studies have found high individual differences in response to stressors (Benus *et al.* 1987; Sapolsky 1990; Chabot *et al.* 1996; Cockrem & Silverin 2002; Campbell *et al.* 2003; Monclús *et al.* 2006). The high variability may also be an artefact of sample size. In fact, comparison of odours effectiveness was difficult because of the large number of scents used relative to the small number of rabbits. In order to determine clear differences in response to the various scents, it would be ideal to increase the number of subjects in future studies to improve the power of statistical analyses.

Conclusions

Rabbits were in general responsive to all the scented treatments used in the study, showing a long-lasting rapid breathing pattern after introduction of all the odours, indicating an increase in alertness. Rabbits seem to become wary at any sudden change in their environment. However, a stronger response was observed with cat and quoll scents. This does not necessarily suggest specific predator recognition as differences could be due to different intensity of the olfactory stimuli. Nevertheless, the effects of noxious or pungent odours on rabbits clearly demonstrate that their perception of immediate changes in their environment is mediated by anxiety.

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Chapter 4: Measurement of the physiological response of tammar wallabies (*Macropus eugenii*) to predator scents

Abstract

This study determines the physiological responses of an Australian native terrestrial herbivore to olfactory cues suggesting the presence of native and introduced predators. Changes in the metabolic rate and in the ventilatory variables of tammar wallabies (*Macropus eugenii*) were recorded after presentation of biological non-predator odours (water and horse, *Equus caballus* urine) and the scent of possible predator scents (cat, *Felis catus*; fox, *Vulpes vulpes*; quoll *Dasyurus hallucatus* and snake, *Aspidites melanocephalus*). While there was no variation in the metabolic rate, the ventilatory responses showed a pattern of response similar for all the odours used in the experiment. A difference was found in the values of respiratory frequency and tidal volume recorded before (mean respiratory frequency = 44.96 ± 5.12 breath min^{-1} and mean tidal volume = 27.44 ± 1.67 mL) and after (mean respiratory frequency = 136.6 ± 11.24 breath min^{-1} and mean tidal volume = 20.65 ± 0.79 mL) the first minute of exposure to the odours. However, these variations quickly diminished over time, with the first minute after introduction of the scents consistently different from the subsequent four. The physiological changes observed in tammar wallabies suggest disturbance as eliciting a first reaction to the scents. However, fox and cat odours provoked a stronger and more prolonged response for respiratory frequency (ratio after/before exposure to fox = 3.58 ± 0.918 and ratio after/before exposure to feral cat = 2.44 ± 0.272) and tidal volume (ratio after/before exposure to fox = 0.84 ± 0.110 and ratio after/before exposure to feral cat = 0.98 ± 0.155) compared to the other scents used in the experiment. Therefore, after investigation, tammar wallabies seemed to possess a mechanism for the recognition of predator odours as responses were restricted only to certain predator scents.

Introduction

Recognition of predators by mammalian prey could be based on olfactory cues (Lima & Dill 1990; Kats & Dill 1998). As a consequence, odours derived from predator glands, hair, faeces, and urine have been used as scent sources in many laboratory and field studies (reviewed in Apfelbach *et al.* 2005), since the chemical components found in these often makes the odour an accurate indicator of risk of predation to the potential prey (Jedrzejewski *et al.* 1993). Indeed, predator scents seem to offer an important approach to investigate fear and anxiety in prey animals (Blanchard *et al.* 2003). However, there have been contrasting results. Tammar wallabies (*Macropus eugenii*) and red-necked pademelons (*Thylogale thetis*) did not modify their feeding behaviour in response to predator scents (Blumstein *et al.* 2002b), but swamp wallabies (*Wallabia bicolour*) reduced browsing when confronted with predator odours (Montague *et al.* 1990).

Predators can represent a strong alerting stimulus that may elicit a physiological stress response in prey animals (Dell'omo *et al.* 1994). For instance, anxiety has been observed in rats (*Rattus norvegicus*) after exposure to a cloth impregnated with cat (*Felis catus*) odour (Cohen *et al.* 2000), while cat faeces provoked freezing, agitation and escape attempts (Sullivan & Gratton 1998). Mongolian gerbils (*Meriones unguiculatus*) have shown physiological arousal after sensing the odour of the blood of conspecifics stressed by the presence of a cat (Cocke & Thiessen 1986). In addition, mice (*Mus musculus*) presented with the main constituent of weasel (*Mustela nivalis*) anal secretion have shown increased corticosterone levels (Kavaliers *et al.* 2001). Changes in respiratory rate have also been reported in crayfish (*Procambarus clarkii*) after exposure to stressful sensory stimuli, without an increase in ambulatory activity (Schapker *et al.* 2002). Therefore, alteration of physiological parameters can be expected in response to an olfactory cue of predation.

Some prey species show generalised avoidance of predator odours without having experienced contact with them in evolutionary time, nevertheless some others need long-term exposure to predators before showing responses (see review in Apfelbach *et al.* 2005). Indeed some anti-predator behaviours are species specific and animals may not show avoidance of predators they have been sympatric with only for a short time (Banks *et al.* 2003). Unfortunately, in Australia many prey species are facing entirely new kinds of predators introduced by humans (McLean *et al.* 1996). In fact, the great number of extinctions experienced during the last century can be largely attributed to the introduction of exotic predators such as the fox (*Vulpes vulpes*) and the feral cat (McLean *et al.* 2000).

The tammar wallaby is a medium-sized Australian macropodid marsupial (Smith & Hinds 1995), which feeds in open grassy areas in aggregation with other individuals (Blumstein *et al.* 1999; Blumstein *et al.* 2002a). Although nowadays its range is restricted on the mainland, it is still perceived as an agricultural pest in some areas (Wright & Stott 1999). While natural predators of the tammar wallaby are few, the feral cat is believed to have made a significant contribution to the disappearance of most populations (Smith & Hinds 1995). In addition, fox removal has shown to be effective for the recovery of some tammar populations in Western Australia (Morris *et al.* 1998).

The tammar wallaby has been used intensively as a model for a broad range of studies on physiology and breeding of macropod marsupials (Hinds 2008). Recently, numerous studies on its behaviour helped to better understand its anti-predator strategies and the use of predator cues (Griffin *et al.* 2001; Blumstein *et al.* 2002b; Blumstein *et al.* 2004). In fact, acoustic, visual and olfactory predator recognition in tammar wallabies has been tested before (Blumstein 2002) and results have shown that tammars can respond to the sight of predators by reducing feeding and increasing vigilance but not to recognise predator acoustic stimuli (Blumstein *et al.* 2000). Furthermore, they seem unable to detect predators by their scent (Blumstein *et al.* 2002b). The potential inability of tammars to respond to predation risk from exotic predators may explain their particular vulnerability to feral animals and may have contributed to their rapid decline. It is therefore important to better investigate the role of olfaction in tammars avoidance of predators and to determine their physiological response to predator scent exposure.

Methods

Study animals

Eight male tammar wallabies were wild-caught at Tutanning Nature Reserve (32° 32' S; 117° 19' E). Subjects were distinctly ear-tagged and housed in pairs in outdoor enclosures at University of Western Australia animal yards, Perth, WA. They were fed with kangaroo pellets, fresh vegetables and could also forage on natural vegetation. Water was available *ad libitum*. Tammars experienced natural weather and photoperiod for Perth during April-August.

Experimental procedures

The experiment was conducted during the tammars' inactive phase (daytime) and after the animals had been fasted for 24 hours. A wallaby was removed from its enclosure, weighed to ±1g and placed in a 60 L Perspex metabolic chamber located inside a controlled-temperature room, set to a temperature between 28 and 31°C, within the zone of thermalneutrality (Dawson *et al.* 1969). When the animals had attained a quiet resting state, 2 mL of liquid or 2 cm x 6 cm of solid scent source were introduced in random order into the inlet airline of the chamber for 5 min. Separate tubes were used exclusively for each type of odour and only one scent was used at a time, with a minimum of 1 hr between presentation of successive odours.

During this time, metabolic rate (MR) and ventilation were monitored until values were indicating the maintenance of the basal MR (BMR) and the wallaby was observed using a videocamera to ensure it had retuned to a resting state after the scent had flushed out of the chamber. Washout time was determined to be 20.4 min after Lasiewski *et al.* (1966). At the conclusion of the experiment, the tammars were removed from the chamber and their body temperature was measured by a plastic-sheathed thermocouple, connected to an Omega HH-25TC thermocouple meter. The animals were then weighed to ±1 g and finally returned to their enclosure. Body mass of a wallaby on an experimental day was calculated to be the mean of masses before and after the experiment.

Metabolic measurements

MR was determined by standard flow-through respirometry, where oxygen consumption (VO_2, mL O_2 g^{-1} h^{-1}) and carbon dioxide production (VCO_2, mL CO_2 g^{-1} h^{-1}) were measured for excurrent air using the chambers as open-flow respirometers. Two Aalborg GFC37 mass-flow controllers were used to regulate the flow of ambient air in the chambers at 13.5 L min^{-1}. A subsample of excurrent air was dried with drierite to remove water vapour and O_2 was measured with a Servomex 570A or a Servomex 0A14 analyser, while the percentage of CO_2 with a Hartmann & Brauns Uras 10E or a Heraeus-Laybold Binos-C analyser. The gas analysers were interfaced to a PC via RS232 serial ports (Thurlby Thandar or Brymen TBM859CF multimeters for O_2 and Brymen BM202 multimeters for CO_2). A custom-written data acquisition software (Visual Basic V6; P.Withers) was used to record O_2 and CO_2 every 20 seconds throughout the experimental period. The metabolic system was calibrated using compressed nitrogen (N_2; 0% O_2) and dry ambient air (20.95% O_2) for the O_2 analysers and compressed N_2 (0% CO_2) and a certified gas mix (0.53% CO_2; BOCS, Perth, Western Australia) for the CO_2 analysers. Baselines of background oxygen, carbon dioxide and water vapour levels were established for at least 20 min before and after each measurement. Metabolic rate (MR) before and after the introduction of the scents was recorded and averaged for periods of 20 min and a custom-written Visual Basic (V6) program (Withers, P.) was used to calculate VO_2 and VCO_2 after Withers (2001).

Ventilatory measurements

The metabolic chambers served as whole-body plethysmographs (Malan 1973; Dawson *et al.* 2000; Larcombe 2002; Cooper & Withers 2004) to measure respiratory frequency (f_R, breaths min^{-1}) and tidal volume (body temperature and pressure saturated, BTPS, V_T, mL $breath^{-1}$) of the wallabies. Changes in pressure of the chamber resulting from the warming and humidifying of inspired air were measured using a Sable System PT-100 pressure transducer whose analog voltage outputs were converted to a digital signal using a Pico Technology ADC 11 data logger, and were recorded on a personal computer every 2 $msec^{-1}$ for approximately 20 sec using PicoScope. The plethysmography systems were calibrated after Szewczak and Powell (2003). Changes in respiratory variables were calculated at 1 min intervals for 5 min. Calculations were made using a custom-written Visual Basic (V6) program (Withers, P.) after Malan (1973) and Cooper & Withers (2004).

Scent sources

The presence of four potential predators was simulated during the experiment using skin (black-headed python, *Aspidites melanocephalus*), fur (fox), urine (feral cat) and scats (northern quoll, *Dasyurus hallucatus*). Horse (*Equus caballus*) urine was used as biological control, while distilled water was used as an odourless control. Feral cat urine samples were obtained from Department of Environment and Conservation (DEC), collected as a by-product of the cat research program. Quoll scats were obtained from a captive individual maintained at the University of Western Australia fed on meat. Snake skin and horse urine were donated by private owners. Fox skin was obtained from fresh road kill victims, found by driving on country roads in the early mornings. Although different sources of odour could lead to variability in the response, previous studies have shown that usually prey react to predator odours independently of the source (Muller-Schwarze 1972; Melchiors & Leslie 1985; Swihart *et al.* 1991; Epple *et al.* 1993).

Data Analysis

All statistical analyses were accomplished using StatistiXL for Microsoft Excel Version 1.7 (Nedlands, Western Australia). All values are presented as means ± standard error (SE; N=8), unless stated otherwise, where N is the number of animals and n is the number of measurements.

Before and after exposure differences

MR and ventilatory values were tested for a significant change after exposure to the various scents using two-tailed paired *t*-tests to determine if MR, f_R and V_T of the tammars after exposure to each scent were significantly different than before. Any increase in the rate of VO_2, VCO_2 and f_R or a decrease in V_T was used as a measure of physiological response to the scent introduced in the chamber. Statistical adjusting of the P value for multiple tests is considered unnecessary and inappropriate in this case (Aickin & Gensler 1996; Perneger 1998; Bender & Lange 2001) because the results of the *t*-tests were not compared to one other.

Odour differences and time effect

One-way analysis of variance (ANOVA) with Student-Newman-Keul (SNK) *post hoc* tests were used to compare the effects of the scents on the MR of the tammar wallabies, while two-way ANOVA with SNK *post hoc* tests were used to detect differences in f_R and V_T in the response to both various odours and time. This was performed on both before, after exposure values and the after/before ratio, as absolute differences and response ratios are differently affected by variation within individuals (Martin & Bateson 2007). ANOVA was then used to compare between regressions of responses to the different scents over time. Time effect on MR during the exposure to the different odours could not be analysed as MR values recorded in the experiment were represented by 20 min averages and not by single minutes.

Results

The mean body mass of the wallabies before and after the experiments was 5.08 ± 0.043 kg (N=8; n=76). While resting, MR, f_R and V_T were regular and stable (Fig.4.1A) and tammars would usually lay on their side with legs extended or crouch with rear legs and tail forward (Fig.4.2 A and B). After introduction of an odour, wallabies would stand, rotate ears and initially move to face the inlet airline (Fig.4.2C), with an increase in f_R and a decrease V_T (Fig.4.1B).

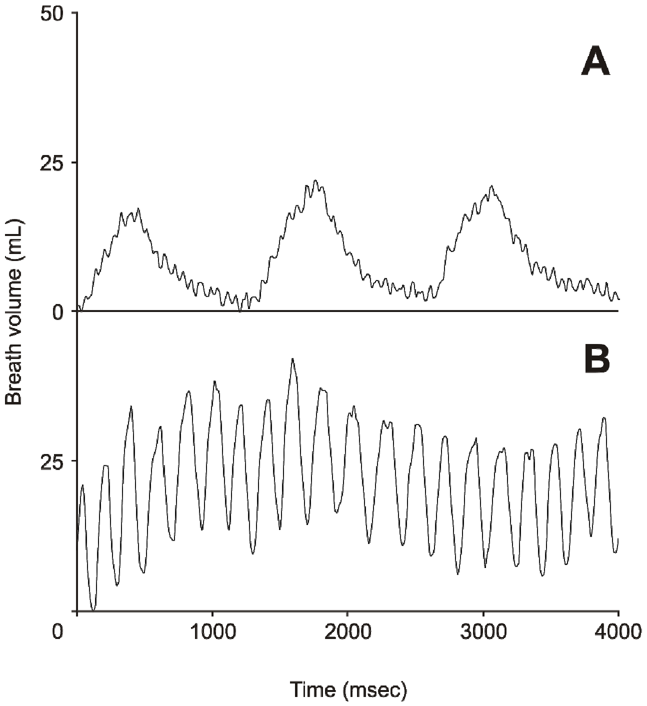

Figure 4.1: Example of breathing traces for tammar wallabies. A) Before exposure to the odours and B) during the first minute after exposure to the scents.

In more than one occasion individuals were observed to face away from the air connection and crouch in a far corner of the metabolic chamber after inspection of fox scent (Fig.4.2D).

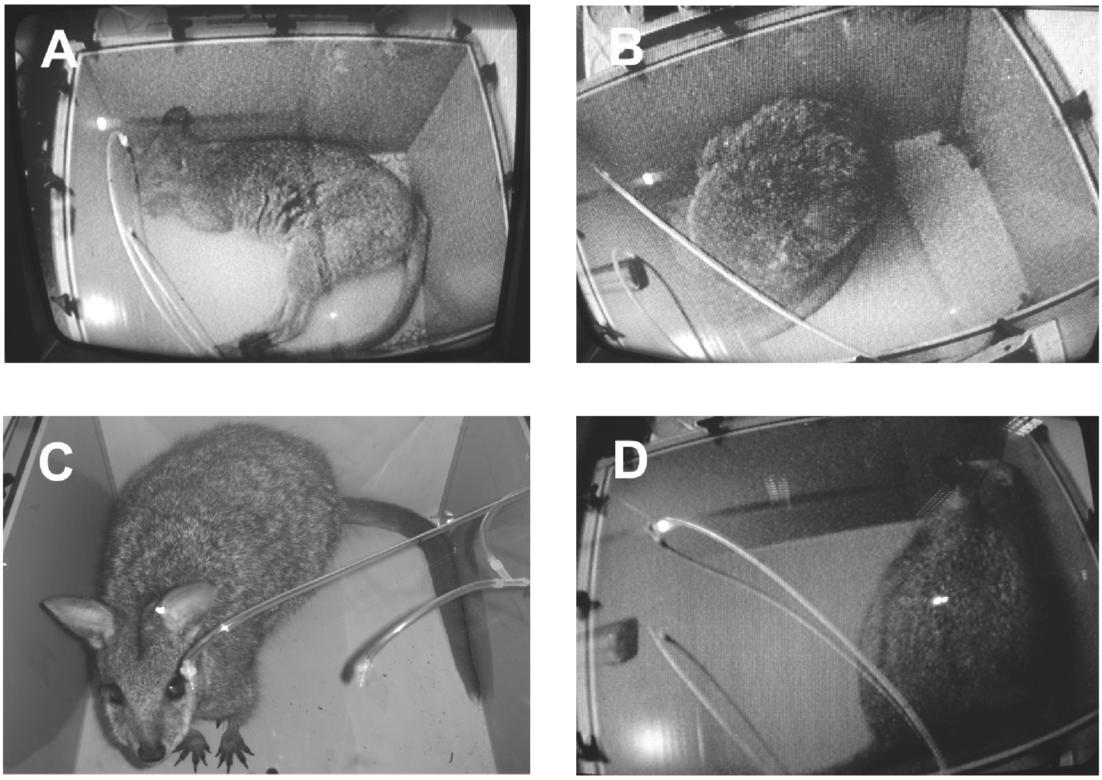

Figure 4.2: Tammar wallabies in metabolic chamber. A) laying; B) in crouched resting posture; C) investigating scents and D) after exposure to fox and cat scents (photos by Sylvie Schmidt).

Metabolic Response

Before and after exposure differences

BMR of tammar wallabies in this study was 1389.82 mL O_2 h^{-1}. Mean VO_2 for resting tammars was 0.353 ± 0.014 mL O_2 g^{-1} h^{-1} and was 0.355 ± 0.012 mL O_2 g^{-1} h^{-1} after exposure to the scents. Mean VCO_2 was 0.27 ± 0.007 mL CO_2 g^{-1} h^{-1} before exposure and 0.28 ± 0.006 mL CO_2 g^{-1} h^{-1} after. *T*-tests performed on each scent showed that values of VO_2 ($P \geq 0.188$) and VCO_2 ($P \geq 0.102$) were not significantly higher after exposure to any treatment compared to before (Fig.4.3A and B).

Odour differences

ANOVA showed no significant effect of either predator or non-threatening odours on the MR of the wallabies. Both VO_2 ($F_{5,42}=1.248$; $P=0.304$) and VCO_2 ($F_{5,42}=1.025$; $P=0.415$) did not differ significantly after introduction of any of the scents (Tab.4.1).

Breathing Response

Before and after exposure differences

Mean resting f_R was 44.9 ± 5.12 breath min^{-1} over all the experiments, while mean f_R was 136.6 ± 11.23 breath min^{-1} after the first minute of exposure to the scents. f_R was significantly higher in the first minute after introduction of all the scents ($P \leq 0.019$) compared to f_R before exposure (Fig.4.3C).

Mean V_T was 27.4 ± 1.67 mL before the introduction of the scents and was 20.6 ± 0.79 mL after exposure. V_T was significantly lower in the first minute of exposure to horse odour ($T_7=3.885$; $P=0.006$), compared to before introduction (Fig.4.3D). There was no significant before-after exposure difference in V_T for the other scents ($P \geq 0.072$; Fig.4.3D).

Odour differences

When considering all 5 min of the experiments, there was a significant effect of odour on values of f_R recorded before exposure ($F_{5,210}=4.004$; $P=0.002$). f_R before introduction of snake scent was significantly higher than the f_R recorded before exposure to the other odours (SNK $P \leq 0.048$). However, no statistical difference between scents was found in the f_R after exposure ($F_{5,210}=2.072$; $P=0.070$) or in the after/before ratio ($F_{5,210}=1.989$; $P=0.082$).

Since tammars reacted strongly to all the scents introduced in the chamber during the first minute, it was impossible to show a difference in the response to the odours. Consequently, the two-way ANOVA was repeated but values recorded in the first minute of introduction of the scents were excluded. Again values before exposure to the scents were significantly different ($F_{5,168}=3.203$; $P=0.009$), with f_R before introduction of snake odour significantly higher (SNK $P \leq 0.014$) than the other scents. This difference was also reflected in the values after introduction of the various odours ($F_{5,168}=4.213$; $P=0.001$), with snake scent again resulting in higher f_R (SNK $P \leq 0.009$). It was not possible to determine actual responses by examining after exposure f_R, since the before effect could not be eliminated. Therefore, ratio of after/before responses was examined to account for this effect. ANOVA indicated a difference in the ratio after/before exposure ($F_{23,168}=2.226$; $P=0.002$) due to the various odours ($F_{5,168}=5.416$; $P<0.001$). *Post hoc* tests revealed that fox odour elicited a significantly stronger response compared to quoll, snake and distilled water (SNK $P \leq 0.04$; Tab.4.1), while the response to feral cat odour was stronger than the one observed in response to quoll scent and distilled water (SNK $P \leq 0.029$; Tab.4.1). However, responses to fox, feral cat and horse scents were not significantly different (SNK $P > 0.05$).

The two-way ANOVA model for odour was significant ($F_{5,210}=2.411$; $P=0.038$) for V_T, with higher values before the introduction of cat odour (SNK $P=0.015$) compared to the other scents. This difference was also reflected in the after values ($F_{5,210}=5.172$; $P<0.001$; SNK $P<0.001$), so the ratio of after/before responses was analysed instead. No significant effect of odour was found in the after/before ratio ($F_{29,210}=0.709$; $P=0.864$) in the first minute of exposure. When the first minute of the experiment was ignored and only data from min 2 to min 5 were considered in the analyses, then no significant differences were found before exposure to any of the scents ($F_{23,168}=0.419$; $P=0.992$). After exposure, odour significantly affected V_T ($F_{5,168}=4.768$; $P<0.001$). *Post hoc* tests revealed that all treatments elicited a significantly different response compared to the one observed to distilled water (SNK $P \leq 0.017$). However, there was no significant effect of odour on the after/before ratio ($F_{5,168}=1.568$; $P=0.172$; Tab.4.1).

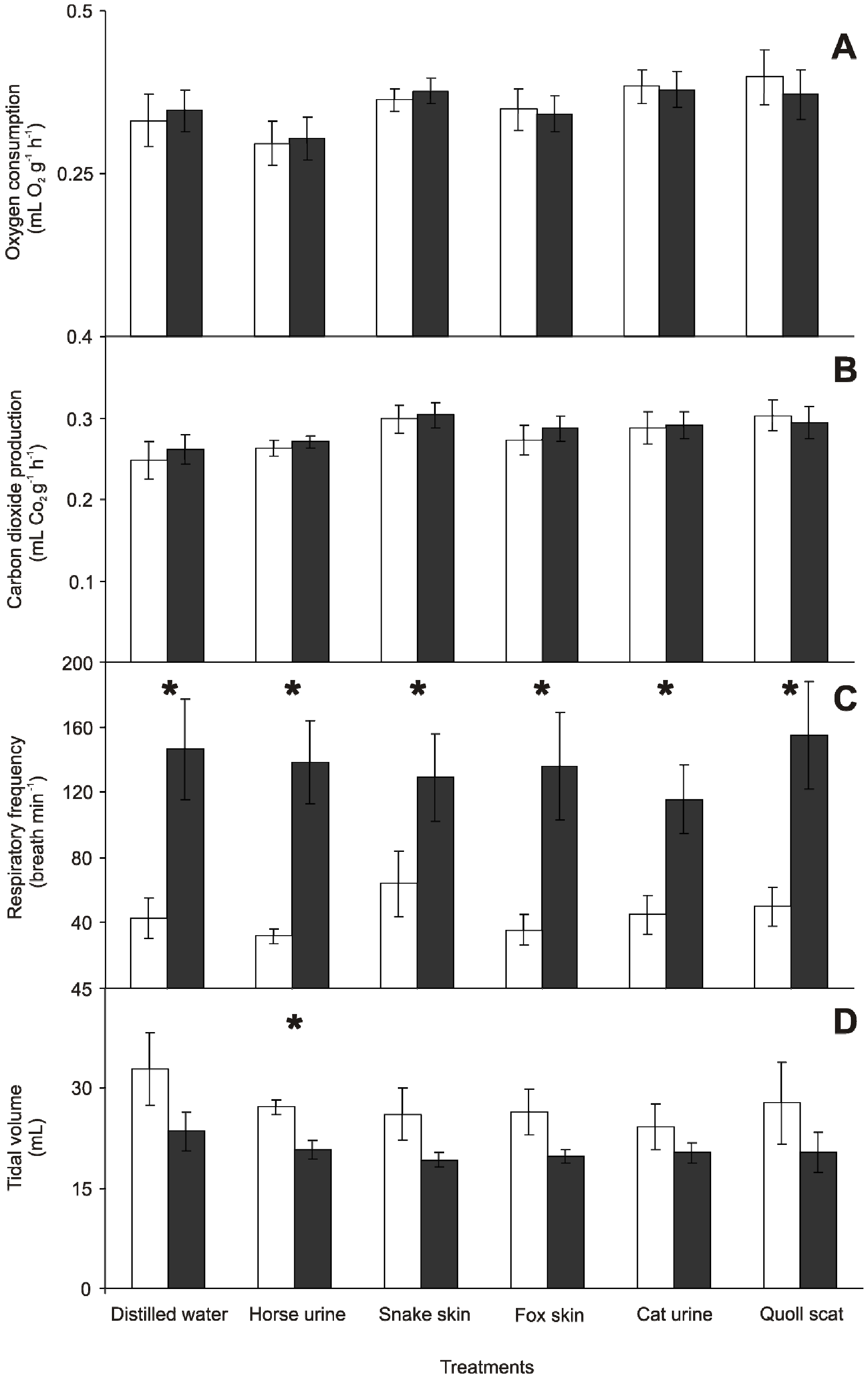

Figure 4.3: The effect of various odours on A) oxygen consumption, B) carbon dioxide production, C) respiratory frequency and D) tidal volume for wallabies in the first minute of exposure to the scents. White bars represent values before and black bars represent values after exposure. Asterisk indicates significant difference between before and after values at $P<0.05$. Values are mean ± SE (N=8).

Table 4.1: Mean ratio ± SE (N=8) of oxygen consumption, carbon dioxide production, respiratory frequency and tidal volume for tammar wallabies during the second minute of exposure to different odours. Asterisk indicates significant difference from other scents at $P<0.05$.

	Oxygen consumption	**Carbon dioxide production**	**Respiratory frequency**	**Tidal volume**
distilled water	1.08 ± 0.056	1.07 ± 0.038	1.81 ± 0.463	0.87 ± 0.092
horse urine	1.03 ± 0.024	1.03 ± 0.023	2.40 ± 0.492	0.80 ± 0.082
snake skin	1.04 ± 0.036	1.02 ± 0.025	2.27 ± 0.370	0.79 ± 0.072
fox skin	1.04 ± 0.051	1.06 ± 0.040	3.58 ± 0.918*	0.84 ± 0.110
cat urine	0.99 ± 0.034	1.02 ± 0.040	2.44 ± 0.272*	0.88 ± 0.114
quoll scat	0.94 ± 0.046	0.97 ± 0.037	1.71 ± 0.245	0.98 ± 0.155

Time effect

The two-way ANOVA model for time (5 min) indicated a significant effect on the f_R of both time after introduction of the scents ($F_{4,210}$=13.90; P<0.001) and the after/before ratio ($F_{4,210}$=17.03; P<0.001). In both cases, *post hoc* tests revealed that the first minute of the experiment was significantly different from the other four (SNK P<0.001). Linear regression confirmed that increases in f_R after exposure to all the scents significantly diminished over 5 min (P≤0.008), except in response to feral cat odour (F_{38}=3.441; P=0.071; R^2=0.083; Fig.4.4A). Comparisons between regressions showed that slopes of f_R and time in response to horse ($F_{1,77}$=4.292; P=0.042) and fox ($F_{1,77}$=4.380; P=0.040) were significantly less steeper than the one observed in response to feral cat. There was no significant effect of time for V_T ($F_{4,168}$=2.209; P=0.069; Fig.4.4B).

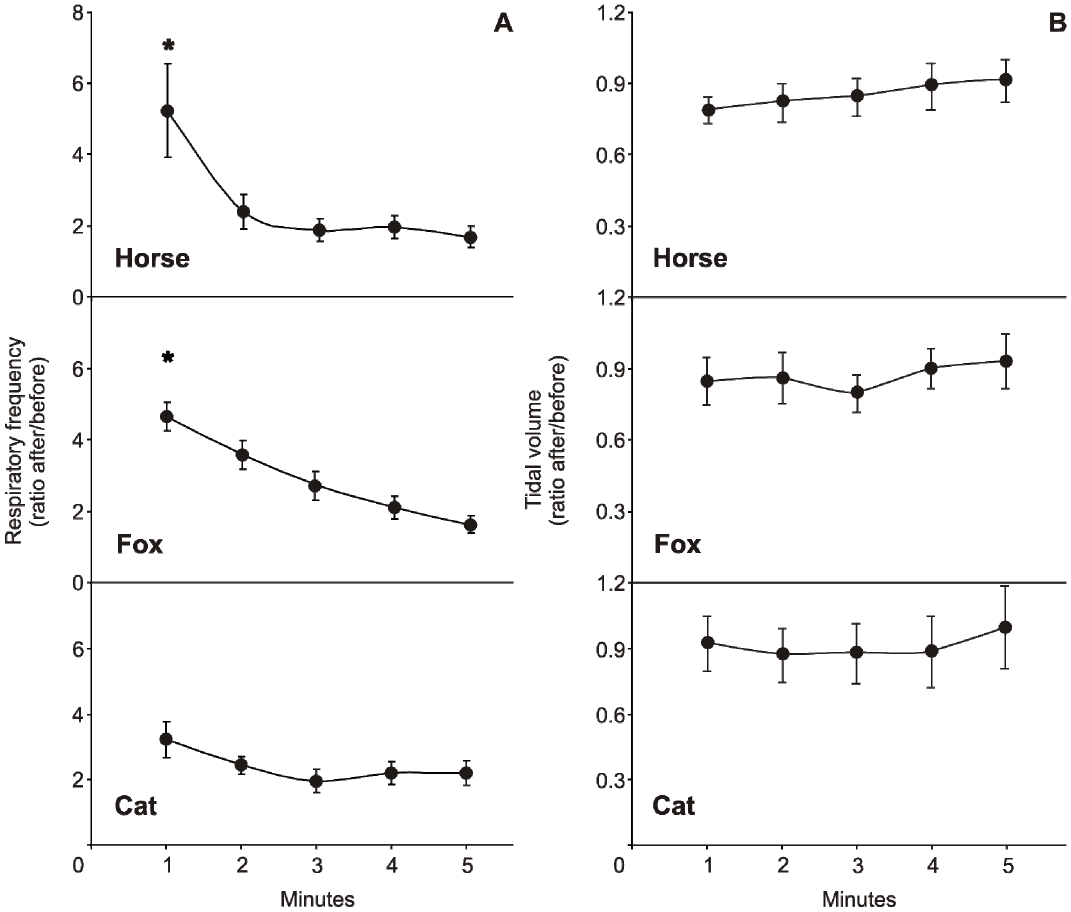

Figure 4.4: Examples of the effect of time on A) respiratory frequency and B) tidal volume responses to different odours for tammar wallabies. Response to a control (horse urine), to fox skin and to cat urine exposure. Asterisk indicates significant difference from other minutes at P<0.01. Values are mean ratio ± SE (N=8).

Discussion

BMR of tammar wallabies in this study was 1389.82 mL O_2 h^{-1}, consistent with BMR previously measured for tammars of 1390 mL O_2 h^{-1} (Dawson *et al.* 1969). Over all the measurements, mean resting f_R was 44.9 ± 5.12 breath min^{-1} and previous studies on tammar wallabies showed resting f_R to be 48 ± 22 breath min^{-1} at 30.5° ± 0.7 (Dawson *et al.* 1969). Most data collected in former studies used shorter measurement durations and this may have affected the results (Cooper & Withers 2009), this almost certainly explains the differences in values found between my study and earlier ones. In this experiment tammars were allowed to settle in the metabolic chamber before the beginning of the measurement and comparison of values indicates that were relaxed and resting comfortably at the time of the experiment.

Metabolic Response

Tammars' VO_2 and VCO_2 did not show any variation after exposure to control scents nor to potential predator odours. MR was not affected by any of the treatments used in the experiment. The 20 min average of MR after introduction of the scents clearly did not contain enough elevated values to significantly show a change in VO_2 and VCO_2. In order to avoid over or underestimation of MR, it was however necessary to allow enough time for expired air to mix and reach the equilibrium with the rest of the chamber before measuring VO_2 and VCO_2 levels. Therefore, shorter measurements of MR were not possible. This may explain why MR has not often been used to quantify alertness in response to a brief stimulus.

No differences in MR were recorded in the responses to exposure to the various scents. Even if MR has rarely been used to reveal fear responses, caribou (*Rangifer tarandus*) have shown increases in VO_2 after visual, acoustic and tactile stimulation (Floyd 1987) and wapiti (*Cervus elaphus canadensis*) had the same pattern of response when exposed to predator odours (Chabot *et al.* 1996). It was anticipated that some odours could provoke an increase in MR, however in the wapiti experiment, a continuous-flow mask was used to measure changes in VO_2 every 2 sec (1 min averages), giving an almost instantaneous pattern of response. The results obtained in my study suggest that MR is not necessarily the best indicator of short-term responses to stimuli when the experiments are conducted in a metabolic chamber, however it may be if the animal can tolerate the use of a mask to perform the measurements.

Ventilatory Response

Tammar wallabies responded to the introduction of a scent in the chamber with a rise in f_R during the first minute of exposure to all the scents. V_T was only slightly affected by the odours, with changes occurring only in response to exposure to horse. The increased f_R and high V_T seem to indicate an investigative approach to the scents, as fast but deep breaths allow a better odour detection. In fact, tammars were observed to orientate toward the stimulus and approach the scent source straight after odour introduction. This exploratory behaviour is similar to that observed in other studies (Muller-Schwarze 1972; Caine & Weldon 1989; Brown *et al.* 2000; McGregor *et al.* 2002; Monclús *et al.* 2006). For example, rodents displayed similar predator odour assessment, closely exploring the scent sources (Blanchard & Blanchard 1989; Williams *et al.* 1990; Kemble & Bolwahnn 1997). In addition, time had a significant effect on the respiratory reaction to the odours, as the increase in f_R observed in the first minute of exposure to all the treatments, quickly decreased in the second minute. This seems to confirm the hypothesis that scent investigation was responsible for the first reaction recorded. If considering just 4 min of exposure to the scents (from min 2 to min 5) and therefore ignoring the scent investigation, then the greatest responses occurred with fox and cat odours. After the initial odour inspection, tammars seemed to restrict their reactions to these particular scents. In fact, a fairly fast return of f_R towards normal levels was noticed during exposure to control scents (water and horse) and a more gradual decrease during exposure to predator scents (snake, quoll and fox), while high levels of f_R were maintained during the 5 min exposure to feral cat scent.

All the predator odours tested in this experiment were those of established or historical predators of tammar wallabies. The diet of the feral cat has largely shown to include marsupials of the *Macropus* genus (Catling 1988; Paltridge *et al.* 1997; Molsher *et al.* 1999; Paltridge 2002) and the fox represents the most likely predator of macropods on the mainland (Kaufmann 1974), considered the principal cause for the decline of wallabies in Western Australia (Kinnear *et al.* 1988). Moreover, both quolls (Belcher *et al.* 2008) and pythons (Blumstein *et al.* 2004) have been referred to as wallaby predators. Predator odour recognition could therefore be expected for all the predators used in this study, however the wallabies showed different responses to diverse predators. It was predicted that a response to predator odour was more likely to occur with the scent of predators which had a long evolutionary contact with tammar wallabies (Dickman & Doncaster 1984; Dickman 1992). However, tammars reacted more to introduced rather than to native predators. Various studies demonstrated that occasionally prey show responses to predators that have not coevolved or are unfamiliar with (Epple *et al.* 1993; Boag & Mlotkiewicz 1994; Rosell & Czech 2000). For example, Alpine goats (*Capra hircus*) suppressed feeding in reaction to exotic predator scents (Weldon *et al.* 1993). This is also consistent with findings on hare wallabies (*Lagorchestes hirsutus*) which responded cautiously to the view of an unknown predator (McLean *et al.* 1996). Since a response to horse scent was also observed during the experiment, the results obtained may also be an example of neophobia of introduced unknown species. In fact, the rise in f_R and the reduced V_T recorded after exposure to horse scent seemed to be consistent with a stress response. Macropods have formerly been recorded showing neophobia, spending more time close to a potential predator (dog) scent, than to an unknown herbivore odour (Blumstein *et al.* 2002b).

It has been previously noted that prey with past experience of a predator may show higher responses to predator scents (see Apfelbach *et al.* 2005). McLean *et al.* (2000) showed that bettongs (*Aepyprymnus rufescens*) and quokkas (*Setonix brachyurus*) learned to be cautious of dogs and foxes after chase-training. Blumstein (2002) suggested the existence of an experience-based mechanism for predator recognition in tammar wallabies. In two of his studies, tammars isolated from predators did not show visual or auditory predator recognition (Blumstein *et al.* 2004), while the ones who encountered predators on a consistent basis responded to even unknown predators, such as the fox and the feral cat (Blumstein *et al.* 2000). In addition, other studies showed that both visual (Griffin *et al.* 2001) and olfactory (Blumstein *et al.* 2002b) predator recognition may need to be learned in predator naïve tammar wallabies. Animals used in this experiment were wild caught on mainland Western Australia and therefore there was no record of their previous experience with predators. However, it is likely they faced some kind of predation risk throughout their free-ranging life as both mammalian and avian predators are present at

Tutanning Nature Reserve (Blumstein & Daniel 2002) and in particular the presence foxes is well documented (Kinnear *et al.* 2002). Some tammars may therefore have been previously confronted by foxes or cats, as suggested by the retreat, avoidance and prolonged reactions observed in response to their odours after initial investigation. Tammars' behavioural and physiological reactions after exposure to these predator scents clearly seemed to resemble fear.

It is known that predation risk varies with the predator type and previous studies have shown that prey are able to discriminate between more and less dangerous predators by showing greater responses or avoiding cues which represent higher risks, usually more abundant or efficient predators (Helfman 1989; Licht 1989; Smith & Belk 2001; Stapley 2003). For example, bank voles (*Clethrionomys glareolus*) showed discrimination and differential responses to seven species of predators (Jedrzejewski *et al.* 1993) and water voles (*Arvicola terrestris*) reacted more to predators which have greater impacts on their populations (Barreto & Macdonald 1999). Although some species of quolls represent a threat for wallabies, it is unlikely that northern quolls would because of their small size (Oakwood 2008). In addition, since both northern quolls and black-headed pythons have distributions which do not currently overlap with the tammars' (Cogger 1996; Van Dyck & Strahan 2008), the wallabies may not perceive them as representing a significant risk. On the contrary, tammars, feral cats and foxes have been sharing habitat for a period of time that may be sufficient for scent recognition to take place. It is therefore not surprising that wallabies showed a stronger reaction to scent of predators that are common in their home ranges as they represent a greater potential predatory threat.

Conclusions

The results of this study validate previous findings that tammar wallabies possess some kind of mechanism for odour recognition. Although the animals initially showed physiological responses to all the scents, the presentation of particular predator odours (cat and fox) elicited stronger reactions. This suggests that after the initial scent investigation, the wallabies narrowed their responsiveness only towards odours which represented a possible threat. Tammars used in this study may have already possessed predator recognition ability due to previous experience with predators and in addition, may have reacted to horse scent considering it a possible novel danger.

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Chapter 5: Predator faecal odour does not influence trappability of two species of marsupials

Abstract

Predators cause changes in the spacing behaviour of many prey species. This chapter investigated if habitat use by brushtail possums (*Trichosurus vulpecula*) and southern brown bandicoots (*Isoodon obesulus*) in their natural environment is influenced by the odour of potential predator. Trapping success was compared between traps with predator scents and control substances in two different experiments. The first measured trapping success of single clean traps and traps scented with different predator odours, while the second offered three diverse choices (historical predator, introduced predator and control) simultaneously. Bandicoots were in general trapped more than possums. Male and female bandicoots were trapped with equal frequencies in both experiments, while female possums were trapped more than males in experiment two. Frequency of capture was not affected by the body mass of the individuals. No pattern of avoidance was observed for either species in both experiments and there was no effect of gender for predator avoidance. The lack of avoidance response observed in this study, suggested that neither southern brown bandicoots nor brushtail possums recognised the odour of potential predators, probably as a consequence of living in a predator-free environment. Loss of anti-predator behaviour could have major consequences for the release of captive-bred individuals or during translocation of populations from a predator-free reserve to the wild.

Introduction

Predation risk can influence prey species and induce a change in their behaviour so that an encounter with a predator is less likely to occur (Lima 1998). Habitat shift as a result of predator presence has been shown in various studies. Doncaster (1994) noted that hedgehog (*Eruinaceus europaeus*) distribution was strongly influenced by the occurrence of badgers (*Meles meles*) and Suhonen *et al.* (1994) found that kestrel (*Falco tinnunculus*) nest position was influencing habitat choice of small birds. Experiments on different species of gerbil have consistently shown shifts in microhabitat and reduced activity in risky habitat (Kotler *et al.* 1991; Kotler *et al.* 1992; Kotler *et al.* 1993a; Kotler *et al.* 1993b; Abramsky *et al.* 1996; Abramsky *et al.* 2002). Prairie voles (*Microtus orchrogaster*) had smaller home ranges (Desy *et al.* 1990) and hares (*Lepus americanus*) altered their habitat use under predation risk (Hik 1995).

An estimation of the risk of predation by only direct contact with predators is dangerous for small animals (Fuelling & Halle 2004). For this reason, various studies have investigated prey sensitivity to indirect predator cues and have shown that prey react by displaying anti-predator responses to predator odours including changes in habitat preference (reviewed Apfelbach *et al.* 2005). For example, studies on voles showed an avoidance of areas marked with predator scents (Jedrzejewski & Jedrzejewska 1990; Barreto & Macdonald 1999; Perrot-Sinal *et al.* 1999). In Western Australia, house mice (*Mus domesticus*) exposed to mammalian predator scents used denser vegetation as they perceived higher predation risk (Dickman 1992). Also native Australian animals such as the bush rats (*Rattus fuscipes*), giant white-tailed rats (*Uromys caudimaculatus*) and fawn-footed melomys (*Melomys cervinipes*) respond to predators by avoiding their scents (Hayes *et al.* 2006). Over all, a lack of appropriate responsiveness to a potential dangerous stimulus may result in a high risk of predation and therefore may be selected against (Edmundus 1974).

Some studies have examined the response of prey to predator odours by comparing the trappability of target species in the presence of a predator scent or in its absence. In general, prey species avoided predator scented traps (Dickman & Doncaster 1984; Calder & Gorman 1991; Wolff & Davis-Born 1997; Borowski 2002). Dickman (1992) demonstrated that house mice avoid traps with predator odours. Weasel

(*Mustela nivalis*) scent (Stoddart 1976, 1980), tiger (*Panthera tigris*) odour (Stoddart 1982) and stoat (*Mustela erminea*) anal gland secretion (Gorman 1984) all reduced the number of voles trapped in different experiments. In addition Sullivan *et al.* (1988) showed that fox (*Vulpes vulpes*) faeces provoked trap avoidance and reduced feeding by voles. In regard to Australian native fauna, swamp rats (*Rattus lutreolus*), eastern chestnut mice (*Pseudomys gracilicacaudatus*) and bush rats have all been trapped less in predator scented traps then in unscented ones (Russell & Banks 2007). Despite these results, studies using olfactory cues to examine space use and general movement patterns of marsupials under risk of predation in the wild are still scarce.

This chapter aimed to test if the use of space by brushtail possums (*Trichosurus vulpecula*) and southern brown bandicoots (*Isodoon obesulus*) is influenced by potential predator scents. Trapping success of the two species in either tainted or clean traps was used as variable representing spacing behaviour and as a measure of habitat use. In addition, I examined prey reaction if challenged with a predator rich environment, presenting simultaneously more than one predator cue as well as a non-scented option.

Brushtail possums travel regularly on the ground for feeding and dispersal (see review by Green 1984), therefore they are vulnerable to terrestrial predators (Vernes *et al.* 2001; Johnson *et al.* 2006). In fact, there is evidence that fox and cat (*Felis catus*) presence affects habitat use by possums (Molsher *et al.* 1999; Pickett *et al.* 2005). Introduction of foxes and cats in Australia is also considered one of the major causes of the decline of the bandicoots (Morton & Baynes 1985; Burbidge & McKenzie 1989). In addition, dingoes (*Canis lupus dingo*; Vernes *et al.* 2001; Isaac 2005) and quoll species (Belcher *et al.* 2008; Oakwood 2008; Serena & Soderquist 2008) are considered natural predators of both possums and bandicoots. Therefore, effective avoidance mechanisms against all these predators are expected for both prey species examined here. Brushtail possums have responded to predator scents in various studies with changes in browsing and vigilance (Montague *et al.* 1990; Woolhouse & Morgan 1995; Gresser 1996; Morgan & Woolhouse 1997). In addition, brushtail possums showed physiological responses to dingo odour in laboratory experiments (see Chapter 2). It is not clear if the reaction observed could be considered a response to a noxious odour or a fear response. Bandicoot species have shown to be sensitive to some predator odours, exhibiting a decrease in mobility, feeding and time spent near the scent sources (Russell 2005). However, they were also found unresponsive to the presence of predator scents in traps (Russell & Banks 2005). It is therefore necessary to observe possum and bandicoot behaviour in the wild to better understand previous results.

Methods

Study animals and site

Brushtail possums and southern brown bandicoots were live-trapped at Harry Waring Marsupial Reserve (32° 9’ S; 115° 49’ E) in Perth, Western Australia, with treadle-operated traps baited with bread, peanut butter and oats. Traps were set at dusk, covered with a Hessian sack and checked twice per night. For each capture, species and sex were noted and animals caught were individually ear-tagged.

Trapping procedures

Experiment 1

The experiment was carried out on a trapping grid consisting of 3 transects spaced about 200 m apart, each with 12 traps sites at 20 m intervals (Fig.5.1A). A single trap was set at each site (36 trap stations in total) with a randomly assigned treatment (quoll *Dasyurus maculatus*, fox, cat, dingo, horse, *Equus caballus* or no scent). Trapping sessions were conducted for four consecutive nights when weather conditions were similar and traps were checked twice per night at 2 hrs intervals. Scents were removed from the traps and replaced with different ones at each round. Each treatment was presented 40 times during the whole experiment, with a total of 240 trapping events.

Experiment 2

The 36 wire cage traps were distributed over one of the three 12-site transects, forming 12 trap stations (Fig.5.1B). Three traps were set simultaneously at each site and placed 1 m apart. One trap contained an introduced predator scent (fox), one a historical predator odour (dingo), while the third one was left empty. Positions of traps were randomised at each trap station. Traps were set for three consecutive nights with no prebaiting period and were checked twice during each experimental night. A different transect was used every night to avoid recapturing the same individuals. Each treatment was presented 72 times, with a total of 216 trapping events. The body mass and the gender of animals trapped was recorded.

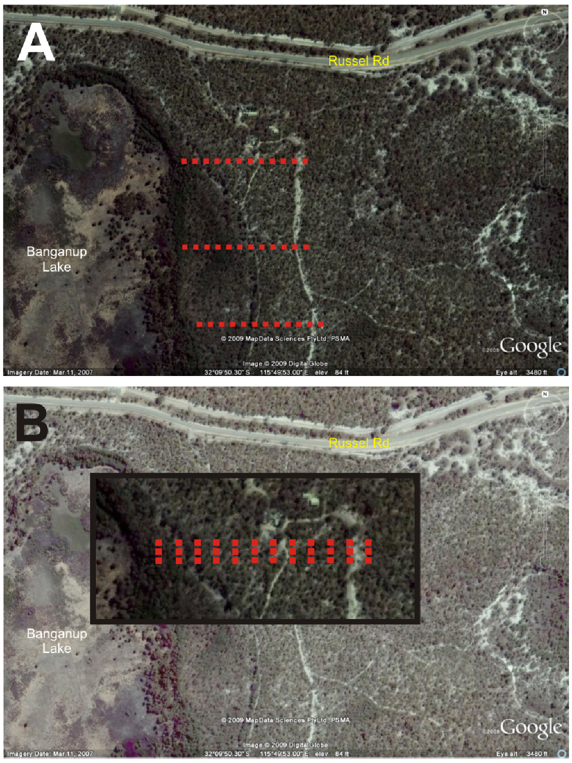

Figure 5.1: Trapping grid maps of Harry Waring Marsupial Reserve showing trap sites of one day for A) experiment one and B) experiment two. Each red square represents one trap (modified from Google Earth).

Scent sources

Figure 5.2: Example of trap baited with food. Burley cage at the entrance contains the scent.

Cat, fox, quoll and dingo faeces were used as predator cues, while horse faeces was used as a control. Fresh scats were obtained daily from captive animals maintained at Caversham Wildlife Park, Perth, WA and from private owners. Scats were collected within 12 hours of deposition and used the same day of collection. Scents were placed in large plastic Burley cages (MAKO) suspended at the entrance of the traps (Fig.5.2). Non-scented control traps were left untreated, but empty Burley cages were still placed at the traps' entrance to control for visual disturbance.

Data Analysis

In order to avoid pseudoreplication, only the first capture for each individual was included in the statistical analysis. In both experiments, general trapping success and avoidance were analysed with Chi-square goodness of fit to describe the discrepancy between observed and expected trapping frequencies in predator scented traps, those treated with a control and in the ones left unscented. Trappability differences in predator scented and control traps were evaluated. Capture of females and males of each species were also tested using Chi-square goodness of fit analyses. Total number of bandicoots and possums trapped were then compared with two-tailed *t*-tests to determine if one of the two species was most successfully trapped in each experiment. Differences in number of captures between males and females for each species were evaluated in both experiments with two-tailed *t*-tests to examine if trapping success was sex biased. Analysis of variance (ANOVA) was used to examine if trappability in experiment two depended on body mass. Since body size can vary with the gender of the individuals in both species (Kerle & How 2008; Paull 2008), gender was included in the model as covariate. All statistical analyses were completed using StatistiXL for Microsoft Excel Version 1.7 (Nedlands, Western Australia).

Results

Experiment 1

During the first experiment, 55 bandicoots (24 females, 31 males) and 13 possums (9 females, 4 males) were trapped over all four nights. In general, there was no effect of scent on capture success (X^2_5=2.453; P=0.784) or trap avoidance (X^2_5=2.154; P=0.827). The frequency of capture in predator, control treated and unscented traps did not differ (Fig.5.3A) for both bandicoots (9 in quoll, 8 in dingo, 10 in cat, 10 in fox, 9 in horse and 9 in no scented traps; X^2_5=0.309; P=0.997) and possums (1 in quoll, 5 in dingo, 3 in cat, 1 in fox, 1 in horse and 2 in no scented traps; X^2_5=5.923; P=0.314). However, bandicoots were captured significantly more often than possums (T_5=8.174; P<0.001). Neither females (X^2_5=2; P=0.849 for bandicoots and X^2_5=6.333; P=0.275 for possums) nor males (X^2_5=1.710; P=0.888 for bandicoots and X^2_5=2; P=0.849 for possums) were affected by the scents. Number of females captured was not significantly different from the number of males for both bandicoots (T_5=1.151; P=0.302) and possums (T_5=1.536; P=0.185).

Experiment 2

In the second experiment, 41 bandicoots (18 females and 23 males) and 15 possums (11 females and 4 males) were captured over the three nights. As in experiment one in general neither trapping success (X^2_2=0.036; P=0.982) nor trap avoidance (X^2_2=0.731; P≥0.694) were affected by scent (Fig.5.3B). There was no effect of scent on trap success for all individuals for both bandicoots (15 in dingo, 13 in fox, 13 in no scented traps; X^2_2=0.195; P=0.907) and possums (4 in dingo, 5 in fox, 6 in no scented traps; X^2_2=0.400; P=0.819). However, also in this experiment possums were trapped significantly less than bandicoots (T_2=7.211; P=0.019). There was no effect of scent on either females (X^2_2=1.333; P=0.513 for bandicoots and X^2_2=0.182; P=0.913 for possums) and males (X^2_2=0.348; P=0.840 for bandicoots and X^2_2=5; P=0.779 for possums). Numbers of females trapped did not differ significantly from number of males for bandicoots (T_2=0.945; P=0.444), while significantly more female possums were captured (T_2=7; P=0.020). Body mass did not have a significant influence on trappability, in fact the mean mass of animals captured in different scented traps was not statistically different (F_2=0.18; P=0.835; Fig.5.4). However, a significant interaction between body mass and gender was detected (F_1=10.0; P=0.003).

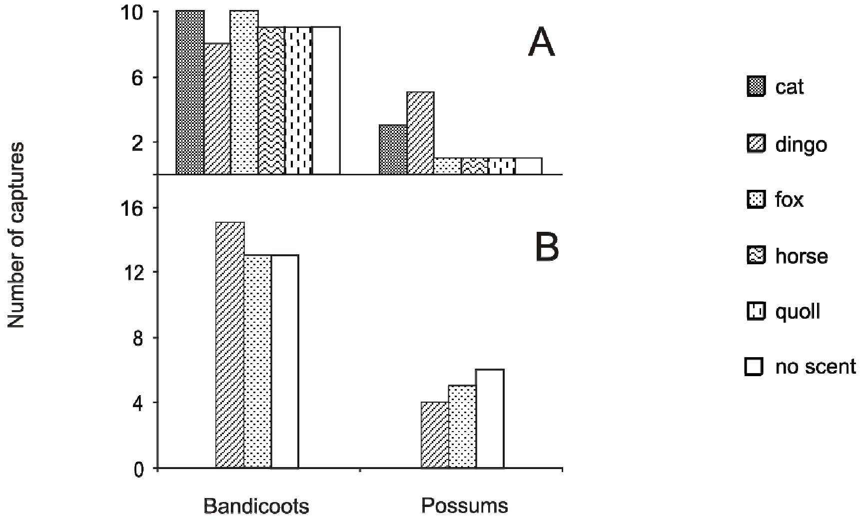

Figure 5.3: Number of captures for southern brown bandicoots and brushtail possums in traps tainted with different odours in A) experiment 1 and B) experiment 2.

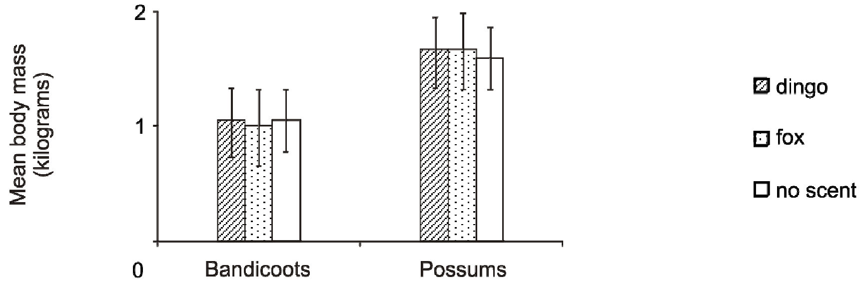

Figure 5.4: Mean body mass ± SE (N=56) of southern brown bandicoots and brushtail possums captured in traps tainted with different odours.

Discussion

The results of the single trap design indicated that the species studied did not avoid predator scents. Trapping success and avoidance did not differ for traps with the odour of potential predators and controls. This result was confirmed by the responses observed in the three-choice experiment. When animals were presented with the immediate multiple choice between traps with dingo or fox odour or no scent, they were given the option to avoid predators by selecting the unscented trap or to preferentially respond to the different dangers posed by different predators. However, they showed no avoidance of either predators. Both trapping methods used in this study are considered reliable; trap choice designs have been used in many studies with predator odours and three-choices experiments with traps tainted with different odours and unscented controls have also been used extensively in other research (Dickman & Doncaster 1984; Gorman 1984; Calder & Gorman 1991; Banks 1998; Russell & Banks 2005). In addition, Russell & Banks (2007) found no difference in the results obtained using single-trap or trap-choice techniques, therefore these two methods can be equally used to test differences in trapping success. In general possums and bandicoots exposed to olfactory cues from predators did not avoid the treated traps nor did they show a preference for unscented ones.

In both experiments, possums were trapped generally less than bandicoots. It is improbable that the reason is a mismatch between brushtail possums' ground activity and the timing of the experiment, as in previous studies possums have shown to reach a peak in feeding activity on the ground in the fifth hours after sunset (MacLennan 1984) and the experiments were carried out in this time frame. However, activity pattern and time spent on the ground are influenced by moonlight. In a study by MacLennan (1984), possums were less active on the ground when it was darker and this seemed to be associated with predator avoidance. Trapping experiments in this study were carried out during full moon nights and it is therefore unlikely that possums were caught less for this reason. A more probable explanation is that brushtail possums have bigger home ranges than bandicoots (Lobert 1990; Harper 2005), therefore the abundance of the latter in a certain area is simply higher than the one of possums.

Number of male and female bandicoots captured did not differ in both experiments. However, female possums were trapped more than males in experiment two. This was unexpected because dispersal in the brushtail possum has shown to be sex biased towards males (Green & Coleman 1986; Ji *et al.* 2001) and although gender has shown not to influence total time spent on the ground, male possums have been reported to undertake more terrestrial feeding than females (MacLennan 1984). However, male possums have larger home ranges than females (Green 1984; Green & Coleman 1986; Statham & Statham 1997) and therefore would be expected to occur at a lower density. In addition, dominant males tend to exclude young ones from their home territories (Biggins & Overstreet 1978). Subsequently the number of male possums present in a particular trapping area may have been reduced by the occurrence of other dominant males in the same zone.

Predator responses can be sexually dimorphic (Blanchard *et al.* 1991) and reactions to predator odours have shown to vary with gender (Perrot-Sinal *et al.* 1999). A difference in avoidance of traps scented with predator odours has been recorded between genders of rodents with a bias towards females (Stoddart 1980), while another study found a bias towards males (Dickman & Doncaster 1984). Therefore gender-based differences in predator avoidance are still unclear but in this study there was no gender difference in response to the scents.

It has been argued that faecal wastes may be ignored by prey with immediate foraging needs (Jones & Dayan 2000; Jonsson *et al.* 2000) as they represent only the mere possibility of an encounter with a predator and not an imminent threat (Banks *et al.* 2003). In fact, effects of predation risk on behaviour are known to be condition dependent and vary with the animal size (McNamara & Houston 1987). For this reason, body mass of the animals trapped was used as index of body condition and used to examine differences in response to different predator scents. However, trappability was not influenced by the mass of the individuals either for possums or for bandicoots. The interaction between body mass of the animals and their gender was expected for both species, as males are generally bigger than females (Kerle & How 2008; Paull 2008).

In this study, exposure to predator odours clearly did not cause any avoidance from the scent sources by either of the species studied, as implied if the odours were considered potentially threatening. Although predator faecal odours were found to be an efficient predation cue for some Australian native animals provoking a repellent effect on prey and causing a space shift to a safer location (Hayes *et al.* 2006; Russell & Banks 2007), other field studies examining responses to predator odours showed that predator scents were not avoided by prey. For example, there was no difference in the trapping success of the wood mice (*Apodemus sylvaticus*) in traps with predator odours compared to unscented ones (Stoddart 1976); bank voles (*Clethrionomys glareolus*) trappability did not decrease with the presence of predator scents (Jonsson *et al.* 2000) and feeding of *Rattus* species (Bramley & Waas 2001), oldfield mice (*Peromyscus polionotus*; Orrock *et al.* 2004), short-tailed voles (*Microtus agrestis*; Koivisto & Pusenius 2003) and other rodents (Novallie *et al.* 1982) were not affected by predator odours. For Australian fauna, bush rats and brown antechinus (*Antechinus stuartii)* showed no change in trapping rate in presence of dog (*Canis lupus familiaris*) scent (Banks *et al.* 2003).

Considering specifically the scents used in this study, no response to fox faecal odour or its compounds were observed in shrews (*Sorex araneus*; Dickman & Doncaster 1984), deer mice (*Peromyscus maniculatus*; Sullivan *et al.* 1988), roof rats (*Rattus rattus*; Burwash *et al.* 1998) and house mice (Powell & Banks 2004). In Australia, bush rats did not respond to fox faecal odour (Banks 1998). Brown antechinus and long-nosed bandicoot (*Parameles nasuta*) did not avoid either quoll or fox faecal scent (Russell & Banks 2007). The scent of cat faeces did not affect feeding rate of brushtail possums (Bramley & Waas 2001). No change in behaviour of tammar wallabies (*Macropus eugenii*) and red-necked pademelons (*Thylogale thetis*) was observed in response to dingo faecal scent (Blumstein *et al.* 2002). In particular, a study by Russell and Banks (2005) found that both southern brown bandicoots and brushtail possums were not affected by the presence of predator scents in traps, moreover they did not show any avoidance specifically for two of the scents used in this study (quoll and fox faeces). However, other bandicoot species have been reported to decrease their foraging activities in the presence of quoll and fox odours (Russell 2005) and brushtail possums have been previously observed to suppress feeding in presence of fox scent (Gresser 1996); though in another study avoidance of predator odours was not different from the one achieved with an egg-based repellent (Woolhouse & Morgan 1995). In all these studies however, the treatments may have acted by altering the food palatability more than the risk of predation (Jones & Dayan 2000).

In some instances, prey seemed to be attracted by the odour sources. Novallie *et al.* (1982) observed that feeding by antelope species was not simply unaffected by predator scents but animals were observed to frequently and closely sniff the predator odours. Possums and bandicoots were caught more in traps scented with quoll odour than in clean ones (Russell & Banks 2005). Olfactory cues placed inside traps might actually draw prey closer before repelling them, causing animals to be instantly trapped and falsely influencing the results. In this study, positioning the scents at the entrance of the traps ensured that scent assessment could be performed before venturing into the traps, so that animals avoiding predator scents were not wrongly captured.

Subjects of this study were living in a predator free environment and this may be why they have not shown an innate aversion to predator odours. At Harry Waring Marsupial Reserve, animals are protected by predator proof fences (Wicks & Clark 2005), therefore predation risk is probably insufficient to develop odour avoidance in possums and bandicoots. This is consistent with findings by Russel & Banks (2005), who observed that brushtail possums living in a predator-scarce environment did not avoid traps tainted with predator odours (fox and quoll). Predator naïve populations of rodents (Kavaliers 1990; Dickman 1992), fish (Magurran 1989) and ungulates (Berger 1998), have shown the same loss of predator scent avoidance. In addition, red-necked pademelon seemed to have lost predator odour recognition in only one generation of isolation (Blumstein *et al.* 2002). Therefore, it is expected for prey to quickly lose their experience-dependent anti-predator behaviours, such as recognition of a predator by its odour in habitats without predators, since it represents an unnecessary strategy in absence of real risk of predation (Blumstein 2002). However, both southern brown bandicoots (Russell & Banks 2005) and brushtail possums (Bramley & Waas 2001) failed to respond to predator scents even in predator-rich environments. The lack of avoidance of predator odours may indicate that these marsupials do not use olfaction for predator recognition.

Conclusions

These results show no evidence that space use by brushtail possums and southern brown bandicoots is influenced by the odour of potential predators. It seems that these marsupials do not perceive increased predation risk by odour cues. This may indicate that predator odour avoidance has not evolved in these species and that they have poor possibilities of escaping potential predators. Alternatively, living in a predator free environment has modified their responses to predator scents as predator odour recognition is no longer required in the populations studied. Loss of anti-predator behaviours should be regarded as a limitation when planning the release of individuals born in captivity and the reintroduction or the translocation of populations without predator experience.

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Chapter 6: The influence of predation risk on the behaviour of the western grey kangaroo, *Macropus fuliginosus*

Abstract

Predation risk influences foraging decisions and time allocation of prey species. Furthermore, habitat shifts from potentially dangerous to safe areas have been reported in numerous studies. In this chapter, an experiment was carried out on a wild population of western grey kangaroos (*Macropus fuliginosus*) to test the efficacy of predator scents to influence time allocated to different behaviours, to dissuade feeding from habitual areas and to induce changes in habitat use. Kangaroos were exposed to the odour of an historical predator, the dingo (*Canis lupus dingo*), an introduced predator, the red fox (*Vulpes vulpes*) and two control treatments simultaneously. Feeding and vigilance rate were compared before and after exposure to the scents and specific behavioural responses to the odours were recorded and quantified. Kangaroos did not increase their anti-predator scanning behaviour in predator scented areas. However, they showed strong investigative behaviour by approaching and sniffing the odour sources. They exhibited clear avoidance responses to predator scents, modifying their space use by moving away or escaping from the odours. Kangaroos were deterred from areas treated with predator odours and shifted to control sites, although preferred feeding patches were not completely abandoned.

Introduction

To prevent an encounter with a predator prey typical modify their use of space, moving from risky to safer areas (Lima & Dill 1990) or become more vigilant, increasing the time they visually scan the surroundings (Edmundus 1974). Under risk of predation prey reduce foraging time, increase scanning rates (Nelson & Mach 1991; Kotler *et al.* 1994) and shift to safer areas (Lima 1986; Sih 1986; Formanowicz & Bobka 1989; Lima 1998). For example, blue sheep (*Pseudovis nayaur*; Gurung 2003), dairy cattle (*Bos taurus*; Welp *et al.* 2004) and deer species (Altendrof *et al.* 2001; Laundre *et al.* 2001; Lingle & Wilson 2001; Childress & Lung 2003; Winnie & Creel 2007) have shown to be highly vigilant in presence of predators. Other studies have demonstrated shifts to protected habitats by prey under perceived risk of predation (Brown 1988; Bowers & Dooley 1993; Korpimaki *et al.* 1995). For example, elk (*Cervus elaphus*) moved from open areas to cover when wolves (*Canis lupus*) were present (Creel *et al.* 2005) and shrimp (*Atya lanipes*) migrated to pools without predators (Crowl & Covich 1994).

Predator odours have often been shown to elicit anti-predator responses in prey, similar to those observed in high predation risk situations (Apfelbach *et al.* 2005). Monclús *et al.* (2006b) found an increase in the time European rabbits (*Oryctolagus cuniculus*) spent vigilant when faced with a predator odour. Red-bellied tamarins (*Sanguinus labiatus*) exposed to predator faeces showed a similar response (Caine & Weldon 1989). Feeding rate has been reduced in mountain beavers (*Aplodontia rufa*) exposed to predator odours (Epple *et al.* 1993), while black-tailed (*Odocoileus hemionus colombianus*; Sullivan *et al.* 1985b) and white-tailed deer (*Odocoileus virginianus*; Swihart *et al.* 1991; Seamans *et al.* 2002) suppressed foraging where predator scents were present. The abundance and activity of Northern pocket gophers (*Thomomys talpoides*) was significantly reduced in areas treated with a predator odour (Sullivan *et al.* 1990) and meadow voles (*Microtus pennsylvanicus*) reduced spatial movement after exposure to fox (*Vulpes vulpes*) scent (Perrot-Sinal *et al.* 1999). Therefore predator odours have the potential to influence the activity pattern and distribution of prey species.

Anti-predator behaviour is established in macropod species (Blumstein *et al.* 1999; Coulson 1999; Blumstein *et al.* 2003) and studies have shown that they have a well developed olfactory system (Salamon 1996; Hunt *et al.* 1999); individual discrimination has been demonstrated in macropods (Blumstein *et al.* 2002a) and both red (*Macropus rufus*; Hunt e*t al.* 1999) and western grey kangaroos (*Macropus fuliginosus*; Jones *et al.* 2003) seem to use olfactory cues to avoid potentially dangerous food. For these reasons, and since kangaroos have always lived under heavy predation (Blumstein & Daniel 2002), predator odour recognition may have evolved. Only few studies have explored how macropods detect and respond to olfactory cues of predation risk. For example, recent studies have shown that anti-predator behaviour of the eastern grey kangaroos (*Macropus giganteus*) increased after exposure to predator scents (Brook *et al.* unpublished data) and that western grey kangaroos reduced feeding activity in response to dingo (*Canis lupus dingo*) odour (Parsons *et al.* 2007). However, it is still not clear if reduced foraging levels indicate the existence of an association with predation risk or if instead are due to reduced palatability of food. In addition, most studies have used captive (Ramp *et al.* 2005) or semi-wild (Parsons *et al.* 2007) individuals, and since captivity has been reported to influence anti-predator behaviour (Miller *et al.* 1990), it is important to explore reactions to predator odours in wild prey populations.

In this experiment I exposed a free-ranging population of kangaroos to predator (dingo and fox) odours in order to determine if they would perceive a predator presence by odour cues, and if this would alter their feeding behaviour and habitat use. I hypothesised that predator odours would provoke an increase in vigilance and a change in the kangaroos' distribution. In addition, this study examined if kangaroos' behaviour differed in response to different predator species (dingo or fox). Kangaroos are the main prey of dingoes (Whitehouse 1977; Caughley *et al.* 1980; Robertshaw & Harden 1985; Thomson 1992) but predation risk by foxes is also known to affect their behaviour (Coulson 1999; Banks *et al.* 2000), as foxes have been shown to predate on juveniles (Coulson 2008). In addition, Banks (2001) observed habitat shifts in kangaroos living in areas where foxes were present. Therefore, predation risk in kangaroos can be interpreted as a foraging cost and can be measured by monitoring their distribution and activity rates.

Methods

Study animals and site

The study took place at Whiteman Park, Perth, WA (31° 49' S; 115° 56' E). The park has a high population density of western grey kangaroos, so individuals were easily monitored in their natural environment. 5 min observations of focal animals (focal sampling, continuous recording) were recorded from a hidden position with a Sony HDR-CX6EK Handycam from a distance of at least 50 m. Kangaroos were filmed within 2 hrs of sunset, when grazing is the main activity (Short 1986), on days with similar conditions (no rain or heavy wind), as both temperature (Merril 1991) and wind affect vigilance behaviour (Hayes & Huntly 2005), habitat choice (Yasue *et al.* 2003) and perceived predation risk (Hilton *et al.* 1999). Since all kangaroos were free ranging and not individually marked during the study, to avoid pseudoreplication by monitoring the same individuals, four different locations in the park were chosen to conduct the observations. All were open homogeneous grassy areas with similar characteristics and surrounded by tall vegetation (trees and shrubs) which provided cover. The study at each location consisted of a before-phase, which served as a baseline control, and an after-phase, which included treatment and control areas. Each location was monitored for one day during each phase of the study. Gender, age group (juvenile and adult), distance to the focal animal's nearest neighbour (<1 m, 1-2 m, 2-3 m, 3-4 m, >5 m) and distance to the odour areas (<1 m, 1-2 m, 2-3 m, 3-4 m, 4-5 m) were noted with binoculars at the beginning of each focal sample.

Study design

Before-phase

The first part of the study consisted of recording kangaroo activity at the different locations to determine occurrence of basic behaviours and time allocated to feeding, vigilance, locomotion, grooming and social interactions.

After-phase

In the second part of the experiment each location was virtually divided in four 25m^2 regions: one contained an historical predator odour (dingo), one a novel predator scent (fox), another an herbivore odour (horse, *Equus caballus*) and one was left empty and used as unscented control. Each region was separated by a buffer area of

20 m. Scent sources were placed on tiles to avoid contamination with the ground. All four treatments were used simultaneously and randomly assigned to the regions. Within each region the odour was presented on three tiles spaced 2 m apart diagonally across the 25m^2 region (Fig.6.1). Individual focal samples were recorded for randomly selected animals within the various scent regions.

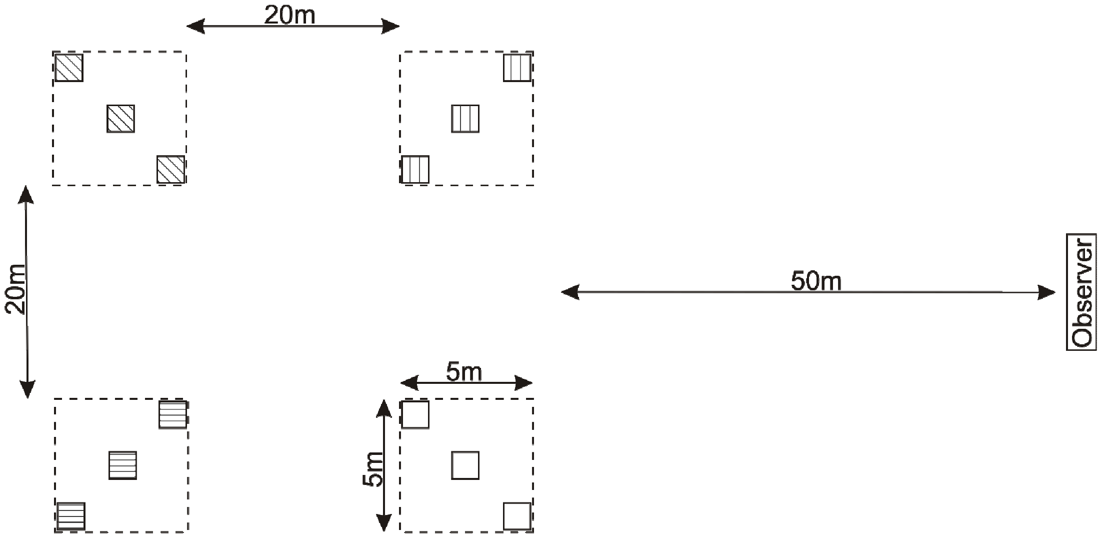

Figure 6.1: Schematic diagram of the design used to present the four different treatments in the after-phase of the study. All the odours were presented simultaneously and each of them was randomly assigned to a region.

Scent sources

Predator odours were fresh faecal wastes obtained daily from dingoes and foxes maintained in captivity at Caversham Wildlife Park, Perth, WA. The herbivorous odour was represented by horse faeces donated by private owners. Faeces were placed on the tiles within few hours of collection. A single bowel motion was used for each tile to resemble odour concentration encountered by animals in natural conditions. In the unscented control region tiles were placed in the same pattern in order to control for visual interference but no odour treatment was presented.

Data Analysis

Behavioural analysis

The software *JWatcher Video* Version 1.0 (www.jwatcher.ucla.edu) was used to score the behaviour of the focal animal samples (Blumstein & Daniel 2007) during both phases of the study. Vigilance measurements were obtained by combining data from kangaroos standing on hind legs with head and ears raised with data from individuals standing pentapedally with head up scanning the surroundings. Kangaroos standing pentapedally and head down biting or chewing food and individuals on hind legs, with tail on the ground regurgitating were combined to generate the foraging category. Self-grooming (pouch cleaning and scratching) and interaction with other individuals (aggressive and affiliative) were observed to occur in short bouts and were not relevant to the experiments, therefore they were combined and considered as ‘other behaviours’. Locomotion included animals hopping slowly or walking pentapedally with head down in search of food. Behaviours directly related to the presence of the scents included approaching (pentipedally walking towards the tiles), investigating (sniffing the scent source on the tiles), moving away (pentipedally walking away from the scent source), fright response (jump) and escaping (fast hopping away from the odours). These behaviours were all considered separately and not combined with others. Time spent in sight and out of view within the focal sample was also noted.

Statistical analysis

Repeated measures analysis of variance (ANOVA) with a Student Newman-Keuls (SNK) *post hoc* test was used to establish if odour influenced the time kangaroos allocated to different behaviours. Data collected in both phases of the experiment were included in the analysis. Treatments (before, dingo, fox, horse and no scent) were factors and total time (sec) engaged in the various behaviours was the repeat. Number of times a behaviour was displayed (occurrence) in the before and after-phase was also analysed with repeated measures ANOVA with SNK *post hoc* tests. As the time allocated to different activities has been shown to vary with gender (Ruckstuhl *et al.* 2003; Pays & Jarman 2008), age group (Berger 1991; Mateo 1996), distance to the nearest neighbour (Poysa 1994; Fernandez-Juricic *et al.* 2004; Fernandez-Juricic 2007; Pays *et al.* 2008) and proximity to the scent sources (Parsons *et al.* 2007), these variables were included in a separate model as covariates. Subsequently a repeated measure ANOVA with *post hoc* tests was carried out on the significant interactions to be able to detect where differences were found. All statistical analyses were completed using StatistiXL for Microsoft Excel Version 1.7 (Nedlands, Western Australia).

Results

A total of 109 western grey kangaroos (50 females, 44 males and 15 unknown) were filmed during the study, 51 in the before-phase and 58 in the after-phase. There was a significant interaction between odour and both occurrence of different behaviours ($F_{36,361}$=3.406; P<0.001; Fig.6.3A) and the time allocated to different activities ($F_{36,361}$=2.883; P<0.001; Fig.6.3B). Occurrence (SNK≥0.131) and time spent (SNK≥0.174) in the various behaviours were not significantly different in the before-phase and in the unscented control areas.

When odours were present, number of feeding events (7.4±1.03 for horse, 5.2±2.08 for fox and 5.9±1.33 for dingo; SNK≤0.010) and time spent foraging (33.7±5.5 sec for horse, 17.7±7.2 sec for fox and 22.2±4.6 sec for dingo; SNK≤0.045) were significantly lower than in the before-phase (11±0.58 feeding events and 49.5±4.3 sec feeding) and in unscented areas (10±0.67 feeding events and 35.5±4.6 sec feeding). Time allocated to locomotion for food searching was not affected by the scents (120.6±8.9 sec for before, 115.2±15.7 sec for no scent, 100.6±16.3 sec for horse, 63.9±20.7 sec for fox and 70.3±20.9 sec for dingo; SNK≥0.076) but the occurrence of locomotion was significantly lower when the odours were present (4.3±0.62 for horse, 2.5±1.07 for fox and 3.2±0.65 for dingo; SNK≤0.022) compared to the before-phase (6.1±0.42) and the unscented control (6.5±1.37).

There was no significant difference between before, scented and unscented areas in number of scanning events (6.3±0.62 for before, 7.3±1.05 for no scent, 7.3±0.82 for horse, 4.3±1.58 for fox and 5.9±1.04 for dingo; SNK≥0.374) and time spent vigilant (85.4±8.3 sec for before, 100.7±18.7 sec for no scent, 85.9±11.2 sec for horse, 48.6±18.5 sec for fox and 60.2±15.8 sec for dingo; SNK≥0.134). No difference was also found in occurrence (1.1±0.16 for before, 1.8±0.65 for no scent, 1.2±0.28 for horse, 0.22±0.05 for fox and 0.81±0.23 for dingo; SNK≥0.077) and time spent in 'other behaviours' (20.9±4.5 sec for before, 30.3±9.8 sec for no scent, 18.8±8.8 sec for horse, 17.1±5.2 sec for fox and 17.4±8.2 sec for dingo; SNK≥0.239).

In both predator and herbivorous scented areas, number of contacts (0.9±0.25 for horse, 0.8±0.26 for fox and 1.1±0.35 for dingo; SNK≥0.536) and time spent approaching (6.7±1.9 sec for horse, 4.2±1.2 sec for fox and 9.2±3.9 sec for dingo;

SNK≥0.251) the odours were the same, and so were occurrence (1.6±0.43 for horse, 1±0.23 for fox and 1.1±0.30 for dingo; SNK≥0.131) and time (6.9±1.8 sec for horse, 4.3±1.4 sec for fox and 4.1±1.3 sec for dingo; SNK≥0.197) allocated to investigation of the various scents.

Some kangaroos were observed to show a specific response after investigation of the odours, displaying an evident jump followed by a quick escape (Fig.6.2). Mean number of jumps (0.1±0.05 for horse, 0.3±0.05 for fox and 0.4±0.1 for dingo; SNK≤0.012) and fleeing events (0.1±0.05 for horse, 0.4±0.17 for fox and 0.3±0.12 for dingo; SNK≤0.013) were significantly higher in the presence of predator odours compared to areas tainted with horse scent (SNK≥0.131). There was no difference in the number of fright responses (SNK=0.432) and time spent escaping from dingo and fox odours (41.4±17.5 sec for fox and 33.8±13 sec for dingo; SNK=0.144). Number of moving away events was significantly higher (SNK≤0.030) in dingo scented areas (2±0.58) compared to both fox (0.7±0.23) and horse (1.1±0.36), while time spent moving away from areas where dingo and fox odours were placed was the same for both scents (18.3±5.3 sec for fox and 16.1±5.3 sec for dingo; SNK=0.054), but was different from the herbivorous control (10.5±4.6 sec; SNK≤0.017).

There was no difference between the before and after-phase in number of out of sight events during the focal sessions (0.5±0.09 for before, 0.3±0.16 for no scent, 0.9±0.17 for horse, 0.9±0.23 for fox and 0.3±0.19 for dingo; SNK≥0.197) and time spent out of view (4.8±2 sec for before, 1.5±0.7 sec for no scent, 4.8±1.4 sec for horse, 1.48±0.62 sec for fox and 2.9±1.4 sec for dingo; SNK≥0.628).

Gender of the kangaroos and distance to the nearest neighbour did not affect occurrence ($F_{9,95}$=1.822; P=0.074 for gender and $F_{9,95}$=1.906; P=0.060 for nearest neighbour) and time spent ($F_{9,95}$=0.705; P=0.179 for gender and $F_{9,95}$=1.715; P=0.096 for nearest neighbour) in different behaviours. There was no an effect of age on time allocated to different behaviours in the presence of the odours ($F_{9,95}$=1.587; P=0.130). However, scents influenced the incidence of the activities ($F_{9,95}$=3.391; P=0.001), with adult kangaroos showing to be affected by the scents ($F_{36,245}$=3.172; P<0.001). In fact, number of foraging (SNK≤0.016) and locomotion (SNK≤0.046) events were lower in the presence of the odours and number of jumps (SNK≤0.004) and fleeing ($SNK \leq 0.019$) events were higher in areas tainted with predator scents. No significant change in behaviour was found for juveniles when odours were present ($F_{27,53}=1.633$; $P=0.063$).

There was an effect of the distance to the scent on both occurrence of behaviour ($F_{9,95}=2.744$; $P=0.007$) and distribution of time ($F_{9,95}=2.565$; $P=0.011$), with only kangaroos in close proximity (<1 m) to the odour regions being affected by the scents, showing a significant change in occurrence of behaviour ($F_{27,88}=2.369$; $P=0.001$) and time allocation ($F_{27,91}=2.144$; $P=0.004$). In fact, number of foraging ($SNK \leq 0.032$) and locomotion for food searching ($SNK \leq 0.014$) events were significantly reduced in predator scented areas and so was time spent in different activities in general ($SNK \leq 0.046$).

Figure 6.2: Sequential reactions to different odours for western grey kangaroos. Initial investigation of dingo (A1 and B1), fox (C1) and horse (D1) faeces; jump of fear in response to both dingo (A2 and B2) and fox (C2) scents followed by flee reactions (A5, B5 and C5); no escape in response to horse odour (D2) and subsequent feeding in close proximity of the scent (D5).

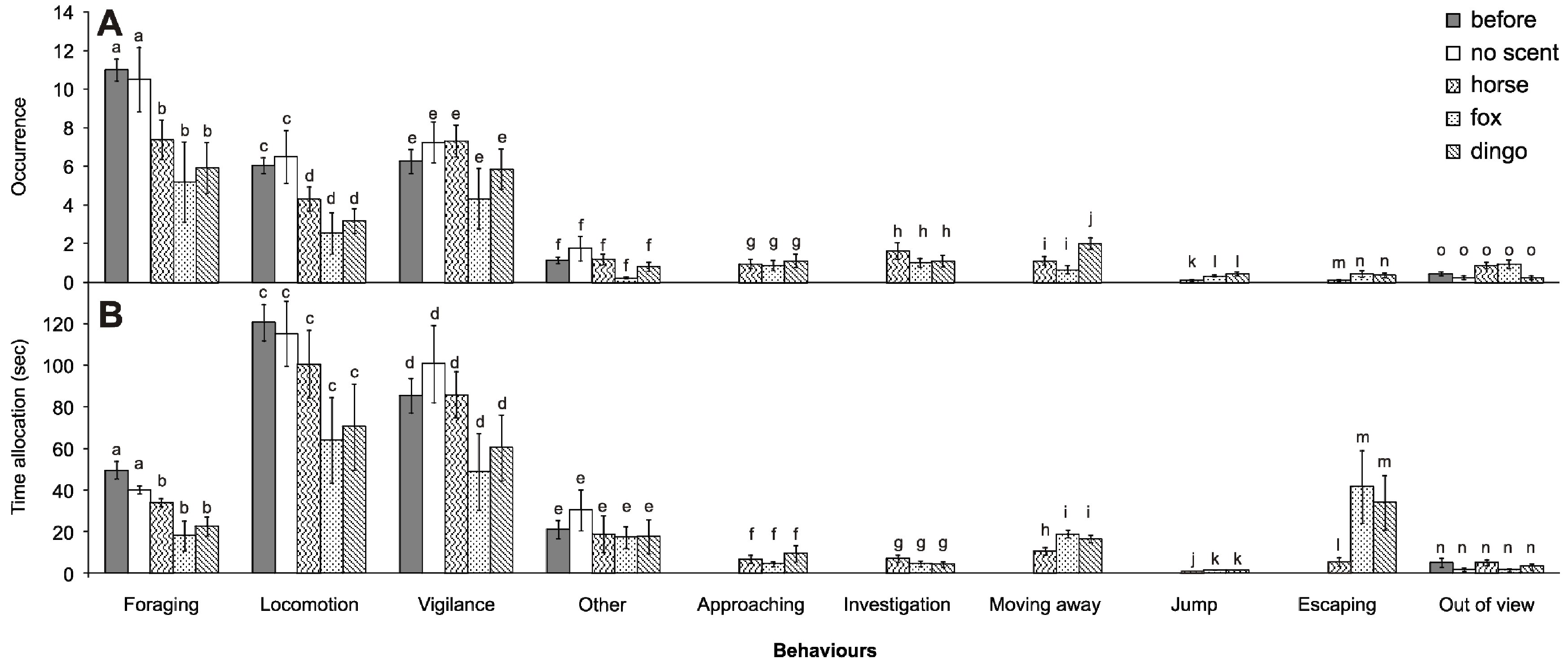

Figure 6.3: Variation of A) mean occurrence and B) mean time allocated to different behaviours for western grey kangaroos as a function of the presence of various odours in the feeding area during 5 min focal samples. Values are mean ± SE (N=109). For each behaviour, different letters indicate significant difference from other odours at P<0.05.

Discussion

Prey under predation risk may trade off feeding and for increased vigilance (Lima 1990) or may respond by moving away from potentially dangerous areas (Lima & Dill 1990). Western grey kangaroos significantly reduced time spent foraging and food searching events when predator odours were present. Black-tailed deer (Melchiors & Leslie 1985), goats (*Capra hircus*; Weldon *et al.* 1993), sheep (*Ovis aries*; Arnould & Signoret 1993) and beavers (*Castor canadensis*; Englehart & Muller-Schwarze 1995) suppressed browsing when exposed to predator faeces. A common assumption is that in a risky situation, foraging is reduced as a consequence of increased vigilance (Lima 1987). In macropods predation risk has been observed to affect vigilance (Coulson 1999; Wahungu *et al.* 2001; Blumstein *et al.* 2002b; Blumstein & Daniel 2003; Blumstein *et al.* 2003), and since kangaroos are highly vigilant animals (Banks 2001), their scanning rate was expected to increase if they perceived the risk of encountering a predator (Brown & Kotler 2004). In addition, eastern grey kangaroos increase vigilance in the presence of predator odours at the expense of feeding (Brook *et al.* unpublished data). However in this study there was no such pattern. In fact, kangaroos did not increase their vigilance in the presence of potential predator odours; the decrease in feeding and food searching resulted from avoidance of the predator scented areas, indicating a tendency to shift from risky to safer sites.

The foraging behaviour of the kangaroos also decreased in the presence of horse odour. Similar deterrence occurred for cattle (Dohi *et al.* 1991) and sheep (Arnould *et al.* 1998) exposed to herbivorous odours and with steers (Engle & Shimmel 1984) and white-tailed deer in the presence of common repellents (Harris *et al.* 1983). In some of the previous studies on predator-based deterrence, feeding suppression may have been caused by the malodorous substances placed on food, more than by risk of predation. In this study the presence of noxious odours in general seemed aversive and disturbed feeding sessions. However, the kangaroos displayed different reactions to the herbivore and the predator odours.

The kangaroos exhibited an initial attraction for all the odours presented, showing strong investigative behaviour by approaching and sniffing the tiles where the scents were placed. Number of contacts made with the odours (approaches) and time spent investigating were the same for both predator and the herbivorous odours. Previous studies found that prey closely investigated predator scents. For example, sheep examined predator odours at close range (Pfister *et al.* 1990); the same pattern of response was recorded in red-necked pademelons (*Thylogale thetis*; Blumstein *et al.* 2002c; Ramp *et al.* 2005), hares (*Lepus americanus*; Sullivan *et al.* 1985a), black-tailed deer (Sullivan *et al.* 1985b) and rabbits (Monclús *et al.* 2006b). In addition, Brook *et al.* (unpublished data) observed inspection of predator odours by eastern grey kangaroos. This kind of behaviour seems necessary in order to assess the risk of predation (Fishman 1999), as information about predator density seems to be obtained from faeces (Lima & Dill 1990). In this study, investigation of the scents was followed by differential responses: the kangaroos moved away or escaped from predator odours, while they usually did not leave after assessment of horse scent. Avoidance of areas tainted with predator odours has been commonly observed in studies on anti-predator responses (see review in Kats & Dill 1998) and risk assessment of predator scent has been typically accompanied by suppression of appetitive behaviour (Kemble & Bolwahnn 1997). This is consistent with the results obtained in this study, where there was a reduction in time spent feeding and an increase in time spent moving away from predator scented areas.

After investigation of predator scents, some kangaroos were observed to show a specific fright response, displaying an evident jump resembling fear, followed by a quick escape. No difference was found in occurrence and time allocated to fleeing in response to dingo and fox. It has been suggested that macropods respond to the olfactory cues associated with predator scents in a generic way and not specifically to certain species of predators (Blumstein *et al.* 2002c). The similarity in the responses observed to dingo and fox odours may be attributed to similar features in their faeces (Stoddart 1980; Dickman & Doncaster 1984). Fleeing after investigation of dingo scent has been observed before in a semi-wild population of western grey kangaroos (Parsons *et al.* 2007). Kangaroos in this study seemed to display responses to the historical predation pressure posed by dingoes (Caughley 1964; Kaufmann 1974; Banks 2001) by escaping after investigating their scent and this might have facilitated an effective avoidance of the odour of recently introduced predators, such as foxes.

In general, when a predator odour was present, there was an increase in the time the kangaroos spent moving away from the treated areas, suggesting that they would change their space use after exposure to predator scents. Spatial avoidance of fox faecal odour was recorded in a study on rabbits (Monclús *et al.* 2005); hedgehogs (*Erinaceus europaeus*) shifted feeding from sites marked with predator scents to untreated or non-predator odour areas (Ward *et al.* 1997); studies on voles showed a modification of use of space in areas where predator scents were present (Jedrzejewski & Jedrzejewska 1990; Perrot-Sinal *et al.* 1999) and the distribution of northern pocket gophers was altered in presence of predator derived odours (Sullivan *et al.* 1988). In this study, the number of moving away and out of sight events recorded during the focal sessions was higher in the presence of dingo odour compared to fox, suggesting that the kangaroos were trying to avoid the dingo scent more often. However, although kangaroos moved away from the predator odours, shifting to areas where these were not present, time spent out of view was the same in the before and after-phase, suggesting that they were not retreating to cover after exposure to predator odours. This is consistent with findings showing that prey would only temporarily modify their use of space in presence of predator odours (El Hani & Conover 1998) or in high predation risk situations (Winnie *et al.* 2006).

Sensory cues used by macropods to assess predation risk vary with sociality and patterns of habitat use. For example, solitary species living in forests, such parma wallabies (*Macropus parma*), red-bellied pademelons (*Thylogale billiardierii*), and tammar wallabies (*Macropous eugenii*) respectively avoided areas with predator odour (Ramp *et al.* 2005), foraged close to vegetation cover (While & McArthur 2005) and sheltered (Blumstein & Daniel 2002) under perceived predation risk. On the contrary, social macropods that forage in open areas, such as red-necked pademelons and Bennett's wallabies (*Macropus rufogriseus rufogriseus*) respectively chose to inspect predator odours closely (Ramp *et al.* 2005) and avoided forest margins in risky situations (Blumstein & Daniel 2002; While & McArthur 2005). For animals foraging in open areas, such as western grey kangaroos, escaping to a refuge at the first sign of predation risk may be too costly, especially when other strategies like aggregation can be used to reduce the danger of being preyed upon (Coulson 1999; Blumstein & Daniel 2003). In previous studies, western grey kangaroos were observed to remain in open areas when under predation risk (Blumstein & Daniel

2002), but they escaped to refuge when approached by a predator (Colagross & Cockburn 1993; Jarman & Wright 1993). Some species may actually consider open habitats as areas with lower danger (Hopewell *et al.* 2005) until a predator is visually detected, as the opportunity to directly observe the predator is greater in the open. This may be the reason why kangaroos moved away from the scent sources but still remained in the open.

Habitat choice may not only be influenced by predation risk alone but also by abundance and availability of resources (Heithaus & Dill 2002). Shifting habitat may reduce the chances of a prey to encounter a predator but will also affect other important needs (Winnie *et al.* 2006), becoming a substantial foraging cost (Winnie & Creel 2007). For example, bottlenose dolphins (*Tursiops aduncus*) reduced the use of profitable feeding patches when tiger sharks (*Galeocerdo cuvier*) were present (Heithaus & Dill 2002, 2006). The same pattern of habitat use was observed in wild dogs (*Lycaon pictus*), which were found to be in low densities in areas where the risk of predation was high, despite the great availability of food (Mills & Gorman 1997). Macropods have been observed to choose foraging sites based on food quality (Southwell 1987; Carter & Goldizen 2003), therefore the cost and consequences associated with a complete change in habitat use in response to predator odours might be too high for kangaroos.

There are numerous interacting factors which may also influence the time allocated to different behaviours (Frid 1997). In this study, gender, age group, distance to the nearest neighbour and distance to the scent sources were all considered as variables. Gender influenced response to predator odour in other studies (Dickman & Doncaster 1984; Jedrzejewski & Jedrzejewska 1990; Perrot-Sinal *et al.* 1999; Monclús *et al.* 2006a). For kangaroos, there was no effect of gender on time allocated to different activities in presence of a predator scent. This was unexpected as in previous studies female eastern grey kangaroos have been shown to be more vigilant than males (Jarman 1987; Colagross & Cockburn 1993; Pays & Jarman 2008). This is probably because large males are known to successfully defend themselves against predator attacks (Wright 1993), while females and young are usually preferred prey (Shepherd 1981). High vigilance levels in females have been reported for other macropods, such as red-necked pademelons (Wahungu *et al.*

2001), but this pattern of response does not seem to apply to the western grey kangaroos studied.

Scanning rate of grey kangaroos is known to be affected by number of individuals in the group (Heathcote 1987; Jarman 1987; Jarman & Wright 1993; Coulson 1999) and red-necked pademelons increased their vigilance in function of their distance from conspecifics (Pays *et al.* 2008). Time allocated to different activities could vary differently with the proximity of companions. For example, sheep preferred foraging in close proximity to other individuals (Dumont & Boissy 2000), while tammar wallabies foraged less when closer to conspecifics (Blumstein *et al.* 1999). In this study distance to nearest neighbour did not affect any behaviour.

There was an effect of age on the occurrence of behaviours in the presence of odours but not on time spent in different behaviours. Diverse anti-predatory responses can be expected for individuals at different ages (Vitale 1989). In this study, adults were affected by the presence of the scents, while juveniles were not. In fact, for adult kangaroos both number of foraging and locomotion events were lower in presence of odours and number of jumps and fleeing events were higher in areas with predator scents. These results seem to indicate that adult kangaroos are more sensitive to potential predatory threats by odour cues. This is consistent with other findings on young grey kangaroos which found that juvenile exhibit less anti-predator behaviours than adults (Heathcote 1987). It is likely that juvenile individuals underestimate the risk posed by a predator cue as they rely on mothers for guidance (Hume *et al.* 1989).

The responses to the odours differed with distance from the source. In fact, both incidence of behaviour and time allocation varied with the distance from the odours. Only kangaroos in close proximity (<1 m) to the treatment regions were affected by the presence of the scents, reducing feeding and searching for food. This is consistent with previous findings on western grey kangaroos, which indicated different reactions in individuals standing between 0 to 6 m from predator odours (Parsons *et al.* 2007). However, no change in behaviour was observed in individuals within the scent areas unless investigation was performed. It has been suggested that gregarious species depend more on visual and auditory cues for predator detection (Ydenberg & Dill 1986) and this may explain why a close assessment of the odour sources was necessary before the kangaroos showed any reaction. The region where tiles were presented with no odour clearly provided adequate control for the scented areas since no behaviour was affected by the visual cues and the amount of time spent in different behaviours in the immediate vicinity of the unscented treatment did not differ from the one allocated to the same behaviours before the exposure to the odours.

Conclusions

Wild western grey kangaroos appeared to associate predator odours with predation risk, as they reacted to the odour of mammalian predators (both historical and recently introduced). A strong effect of predator scents on behaviour has been noted, with flight responses and avoidance of the predator scented sites after risk assessment. These results suggest that kangaroos are scared of predator odours and tend to move away or escape from predator scents. However, responses were recorded only after the kangaroos closely investigated the odour sources and although predator odours were avoided, scented areas were not completely abandoned, suggesting that a long-lasting repellent effect is unlikely.

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Chapter 7: General discussion

The results of my research indicate that exotic and Australian prey react differently to predator odours, and demonstrate that avoidance of predator scent has evolved in some but not all Australian marsupials. Macropods responded to olfactory cues of predation, although investigation of odour sources seemed necessary to achieve recognition. Small (0.3-3 Kg) marsupials appeared more naïve towards both native and introduced predator odours. In general, native Australian mammals appeared more relaxed in their approach towards predator odours compared to the exotic prey studied, which was extremely cautious towards any variation in the environment. A summary of the species-specific responses observed in this study is presented in Table 7.1.

Common brushtail possums (*Trichosurus vulpecula*) responded to the scent of the dingo (*Canis lupus dingo*) in laboratory experiments (Chapter 2) but in the wild, they did not avoid the odour of the same predator (Chapter 5). It is known that captivity influences the perception of a predator presence, and that responses obtained in laboratory may often be misleading and artificial compared to natural conditions (Ward *et al.* 1996; Pusenius & Ostfeld 2002). For example, the design of the metabolic experiments in this study did not permit to the possums to avoid the predator odour if desired and, as the intensity of the exposure to dingo scent may have been greater in the laboratory than in the field, it may also have led to stronger responses.

Other prey have shown greater reactions in captive environment and weaker or no responses when free-ranging. For example, meadow voles (*Microtus pennsylvanicus*) did not avoid short-tailed weasel (*Mustela erminea*) odour in field experiments (Parsons & Bondrup-Nielsen 1996); gray-tailed voles (*Microtus canicadus*) did not show any response to mink (*Mustela vison*) scent under natural conditions (Wolff & Davis-Born 1997) and bank voles (*Clethrionomys glareolus*) did not change their spacing behaviour in the field in the presence of mustelid predator odours (Jonsson *et al.* 2000). However, these results were inconsistent with those obtained in the laboratory for the same predator-prey interactions, where prey species always avoided predator odours. The contrasting results obtained in my laboratory and field studies carried out on brushtail possums clearly emphasise the limitations of studying behaviour only in controlled laboratory settings and underline the importance of conducting experiments also in more natural conditions. However, laboratory and field studies may simply be considered as two different approaches to understand animals' awareness of a risky situation. The former may be useful to examine short-term responses to dangerous stimuli, while the latter permits to investigate longer-term effects and both can help understanding the prey reactions to predation risk.

The reaction to dingo odour observed for possums in the laboratory was short-lived, showing to wane in potency over few minutes. This may indicate that after investigation, possums did not consider the scent as a threat. During the physiology experiment, urine was used to represent the presence of predators, while faeces were used in the wild. This may account for the differential responses obtained in the two studies. A strong avoidance to urine but a weak or no response to faecal odours from the same predator species has been previously described. For example, snowshoe hares (*Lepus americanus*) suppressed feeding when wolverine (*Gulo gulo*) urine was present but faecal odour of the same predator was not effective (Sullivan *et al.* 1985a). However, it has also been suggested that predator urine is not successfully used under field conditions (Orrock *et al.* 2004), as evaporative loss results in reduced responses by prey (Sullivan & Crump 1984; Sullivan 1986). Therefore the use of different odour sources from the same predator may sometimes be necessary. Future studies on brushtail possums should investigate if urine rather than faecal extracts represent for this marsupial a more reliable cue of predation risk.

Rabbits (*Oryctolagus cuniculus*) were only studied in the laboratory (Chapter 3) and were highly responsive to every change in their environment, as suggested by the anxiety shown after exposure to both predator (python, quoll, fox and cat) and control (horse) odours. If the behaviour observed in the laboratory accurately reflects the one of rabbits in the field, this may suggest an ability to cautiously react to an entirely new kind of stimuli and may explain why these animals are so efficient in adapting to new situations and colonising new environments (Banks 2000). If rabbits living in Australia are able to detect olfactory cues of both historical and novel predators and respond cautiously, then they may be at an adaptive advantage compared to the more naïve native prey. As rabbits were alarmed by every potentially dangerous or novel situation, it would be interesting to conduct further studies on their responses to predator odours in the field to test if their habitat use is influenced by perceived predation risk and if so, how this affects their breeding and population dynamics.

Tammar wallabies (*Macropus eugenii*) indicated a mechanism for the recognition of predator odours as responses in the laboratory were restricted to particular predator scents (Chapter 4). However, they initially investigated all the scents submitted and only then showed different responses. This pattern of odour assessment was consistent with that observed in my study on wild kangaroos (Chapter 6). It was surprising to observe that tammars showed stronger reactions to the introduced fox (*Vulpes vulpes*) and cat (*Felis catus*) rather than to native predators, such as python (*Aspidites melanocephalus*) and quoll (*Dasyurus hallucatus*). However, it seems that wallabies possess an experience-based mechanism for predator recognition (Blumstein 2002) and, as subjects used in the study lived in a predator rich environment (Blumstein & Daniel 2002), where the presence of foxes has been reported (Kinnear *et al.* 2002), this would explain why they showed greater responses to predators they have probably faced before.

All the animals I studied in the laboratory reacted to predator stimuli (Chapters 2, 3 and 4). However, responses were always restricted to changes in the respiratory variables and never affected metabolism. Ventilatory rate seems therefore to be a more sensitive indicator of animals' perception of an immediate dangerous situation. In fact, in previous studies respiratory rate has shown to be a reliable index of animals' receptiveness to change, as it was dramatically affected by sensory stimuli (Allen *et al.* 1986; Schapker *et al.* 2002). Findings of my study highlight the importance of using real-time measures of response, such as respiratory variables, rather than longer time-averaged measures such as metabolic rate, to study instant physiological responses of prey species to predation risk when responses to stimuli are measured in a metabolic chamber.

Southern brown bandicoots (*Isoodon obesulus*) and brushtail possums did not avoid predator (quoll, dingo, fox and cat) odours in the wild (Chapter 5). A number of factors may have contributed to this lack of an avoidance response for small Australian prey species. For example, trapping success is not considered the most representative way to measure habitat use after predator odour exposures, as trapping only records an immediate response to a potential risk of predation but it does not take into account longer-term behavioural changes (Powell & Banks 2004). It may be useful to conduct studies concentrating on the variation of prey's home range size after exposure to predator scents using radio-tracking of individuals in the wild, without involving trapping techniques.

Another explanation for the results obtained for wild southern brown bandicoots and brushtail possums is that a certain odour intensity may be necessary to achieve a response. For example, different quantities of scent affected fear responses in rats exposed to cat odour, with rats showing stronger reactions when the scent source was larger (Takahashi *et al.* 2005). Therefore, the intensity of predator odours used in the field may have been too weak to provoke avoidance. However, to the human nose, all scents were easily detectable and distinguishable at distance. Future studies should test different odour concentrations to investigate this hypothesis.

It is also important to consider that the populations of bandicoots and possums studied had never faced high predation risk as they were living in a protected reserve. Animals living in predator-free environments have been previously shown to lose anti-predator responses to predator odours in a generation (Blumstein *et al.* 2002), as olfactory cues have no value without predation risk and the cost of avoiding predator scents may be too great for these animals to be maintained. For marsupials, it seems to be necessary to possess some kind of experience with predators before being able to respond to indirect predation cues (Blumstein 2002). Furthermore, studies have shown that prey species usually avoid the scent of evolutionary known predators (see review in Apfelbach *et al.* 2005), while naïve animals often do not (Kavaliers 1990; Dickman 1992; Berger 1998). This study indicates that a potential lack of anti-predator behaviours should be evaluated before planning the release of captive-bred individuals and the reintroduction or the translocation of populations without prior predator experience. However, both southern brown bandicoots (Russell & Banks 2005) and brushtail possums (Bramley & Waas 2001) failed to respond to predator odours even in predator-rich environments, therefore the lack of avoidance of predator odours may indicate that these marsupials do not use olfaction for predator recognition. Further studies should be conducted on populations under regular predation risk in order to build a better understanding of the effect of potential predator odours on these prey.

The intensity to which prey respond to predator odours may be affected by habitat characteristics and structure (Merkens *et al.* 1991; Verdolin 2006). For example, protective cover is normally considered a low risk habitat for small species as it considerably reduces the chances of being detected by predators (Rohner & Krebs 1996). As the response of bandicoots and possums to predator odours in the wild were studied in sheltered areas and, as habitat features can influence the perceived risk of predation by prey, then this may explain why these marsupials did not respond to predator olfactory cues in the field. Future experiments should compare responses of these species in closed and open habitats to understand if their ability to assess predation risk varies with different habitat types.

Finally, the absence of a visual reinforcement may also explain the lack of response observed in the field experiments on bandicoots and possums, as for many prey species visual stimuli represent a more immediate threat (Ydenberg & Dill 1986; Evans *et al.* 1993; Kemble & Bolwahnn 1997). It would be interesting to determine if any other anti-predator strategy is more commonly used by possums and bandicoots for early detection of predators.

Western grey kangaroos (*Macropus fuliginosus*) studied in the wild showed avoidance of both historical (dingo) and short-term introduced (fox) predator odours (Chapter 6). Consistent with other studies, in the presence of predator faecal odours feeding was reduced (Muller-Schwarze 1972; Melchiors & Leslie 1985; Arnould & Signoret 1993; Weldon *et al.* 1993; Englehart & Muller-Schwarze 1995). However, kangaroos were not completely deterred from areas tainted with predator scents, as they did not move away from treated sites unless they closely investigated the odour source. Nevertheless, a clear fear response to fox and dingo odour was observed after assessment of these predator scents, suggesting that the consequent habitat shifts were due to the perceived predation risk.

The first important application of this study was to understand the effects of predators on prey in order to improve the conservation of Australian native animals. Australia has suffered the highest rate of mammal extinction in the world during the past 150 years (Johnson *et al.* 2006), and the decline of Australian fauna has been mainly attributed to introduced predators, such as foxes and cats (Dickman 1996; Short 1998; Johnson 2006). Ineffective anti-predator responses by Australian prey species have been considered responsible for the rapid contraction in range of some marsupials (McLean *et al.* 1996; Short *et al.* 2002; Russell *et al.* 2003). Therefore, a lack of reaction of native prey to introduced predator odours was expected in this study. However, results were inconclusive in this respect. In fact, tammar wallabies reacted more to short-term introduced than to native predators (Chapter 4) and western grey kangaroos did not show differential responses to the odours of historical or novel potential predators (Chapter 6). This is probably because of the heavy predation pressure that macropods have experienced in evolutionary time (Caughley 1964; Kaufmann 1974; Banks 2001), which may have provoked generalised responses to potential predatory threats. It seems that macropods are able to respond to olfactory cues of predation even when these are from novel predators if animals habitually experience predation risk.

In contrast, although brushtail possums responded to the scent of a long-term introduced predator (dingo) in laboratory experiments and not to a short-term introduced one (dog; Chapter 2), they did not show avoidance for any predator (quoll, dingo, fox and cat) odours in the wild (Chapter 5). In the same way, southern brown bandicoots appeared unaffected by predator scents (quoll, dingo, fox and cat; Chapter 5). It may be that predation pressure has not been strong enough to develop avoidance of native predator odours, and as a consequence, they also do not detect and avoid recently introduced predators (Flannery 1997). However, selection should stimulate prey to use sensory cues in order to improve their knowledge of immediate risk of predation (Ramp *et al.* 2005); therefore it is likely that possums and bandicoots employ other anti-predator strategies to prevent predation risk. This results support previous finding on small Australian mammals (Banks 1998) and strengthen the view that these animals are highly susceptible to introduced predators (Burbidge & McKenzie 1989).

The second practical application of this research was the possibility of using predator odours in the field as a repellent to protect crops and to deter herbivores from certain areas. It has been suggested that predator based repellents could successfully be used for non-lethal reduction of damages caused by wildlife (Epple et al. 1995). Although previous studies have shown that predator scents can reduce feeding damage by herbivores for long periods of time (Sullivan & Crump 1984; Sullivan *et al.* 1985b; Sullivan 1986; Swihart *et al.* 1991; Boag & Mlotkiewicz 1994), in my study, responses to predator odours were clearly not sufficient to provide a total avoidance effect (Chapter 5 and 6). Although both feeding suppression and habitat shift occurred during the study on western grey kangaroos, they appeared to be subtle. Kangaroos moved away from predator scents only if a close investigation was performed, and they were not excluded from the entire study area but only from the close proximity of the odours. This is consistent with other findings showing that preferred feeding areas were temporarily avoided but not completely abandoned even in situations of high predation risk (El Hani & Conover 1998; Winnie *et al.* 2006). However, it has been suggested that repellents are most effective if alternative palatable food sources are readily available (Merkens *et al.* 1991; Nolte *et al.* 1993; Milunas *et al.* 1994). In addition, appetite has shown to play a significant role in repellency (Sih 1980; Verdolin 2006), as demonstrated by previous research indicating that hungry animals would not risk starvation to avoid predation threat (Sih 1982; Andelt *et al.* 1992). These results show that the utility of predator odours as an effective management tool to successfully control herbivore pests on a large scale is unlikely.

In conclusion, the intensity to which prey respond to predator odours have shown to depend on several factors. Findings of this study suggest that although a prey may express a strong response to a predator scent in a laboratory study, once the experimental scale is changed to the field, responses to the same predator may disappear. It is therefore fundamental to always examine variation in animal behaviour at a large scale, including concurrent laboratory and field experiments in order to compare results and understand the real non-lethal impacts of predation (Lima 1998). Nevertheless, the laboratory experiments carried out in this study have shown that recording and monitoring ventilatory variables can help to understand animals' immediate physiological awareness of dangers or disturbances and can assist in predicting behavioural changes in the presence of predation risk.

In general, medium-large sized macropods have been shown responses to both native, long-term and introduced predator odours. This olfactory recognition mechanism may be used by these marsupials to reduce their risk of encountering potential predators. Responses to predator odours seem to involve an initial investigation phase, during which the animal assesses the risk, and subsequently may or may not change its behaviour. In fact, free-ranging animals living in an environment rich of predator odours, may choose to avoid predator cues only after a careful assessment or only for a limited period of time. Additional behavioural studies are needed to evaluate the effects of predator odours on small Australian prey, to determine their ability to discriminate and avoid predator cues and to establish if there are particular environmental situations in which olfactory cues may be most effective. Results of this study restrict the use of predator odours as repellents, however emphasise the importance of examining the role of indirect cues in predator-prey relationships to assess new strategies for animal conservation and management.

Table 7.1: Summary of the physiological and behavioural responses of Australian and exotic prey species to the scent of native and introduced predators in the laboratory and in the field. Where - stands for no response, + stands for significant change and N.A. indicates responses which have not been investigated for these predator-prey relationships.

	Laboratory		**Field**
	Metabolic Rate	**Respiratory Rate**	**Habitat use**
Brushtail possum			
dog	—	—	N.A.
fox	N.A.	N.A.	—
cat	N.A.	N.A.	—
quoll	N.A.	N.A.	—
dingo	—	+	—
Rabbit			
python	+	+	N.A.
fox	+	+	N.A.
cat	+	+	N.A.
quoll	+	+	N.A.
Tammar wallaby			
python	—	—	N.A.
fox	—	+	N.A.
cat	—	+	N.A.
quoll	—	—	N.A.
Southern brown bandicoot			
fox	N.A.	N.A.	—
cat	N.A.	N.A.	—
quoll	N.A.	N.A.	—
dingo	N.A.	N.A.	—
Western grey kangaroo			
fox	N.A.	N.A.	+
dingo	N.A.	N.A.	+

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