MINIMISING NATIVE NON-TARGET UPTAKE OF 1080 FOX BAITS

Sinéad Ella Allsop,

BSc

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Declaration

I declare that this thesis is my own account of my research or is appropriately acknowledged, and contains as its main content work, which has not been previously submitted for a degree at any tertiary educational institution.

Sinéad Allsop

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Abstract

Red foxes (*Vulpes vulpes*) are a significant pest species threatening the survival of endangered and vulnerable native Australian fauna. Lethal baiting with the toxin 1080 is currently the most widely implemented form of control. However, non-target uptake is a problem, even in Western Australia where most native species have a high 1080 tolerance. It reduces the number of baits available to foxes and therefore the efficiency and cost-effectiveness of 1080 fox baiting operations. To investigate bait presentation and the use of aversive tastant agents as potential techniques to reduce non-target uptake of 1080 baits, two separate studies were conducted. Study 1 examined different non-toxic meat bait presentations to find the presentation that had the lowest non-target uptake. Baits were laid on the surface, suspended, buried or wrapped in kangaroo hide. Study 2 investigated the effectiveness of potential deterrents in causing aversion both immediately and accumulatively over time. Nine different deterrents (washing-up liquid, citric acid, wasabi, baking powder, bicarbonate soda, salt, sodium saccharin, Bitrex and chilli) were tested for immediate aversion and chilli and Bitrex were tested for accumulative aversion. A cafeteria-style presentation was used, with deterrents applied to apple baits of control, low and high concentrations. For Study 1 and 2 animal activity and bait take were monitored on remote-sensing Reconyx cameras for later photo analysis. The results of Study 1 suggested no significant effect of bait presentation. However, as hypothesised the longevity and non-target uptake was lowest for buried baits. Study 2 suggested that salt, wasabi and chilli created immediate aversion in the most abundant non-target species, quokkas. However, aversion was only carried-over onto untreated apple baits in wasabi and chilli. These findings suggest that bait presentation and the use of aversive tastant agents may have potential to decrease non-target uptake and be employed in future baiting 1080 fox baiting operations.

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1 CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

GENERAL INTRODUCTION

The successful introduction of the red fox (*Vulpes vulpes*) and its subsequent spread over most of Australia has had detrimental impacts on both native wildlife and livestock (Saunders *et al.* 1995; 2010). In 2009 the total estimated annual loss in the economic surplus in Australia, which can be attributed foxes was $21.2 million (Gong *et al.* 2009). While the importance of fox control for livestock should be recognised, fox predation on diverse Australian species is a more significant issue and is, therefore, often the focus for native species conservation (Fennessy 1966; Hone *et al.* 1981; Saunders *et al.* 1995).

Control of foxes first began only 20 years after their introduction to Australia, in 1893, when the first bounty payment was recorded (Rolls 1969), and control techniques such as shooting, poisoning and trapping were all used (Saunders *et al.* 1995). Currently, lethal baiting with the toxin sodium fluoroacetate ('1080') is the most widely recommended technique for fox control (Saunders *et al.* 2010). The compound 1080 is a synthetically manufactured version of sodium fluoroacetate, which occurs naturally in many native Australian plant species (Twigg 1994; Twigg *et al.* 1996b; Twigg *et al.* 1996a; Twigg *et al.* 1999) As a result, many native fauna species, particularly those from Western Australia, have high tolerance to 1080, thereby offering fox-specific control with limited impact on native species (King *et al.* 1981; McIlroy 1986; King and Kinnear 1991).

A major concern with 1080 baiting is that the fate of baits is often unknown (Koertner 2007). This is a significant issue in eastern Australia, as some native non-target species have relatively high risk of poisoning (Koertner 2007). Although the risks to non-target species may not be as significant in Western Australia, non-target uptake decreases the number of baits available to foxes and therefore the overall efficiency and cost-effectiveness of 1080 baiting programs (Algar *et al.* 2007; Moseby *et al.* 2009b; Moseby *et al.* 2011; Dundas *et al.* 2014). Research is therefore required to improve the efficiency of 1080 baiting campaigns by increasing target specificity and decreasing non-target uptake (Glen *et al.* 2007).

The present study investigated methods to reduce non-target uptake and improve current 1080 baiting operations in Western Australia. The first chapter of this thesis presents a literature review on the red fox in Australia examining their introduction, general biology, threats and the control methods to mitigate these. The chapter ends by stating the general aim of the project.

The second chapter presents the study on the effect of bait presentation on non-target uptake. The third chapter presents the study on the immediate and additive effect of deterrents on creating aversion in non-target species. The thesis is concluded in chapter four with a general discussion and recommendations for future research.

1.1 Origin and Introduction into Australia

As a member of the Canidae family, it is suggested that the European red fox evolved in North America during the Eocene (3.50MYA) (Saunders *et al.* 1995). The species occurs naturally across most of the Palaearctic region (Saunders *et al.* 1995). However, the red fox has expanded its range, including human-assisted colonisation of Australia.

The red fox was first introduced into Australia in Victoria in the late 1870s, with the intention of use for recreational hunting with hounds and horses (Rolls 1969; Kinnear *et al.* 1988; Saunders *et al.* 1995). Except for Tasmania, the tropical north and some smaller islands, foxes were found across the majority of habitat types in mainland Australia by the 1930s (Saunders *et al.* 1995). Only 30 years after its initial release in the northern part of Victoria, it was given the status of 'pest' (Kinnear *et al.* 1988; Saunders *et al.* 1995). While the red fox was not recorded in Western Australia until 1912, it spread to all regions of the state, with the exception of the Kimberly region and most islands, within the next 25 years (Kinnear *et al.* 1988). There is strong suggestion in the literature, particularly from Australian fox diet studies, that rabbits (*Lepus curpaeums*) have been an important food source for foxes and thus the spread of rabbits across the continent assisted the colonisation of foxes (Long 1988; Vertebrate Biocontrol Centre 1992; Saunders *et al.* 1995). In Western Australia it has been suggested that foxes followed a very similar invasion path to the rabbit, only a few years after (Long 1988; Saunders *et al.* 1995). Figure 1.1 taken from Saunders *et al.* (1995) illustrates this theory showing the great degree of overlap in rabbit and fox distributions in Australia (Vertebrate Biocontrol Centre 1992).

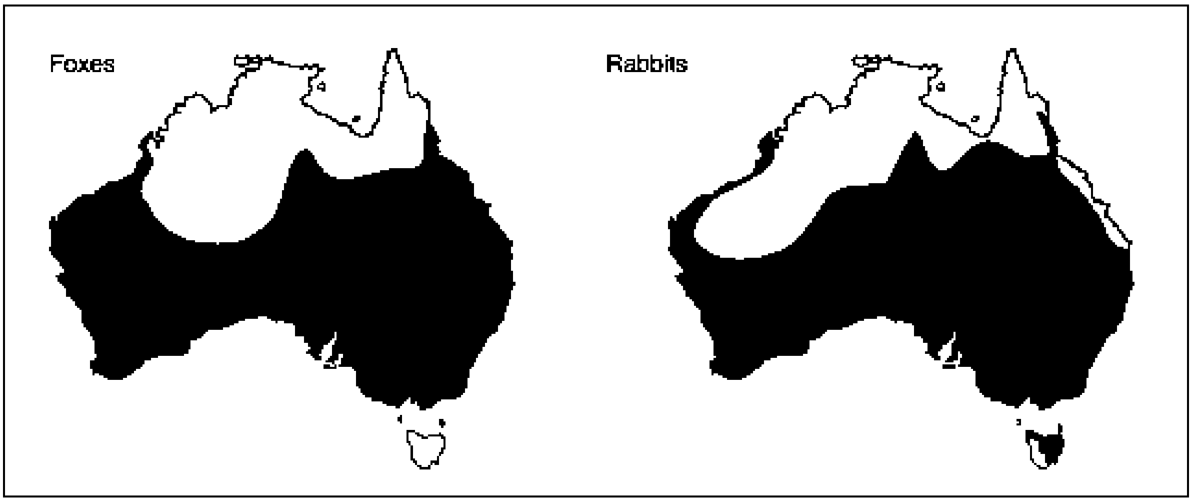

Figure 1.1: The relative distribution of foxes and rabbits in Australia.

1.2 General Biology

In Australia, the red fox can be found in a wide range of habitats including arid, alpine, woodlands and semi-open habitats. However, they are often most common in urban habitats, most likely due to the large range of cover, food and den sites that they provide (Saunders *et al.* 1995). They are absent from some offshore islands and otherwise only seem to be limited by tropical climates and, in some cases, the presence of dingoes (Saunders *et al.* 1995; Reddiex *et al.* 2004). In agricultural areas, foxes are usually abundant due to plentiful cover, food and den sites (Saunders *et al.* 1995).

Foxes are able to occupy and spread into such a wide range of areas due to their high adaptability and rapid reproduction; characteristics common of invasive species (Corbet and Haris 1991). Despite their litters usually being small and females breeding only once per year, survival is high and the majority of adults breed (McIntosh 1963; Ryan 1976; Saunders *et al.* 1995). Another part of this adaptability comes from their wide dietary range. While red foxes are predominantly carnivorous, they are also opportunistic predators and scavengers with no specialised diet requirements (Saunders *et al.* 1995). Their diet can include small-medium sized mammals (Phillips and Catling 1991), birds, reptiles, amphibians, insects, carrion, fruit and human refuse (Saunders *et al.* 1995).

1.3 Impacts of Red Fox

Within their natural home range, urban foxes particularly have become a problem capable of carrying the zoonotic disease rabies, a serious health risk to the general public (Harris *et al.* 1991; Eckert and Deplazes 1999; Jackson *et al.* 2007). Foxes have been implicated to be the most important species for the spread of rabies in Europe (Macdonald 1980; Kaplan *et al.* 1986). In red fox populations in Western Europe and North America, the two main forms of sylvatic rabies are characterised by a high incidence of disease (Wandeler *et al.* 1974). There is also the suggestion that no other wild species would be able to maintain this sylvatic rabies (Saunders *et al.* 1995). The high susceptibility, behaviour and structure of red fox populations enables the disease to spread quickly and be maintained (Saunders *et al.* 1995).

The introduction of rabies is also a major risk in Australia and therefore much research focuses on contingency planning for its potential occurrence (Marks and Bloomfield 1999). However, while rabies has not yet infected foxes in

Australia, the key concern with foxes is the risk they pose as predators on native fauna (Burbidge and McKenzie 1989; Kinnear *et al.* 2002; Jackson *et al.* 2007). The federal Environmental Protection and Biodiversity Act 1999 identifies five species out of the many invasive mammals that have established in Australia as threatening 'the survival or evolutionary development of a native species or ecological community' (Department of the Environment and Heritage 2005). One of these species is the red fox (Reddiex *et al.* 2007). As native species did not co-evolve with foxes, they often have few strategies to avoid their predation, therefore making foxes a serious threat (Finlayson 1961; Saunders *et al.* 1995).

1.3.1 Evidence of red fox impacts

Whilst the significance of their predation on many native species has long been suspected, the emergence of evidence to support this (not simply anecdotal or circumstantial) is relatively recent (Saunders *et al.* 1995). Some of this circumstantial evidence was based on the observation that during and following fox colonisation in Australia, the native mammalian fauna experienced significant changes, specifically with many small-medium sized species becoming rare or extinct and also based on the known predatory behaviour of the red fox. However, there are other factors such as habitat fragmentation and destruction, changes in fire regimes, rabbit plagues, disease and climate change that could have caused these changes (Kinnear *et al.* 1988). For example, while red foxes have been implicated in the reduction or extinction of some native species, at the time of red fox introduction many native species were already in decline, probably due to disease (Abbott 2006; Abbott 2008). To identify the predatory role of the red fox, removal experiments have been conducted in many areas on many endangered or vulnerable native species (Kinnear *et al.* 1988; Saunders *et al.* 1995). For the majority of these, it has been shown that red fox removal leads to a substantial increase in population size and habitat use by the focal native prey species (Saunders *et al.* 1995).

The best examples of the impact of fox predation in Australia often come from Western Australia (Saunders *et al.* 1995). One well-known example focused on rock wallabies (*Petrogale lateralis*), which were once abundant through the southwest of Western Australia (Kinnear *et al.* 1988; Saunders *et al.* 1995). However, in 1979 there were only six known isolated colonies left, all of which were in decline. In the Kinnear *et al.* (1988) study fox numbers were controlled using 1080 baits in two of these colonies, and three of the colonies were left uncontrolled. All of these rock wallaby colonies were assessed before and after fox control. After eight years it was found that the colonies with fox control had increased four to five fold in size, while the others without control were either unchanged or had fluctuated and then decreased. One of the colonies without control declined so dramatically that only one single, barren female remained (Kinnear *et al.* 1988). However, after this study there was a reintroduction into one site as well as the implementation of fox control, which was followed by a successful increase in wallaby population numbers (Kinnear *et al.* 1998).

From the Kinnear *et al.* (1998) study it can be concluded that the fox appears to have altered the range and distribution, as well as contributed to the reduction in population size and extinction, of many native species. It is suggested that foxes have altered the niches of some native species, which are surviving in refugia with exaggerated requirements of a protective shelter and food to be close. It also suggests that if this predation was controlled, the dimensions of the realised niche could be relaxed allowing for greater use of habitat and resources (Kinnear *et al.* 1988).

1.4 Control Methods

Pest control is defined as the efforts of many organisations to mitigate the negative impacts of pests usually through intentional culling (Reddiex *et al.* 2007). It aims to eradicate or maintain population levels at low densities, otherwise known as sustained control (Braysher 1993; Saunders *et al.* 1995; Choquenot *et al.* 1996; Reddiex *et al.* 2007).

1.4.1 Control of rabies in foxes in Europe and North America

In Europe and North America there are two main methods to control rabies. In the past the only option was fox population reduction (Wandeler *et al.* 1988), which involved using traditional control methods such as trapping, shooting, gassing of dens and poisoning. However, currently oral vaccination is often the control practise of choice (Black and Lawson 1970; Baer *et al.* 1971). It is more economically, socially and scientifically acceptable (Saunders *et al.* 1995), and also in North America, and European countries such as France, Germany and Poland, traditional control methods were not effective in reducing the spread of rabies (Linhart 1960; Johnston and Beauregard 1969; Wandeler *et al.* 1974; Toma and Andral 1977; Macdonald 1980). Oral vaccination is commonly distributed in the form of baits (Baer *et al.* 1971) with the aim of lowering the population density of susceptible individuals below a particular threshold. This means that the probability of foxes encountering and infecting non-vaccinated foxes is kept low enough to maintain chain vaccination (Vos 2003). This control method has shown success in West and Central Europe with rabies in foxes almost being eradicated. This means that the vaccination has reached more than 80% of the population (Tischendorf *et al.* 1998; Vos 2003).

1.4.2 Control techniques in Australia

In Australia a variety of techniques to control red foxes are employed (Saunders *et al.* 1995). Bounty schemes were the first recorded control technique (Saunders *et al.* 1995; Saunders *et al.* 2010). They offered financial incentives on proof of destruction of a fox (Rolls 1969; Whitehouse 1977; Lloyd 1980) by presentation of a body part such as a scalp or tail (Saunders *et al.* 2010). However, it has now been recognised that bounty schemes made little to no significant impact on fox control (Saunders *et al.* 2010). Other more common methods that are still used today include shooting, trapping, den fumigation and destruction and exclusive fencing (Saunders *et al.* 1995; DPaW 2013). However, many of these are labour intensive and not practical on large scales (Saunders *et al.* 1995; DPaW 2013). An alternative control technique to lethal control (Saunders and McLeod 2007) is fertility control in the form of immunocontraception (Bradley *et al.* 1996), or chemical fertility control (Marks *et al.* 1996; Marks *et al.* 2001). While there is potential for employment of fertility control in the future, currently more research is needed (Bomford 1990;

Bomford and O'Brien 1992; Saunders *et al.* 1995; Saunders and McLeod 2007; Department of the Environment 2008). Overall, government agencies usually recommend lethal baiting to control fox populations in Australia (Saunders *et al.* 1995; Saunders and McLeod 2007; Saunders *et al.* 2010; DPaW 2013).

1.5 Poison Baiting

1.5.1 Strychnine

Poison has long been considered the most effective technique to control red foxes (Saunders *et al.* 2010). Strychnine, an indole alkaloid derived from seeds from the Southeast Asian plant, *Strychnos nux vomica*, was the first toxin used in poison fox baits (Saunders *et al.* 1995). This toxin prevents normal functioning of muscle tissue through action on the central nervous system. Early symptoms include nervousness, tenseness and a developing stiffness. Stimuli such as a sudden bright light, touch or sound may then trigger violent tetanic spasms, or they may occur spontaneously. These spasms are characterised by the animal finding 'it impossible to stand, and falling rigidly to its side with legs stiff and outstretched, neck and back arched, ears erect and the lips pulled back from the teeth' (Saunders *et al.* 1995, p. 54). When these spasms begin they occur periodically, increasing until they become continuous within about an hour from the start of clinical symptoms. Death results from spasms of the diaphragm and asphyxia (Seawright 1989). Strychnine is considered to be inhumane and not target-specific, including a potential exposure of humans to risk (Saunders *et al.* 1995; Saunders *et al.* 2010). In July 1991, a Working Group of the National Consultative Committee on Animal Welfare recommended that the sale and use of strychnine should be banned in Australia (Department of Primary Industries and Energy 1992; Saunders *et al.* 1995).

1.5.2 Cyanide

Another toxin that has been used for red fox control is sodium or potassium cyanide (Marks and Gigliotti 1996). Cyanide inhibits the oxidative enzyme systems and death results from anoxia (Saunders *et al.* 1995). Symptoms include rapid breathing, an irregular, weak pulse, salivation, muscular twitching and spasms, an unstable stride, coma and eventually death (Seawright 1989). Death is rapid, occurring in a few minutes after most acute intoxications (Jubb 1985) and seldom exceeding 45 minutes even after subacute doses. Individuals that survive to two hours after the onset of clinical symptoms will in most cases recover (Saunders *et al.* 1995). This rapid death after ingestion has led this toxin to be identified as a more humane poison than the commonly used 1080 (Saunders *et al.* 1995; Hooke *et al.* 2006; Gentle *et al.* 2011).

The considerably shorter time between ingestion and death for cyanide compared with 1080 means that carcasses can be found relatively close to bait stations, which is important for carcass retrieval (Busana *et al.* 1998; Marks *et al.* 2004b). Assuming that foxes are not taking multiple baits before the onset of poisoning, carcass retrieval provides an estimation of fox bait uptake and therefore the of the efficiency of baiting operations (Marks and Gigliotti 1996). It is also important for research programs that require carcass samples or estimates of abundance (Algar and Kinnear 1992). Despite the many advantages of cyanide, it is only used by government agencies as a research and management tool, and further research is required before it can be used more broadly in fox control (Saunders *et al.* 1995).

Cyanide's limited use is partly because in powder form is dangerous to operators and non-target species (Saunders *et al.* 2010). However, the use of a mechanical ejector, known as the M-44 ejector, may minimise many of these risks (Connolly 1988). The cyanide powder is ejected directly into the animal's mouth when it pulls on the lure holder with their teeth, activating the spring and hold mechanism (Connnolly and Simmons 1984). M-44 ejectors have shown some success to date (Busana *et al.* 1998; van Polanen Petel *et al.* 2004), and they are currently under consideration for registration to be used as a fox control technique in Australia (Saunders and McLeod 2007). M-44 ejectors are more labour intensive than other fox control techniques (such as spotlight shooting and 1080 baiting), they require use of specialised equipment, and there is still potential to harm to the operator on activation (Gentle *et al.* 2011).

1.5.3 Para-aminopropiophenone

Para-aminopropiophenone ('PAPP') has an antidote, and has had some success as a bait toxin (Marks *et al.* 2004b). PAPP is associated with the clinical condition methaemoglobin (Vandenbelt *et al.* 1944) and is a highly effective and humane toxin that causes a lethal deficiency of oxygen to the brain and cardiac muscles (Marks *et al.* 2004b). A recent study found PAPP to be highly efficient with low susceptibility to non-target species and little effect on the environment

(Humphrys *et al.* 2008). PAPP also showed promising results with the use of M44-ejectors (Marks *et al.* 2004b). While it is not yet available commercially in Australia recent research and developments on its use are extensive (Southwell *et al.* 2013). The many benefits of this toxin suggest that it could potentially be a better option than 1080 in Australia (Savarie *et al.* 1983; Marks *et al.* 2004a; Fisher *et al.* 2005) and much focus is now on convincing land managers of its adoption (Southwell *et al.* 2013).

1.6 Compound '1080'

1.6.1 History of use

Sodium fluoroacetate ('1080') is the current preferred toxin for vertebrate pest control in both agricultural and wildlife management applications in Australia (Peters 1952; McIlroy 1981; Biodiversity Group Environment Australia 1999; Government of Western Australia 2002; Williamson and Bloomfield 2003; Glen *et al.* 2007; Sherley 2007). 1080 was the number designated to the toxin in the USA in the 1940s when it was first being investigated for use in rodenticides (Twigg and King 1991; Eason 2002). In 1944, field trials also began in assessment of its use for predator control for coyotes (*Canis latrans*) (Calver and King 1986). However, today, 1080 use in the USA is restricted to livestock-protection collars to reduce the amount of coyote predation on sheep and cattle (Eason 2002). Since the 1950s, 1080 has also been used for pest control in Australia where it was originally introduced for rabbit control and New Zealand for rabbit and possum control (Calver and King 1986; Eason 2002). 1080 is also used in Mexico and Israel (Eason 2002).

While 1080 is an artificially manufactured toxin, fluoroacetate is a poison agent found in many native plants including species from Brazil (de-Moraes-Moreau *et al.* 1995), South and West Africa (Atzert 1971) and at least 40 species in Australia (Twigg and King 1991; Twigg 1994; Twigg *et al.* 1996b; Twigg *et al.* 1996a; Twigg *et al.* 1999). This naturally-occurring toxin has been shown to be chemically and toxicologically identical to 1080 (Eason 2002). In Australia, these native fluoroacetate bearing species fall into the genera *Acacia*, *Gastrolobium* and *Oxylobium* (Oliver *et al.* 1977; Twigg and King 1991) and are most common throughout southwest Western Australia, but are also found to extend into the Northern Territory and down through the central highlands of Queensland (Everist 1947; McIlroy 1986; Twigg and King 1991). There are no known fluoroacetate-containing plants in South Australia, New South Wales, Victoria and Tasmania (Twigg and King 1991; Saunders and McLeod 2007).

1.6.2 1080 poisoning

The time between 1080 ingestion and beginning of symptoms in mammals is usually between 0.5 to 3 hours (Eason 2002). Individuals that ingest small, sub-lethal doses of 1080 will only have mild symptoms of poisoning and the toxin will be metabolised and excreted within 1 to 4 days, in which time the animal will recover (Egekeze and Oehme 1979; Eason *et al.* 1997). When consumed,1080 is metabolised to form fluoroacetate, which inhibits the action of the enzymes that normally convert citrate to succinate in the tricarboxylic acid (Krebs) cycle, hindering the production of cellular energy (Peters 1952; Fanshier *et al.* 1964; Saunders *et al.* 1995). While the inhibition of these enzymes has been suggested to be the primary cause of 1080 toxicity (Atzert 1971), other cellular enzymes are also impacted after 1080 ingestion (Kun 1982), although their role in toxicoses is still widely unknown (Mehlman 1968; Godoy and del Carmen Villarruel 1974; Taylor *et al.* 1977; Kirsten *et al.* 1978). Despite the exact mode to toxicoses not being understood, there is accumulation of fluorocitrate in the body cells after ingestion of a lethal dose, energy loss and disruption to the central nervous system, and therefore heart functioning. This develops into a lowered activity in the central nervous system, eventually resulting in death from cardiac failure or convulsive respiratory arrest (Saunders *et al.* 1995).

1.6.3 Choice of toxin

1080 is recognised as an odourless, virtually tasteless and water soluble bait toxin to control foxes (Saunders *et al.* 1995). 1080 is low cost, has a high potency and relative ease of application (especially when pre-prepared baits are also used) (McIlroy 1996). 1080 is also generally known to be an efficient, target specific and humane toxin (Government of Western Australia 2002; Williamson and Bloomfield 2003). 1080 does not accumulate in the environment as it naturally degrades forming harmless by-products (Kelly 1965; Bong *et al.* 1979; King *et al.* 1994), its breakdown often assisted by soil microorganisms (Walker and Bong 1981; King *et al.* 1994) or insects (Staples *et al.* 1995) and therefore 1080 baiting operations are unlikely to have any long-term detrimental impacts on invertebrate populations (Sherley *et al.* 1999; Spurr and Drew 1999). 1080 has a good safety profile - there is minimal risk of public 1080 poisoning, as the quantities administered in baits are not near human lethal levels (Jackson *et al.* 2007). Another advantage is that the majority of Australian fauna are less sensitive to 1080 than introduced animals such as the fox (Oliver *et al.* 1977; King *et al.* 1978; McIlroy 1981; McIlroy 1986; Twigg *et al.* 1996a). In general, dogs have an extremely high susceptibility, similar to most other carnivores that are extremely sensitive, while herbivores are less sensitive. Birds, reptiles and fish are also relatively resistant (Atzert 1971; Notman 1989; Eason 2002).

The natural occurrence of fluoroacetate is advantageous for 1080 baiting operations, particularly in Western Australia, where the plants of highest concentrations are most common. Native species have developed higher tolerances to 1080 than the introduced fox and native animals from eastern Australia (King *et al.* 1981; McIlroy 1986; King and Kinnear 1991) reducing non-target risk. It is thought that this has developed through a selective pressure for high tolerance from exposure to fluoroacetate over numerous generations to both the herbivores that are consuming the plants but also the native carnivores and omnivores to avoid secondary poisoning (Glen *et al.* 2007). The low basal metabolic rate of many native Australian species may also contribute to their higher 1080-tolerance compared with non-native species (McIlroy 1984; Twigg 1994).

The sensitivity of some native species to 1080 is important to consider, as the bait is not always taken by the target species. A study in NSW found that 45% of baits were eaten by foxes during the baiting operations (Saunders *et al.* 1997). This meant that the fate of over 50% of baits was unknown, with potential for non-target uptake, the development of bait aversion from sub-lethal doses, and overall loss of cost-effectiveness of the baiting programs (Kay *et al.* 1999). In a more recent study from Western Australia, 99% of camera-monitored fox baits were taken by non-target species; only one bait was taken by a fox despite there being eight separate fox sightings (Dundas *et al.* 2014). Studies have shown the removal of both toxic and non-toxic baits by non-target species including birds (Allen *et al.* 1989), rodents (McIlroy 1982), spotted-tail quolls (tiger quoll; *Dasyurus maculatus*) (Glen and Dickman 2003; Belcher 2004; Murray and Poore 2005) and reptiles (Short *et al.* 1997). Such studies highlight the importance of determining the fate of 1080 baits, particularly identifying the species that is removing them (Koertner 2007).

Non-target uptake is a major problem because, in addition to risking poisoning native fauna, it can significantly reduce the number of baits available to target species, reducing the effectiveness of the baiting program and requiring increased numbers of baits (Algar *et al.* 2007; Algar and Brazell 2008; Moseby *et al.* 2009b; Moseby *et al.* 2011). There is also the issue that in many cases, non-target species do not consume whole baits. If target species then consume these partially-eaten baits, they will receive a sub-lethal toxin dose, which could lead to reduced effectiveness, for example through learned avoidance.

Some native non-target species have been identified as being potentially at risk from 1080 baiting (Glen *et al.* 2007), including the threatened spotted-tail quoll, eastern quoll (*Dasyurus viverrinus*) and brush-tailed phascogale (*Phascogale tapotafa*) (McIlroy 1994). Poisoning of domestic species is also a major concern, especially if baits are moved away from known location bait stations (McIlroy 1986; Jackson *et al.* 2007). Due to their similarity in feeding behaviour to foxes, domestic dogs (*Canis lupus familiaris*) are a particular species at risk of 1080 poisoning (McIlroy 1986; Calver *et al.* 1989; Jackson *et al.* 2007).

Despite the risks that come with 1080 use, the balance of benefits means it is still the toxin of choice (Rolls 1969; Glen *et al.* 2007). Reducing the number of feral predators through baiting will benefit non-target species on the population level, despite the potential poisoning of some individuals (Glen *et al.* 2007). While populations are likely to recover from these potential short-term losses, fox control offers long-term recovery (Glen *et al.* 2007).

The success of fox control is evident as native species population increases across baited areas and in survival of translocated populations. In 1996, the Department of Parks & Wildlife ('DPaW') in Western Australia commenced 'Western Shield', where over 800,000 baits are seasonally aerially deployed over 3.9 million hectares of land they manage for conservation (Department of Conservation and Land Management 1996; Armstrong and Batini 1998; DPaW 2013). Conservation reserves smaller than 20,000 hectares are also ground baited (Department of Conservation and Land Management 1996). As a result of this large-scale 1080 baiting, significant population increases were observed in some native species (Dundas *et al.* 2014), for example, in brush-tailed bettong (*Bettongia penicillata*), Southern brown bandicoot (Quenda; *Isoodon obesulus*), brush-tailed possum (*Trichosurus vuloecula hypoleucus*), chuditch (*Dasyurus geoffroii*) and rock wallaby (*Petrogale lateralis*) (Kinnear *et al.* 1998; Burrows and Christensen 2002; Kinnear *et al.* 2010). Successful reintroductions have also been strongly linked to predator control with their reduction or exclusion increasing the survival of the native species (Short *et al.* 1992; Risbey *et al.* 2000; Moseby *et al.* 2009a; de Tores and Marlow 2012).

While there has been success in 1080 control operations, especially in Western Australia, there is still some unresolved debate about the humaneness of 1080, particularly due to the lag time until death (e.g. Sherley 2007) and the neurological effects of 1080 (Twigg and Parker 2010). However, despite this, in the majority of cases it is still considered conditionally acceptable (Gregory 1996), at least while there is no alternative suitable and effective toxin commercially available (McIlroy *et al.* 1986; McIlroy and Gifford 1992; Sharp and Saunders 2005; Saunders and McLeod 2007; Twigg and Parker 2010). Adding suitable analgesic or anxiolytic drugs to 1080 baits have been proposed as an option to improve welfare standards. However, the cost effectiveness and regulations on this may need investigation (Marks *et al.* 2009). Despite the controversy that comes with its use, lethal baiting with 1080 is currently the most widely available and implemented fox control technique in Australia (Glen *et al.* 2007; Twigg and Parker 2010). Therefore, much research has focused on investigating methods to maximise its effectiveness through improving target specificity and minimising non-target uptake (Glen *et al.* 2007).

1.6.4 Bait choice

The type of bait is one way to improve uptake through improving target specificity to the fox. Meat is the preferred medium for baits with its high palatability to foxes (Kinnear *et al.* 1988). In Australia, there is a wide range of meat baits used, including fowl heads or wings, fresh or dry pieces of meat, offal, lamb tongues, as well as commercially produced baits (Kinnear *et al.* 2002). Mass-produced dried meat baits or Probait sausages are commonly used in Western Australia, while the commercially produced Fox-off bait is the preferred bait in eastern Australia (Saunders *et al.* 2010). Calver *et al.* (1989) found that many non-target species preferred crackle baits to meat baits, which may be because the meat baits used were sundried with a tough crust, while the crackle baits were softer with sharp corners that may have made gnawing easier. The crackle baits could also hold much greater 1080 concentrations per gram making them more of a risk to non-target species. However, bait choice is often not enough to significantly reduce non-target uptake as even in primarily herbivorous species such as the quokka (*Setonix brachyurus*) meat baits offer an accessible and abundant source of protein (Dundas *et al.* 2014).

1.6.5 Number of baits

Minimising the number of baits deployed is another potential method to reduce the amount of non-target uptake (Thomson and Kok 2002). To maximise the efficiency and cost-effectiveness of 1080 baiting operations, it has been recommended that bait delivery must maximise fox uptake while minimising the number of baits used (Thomson and Algar 2000). The use of fewer baits should also minimise potential non-target uptake. Thomson and Algar (2000) recorded uptake of aerially-delivered non-toxic baits at different baiting rates in sites across Western Australia, and showed that bait uptake did not significantly increase at the higher baiting rates (10 bait km^{-2} compared to 5 baits km^{-2}) but the lowest bait uptake by foxes was recorded at the site with only 3.3 baits km^{-2}. This study indicates that reducing the baiting rate below 5 baits per km^{-2} decreases bait uptake by foxes. However, further investigation is required to replicate this study and determine the critical rate for bait rate before uptake by foxes begins to decrease due to reduced probability of bait encounter.

1.6.6 Bait presentations

As well as differences in the type of baits used within Australia, there are also local regulations for bait presentation (Kinnear *et al.* 2002). In eastern Australia, baits need to be buried in shallow depressions of 5-10cm (Saunders and Harris 2000) to reduce non-target uptake (Allen *et al.* 1989). However, in Western Australia and Queensland, where non-target poisoning is unlikely due to higher 1080 tolerances, baits are usually presented on the surface by ground baiting or large areas are baited by aerial deployment (Armstrong and Batini 1998). This however, does not include farmlands in Western Australia where it is recommended that baits are either buried or tethered to reduce non-target uptake, particularly by domestic animals (Department of Agriculture 2007). Buried baits have the lowest non-target uptake (Allen *et al.* 1989; Thomson and Kok 2002; Moseby *et al.* 2011), but also have lower fox takes compared to tethered and untethered baits (Thomson and Kok 2002). While tethering baits decreased the likelihood (Thomson and Kok 2002), burying baits increased the likelihood of fox caching (which is when foxes take and bury baits for later consumption) (Gustavson 1977; Kay *et al.* 1999; Kinnear *et al.* 2002). This reduces the number of baits available to other foxes and if cached baits are later consumed when 1080 has degraded, the fox will ingest a sub-lethal dose (Kay *et al.* 1999). If foxes associate any symptoms of acute poisoning with the bait, they may develop aversion towards baits reducing the number of susceptible foxes and the overall efficiency of control programs (Kay *et al.* 1999; Kinnear *et al.* 2002). Taking this into account, more research is needed to find the optimum bait presentation that makes the most appropriate compromise between increasing target, and reducing non-target, uptake.

While not as commonly used, other bait presentations such as bait suspension and confinement, have also been investigated (e.g. Gustavson *et al.* 1976; Algar and Brazell 2008). Suspending feral cat (*Felis catus*) baits reduced non-target removal, while maintaining target species bait attractiveness and availability (and therefore increasing target specificity) (Algar and Brazell 2008). Bait confinement with the use of animal hide is another suggested bait presentation (e.g. Gustavson *et al.* 1976). It was demonstrated that baits wrapped in sheep hide had significantly increased target (coyote) specificity (Linhart *et al.* 1968; Gustavson *et al.* 1976). These alternative bait presentations should also be investigated for efficiency and use in Australia to increase target specificity and reduce any non-target uptake. However, until more research is done, burying baits is suggested to be the most effective bait presentation in reducing non-target uptake (Allen *et al.* 1989; Belcher 1998; Glen 2001; Körtner *et al.* 2003).

1.6.7 Aversive agents

The addition of aversive tastant agents has been suggested as a way to decrease the attractiveness and deter non-target species from taking 1080 baits intended for foxes (Hone and Mulligan 1982; McIlroy 1994; Glen *et al.* 2007; Dundas *et al.* 2014). These agents are intended to cause species to reject materials such as baits, with their unpleasant gustatory cues including flavours, textures or other taste and associative odour characteristics. They can induce the triggering of either innate aversion or conditioned taste aversion (Riley and Clarke 1977; Conover 1984; Watkins *et al.* 1994; Mason and Clark 1997; Gill *et al.* 1999; Macdonald and Baker 2004; Baker *et al.* 2005). This aversion can be immediate or cumulative developing after continuous exposure (Baker *et al.* 2005). While aversive agents have not been used to deter non-target uptake in fox baiting, they have been used successfully in non-lethal wildlife control to deter browsing activities (e.g. Andelt *et al.* 1994a; Baker *et al.* 2005) and as additives in rodenticides to increase selectivity to the target species (Kaukeinen and Buckle 1992).

Bittering agents and hot sauces are examples of commonly used deterrents (Kaukeinen and Buckle 1992). In one study that looked at both of these deterrents, it was found that while hot sauce at high concentrations was able to effectively deter mule deer (*Odocoileus hemionus*) from browsing, Ani-Spray, a commercial bittering agent containing denatonium benzoate, produced no effect, even at high concentrations (Andelt *et al.* 1994a). However, in another study, pocket gophers (*Geomyidae*) avoided denatonium benzoate at high concentrations (El Hani *et al.* 1998b) showing that species differ in sensitivity and perception of these deterrents, and thus their use needs to be species-specific (Norman *et al.* 1992). Finally, if deterrents are to be successfully used to deter non-target uptake of fox baits, it would have to be ensured that only the non-target species and not the foxes were exposed or that the foxes did not have as high sensitivity as the non-target species being deterred by these agents (Dundas *et al.* 2014). For example, one study tested the use of 'hot pepper' extracts (capsaicin) as an additive to rodenticides and found that the treated pellets were significantly less accepted than the untreated (Jones-Smith 1990; Kaukeinen and Buckle 1992). In this case the aversive tastant agent would be a disadvantage rather than an advantage.

1.7 Summary

In Australia the introduced red fox is recognised for its high threat to the survival of many native species (Marlow 1958; Finlayson 1961; Priddel 1989; Friend 1990; Short and Milkovits 1990; Kinnear *et al.* 2002; Saunders and McLeod 2007). Although there is a wide range of control techniques that have been employed to reduce this threat, lethal baiting with the toxin 1080 is considered to be the most effective (Saunders *et al.* 1995); however, non-target uptake of 1080 baits is a problem, risking poisoning of non-target species and reducing the availability of baits to foxes (Algar *et al.* 2007; Moseby *et al.* 2009b; Moseby *et al.* 2011). Various methods to minimise non-target uptake (Saunders *et al.* 1995) were also discussed, including bait presentation with burying baits suggested to be the most effective presentation (Allen *et al.* 1989; Saunders *et al.* 1995; Belcher 1998; Glen 2001; Körtner *et al.* 2003). Aversive agents have also been suggested, as a way to deter non-target species from taking fox baits, however, this technique has not been explored in previous research (Hone and Mulligan 1982; McIlroy 1994; Glen *et al.* 2007; Dundas *et al.* 2014).

1.8 Overall Aim

The aims of this investigation are to improve the effectiveness of current 1080 fox baiting regimes by minimising native non-target uptake. Specifically:

1. To investigate the bait presentation which minimises non-target uptake; and
2. To investigate tastant agents that will deter native non-target species from consuming otherwise highly palatable baits.

2 CHAPTER 2: STUDY 1: OPTIMUM BAIT PRESENTATION TO MINIMISE NON-TARGET UPTAKE

2.1 Introduction

While usually the fate of 1080 fox baits is unknown (Koertner 2007), in a recent study where it was measured, non-target species accounted for 99% of bait uptake (Dundas *et al.* 2014). Therefore, even in Western Australia where native species have a reduced 1080 poisoning risk, non-target uptake needs to be reduced for the efficiency of 1080 fox baiting programs (Algar *et al.* 2007; Moseby *et al.* 2009b; Moseby *et al.* 2011). Bait presentation has been explored in previous research and has shown to have an effect on non-target uptake (e.g. Thomson and Kok 2002). The research objective for the current study was to investigate the effect of four different presentations (surface-laid, buried, wrapped and suspended) on the persistence of baits in the environment without non-target uptake. As the majority of previous studies have shown buried baits to have the lowest non-target uptake (Allen *et al.* 1989; Thomson and Kok 2002; Moseby *et al.* 2011), it was hypothesised this also to be the case in this study. However, currently there no previous research has explored a comparison of all of these presentation types.

2.2 Methods and Materials

2.2.1 Study sites

This study was conducted at eleven sites (n = 4 for Study 1, n = 3 for Study 2a, and n = 4 for Study 2b) in the State Government-managed (Department of Parks and Wildlife; DPaW) northern jarrah forest, situated southeast of Perth, Western Australia. All sites comprise *Agonis* swamp scrublands and are located along the upper reaches of creek systems (Hayward 2002) within areas of known quokka mainland populations (Sinclair 1998; Hayward 2002; Dundas *et al.* 2014). The majority of these sites are also known to be ground-baited on a monthly basis predominately to protect these quokka populations (Dundas *et al.* 2014).

Study 1 investigated the most effective bait presentation for minimising non-target native species bait uptake. This study was conducted over three months (20 June to 31 August 2014) at four sites near Collie (Table 2.1 and Figure 2.1).

Table 2.1: Study 1 site descriptions

Site	Access	Latitude and Longitude
Gervasse	Off Windy Ridge Road	S33° 21.507' E115° 55.393'
Hadfield	Off Mornington Road where it crosses the power line easement	S33° 10.513' E115° 58.410'
Hamilton	Off Mornington Road North	S33° 15.198' E115° 49.756'
Victor	Off Victor Road	S33° 15.604' E116° 01.096'

Figure 2.1: Study 1 map of study sites taken from Google Earth (2014).

The last site, Victor is the only unbaited site (Hayward 2002). The number of baits laid was not equal for each site (*n* = 10 at Hadfield, *n* = 7 at Hamilton, *n* = 5 at Gervasse, and *n* = 7 at Victor), partly due to difference in the ease of accessibility. One reason for this was that there was 296.2mm of rain in Collie (BOM 2014) during the time this experiment was conducted, making some of the off-road tracks to dangerous to drive on.

2.2.2 Experimental design

Non-toxic kangaroo meat baits weighing approximately 100g were deployed at the four Collie sites. Four bait presentations were tested:

- **Surface-laid** baits placed on a cleared patch of ground.
- **Buried** baits placed approximately 5cm below ground following current guidelines (Saunders and Harris 2000; Department of Agriculture 2007).
- **Suspended** baits were attached by fishing line to hang approximately 60cm off the ground from a piece of structural steel. This was hammered into the ground and affixed to a fence post to increase stability (Figure 2.2). This suspension design was modelled on methods used by Harrison-White (2006) for predator baiting in South Africa and Algar and Brazell (2008) for feral cats.

Figure 2.2: Suspended bait.

- **Wrapped** baits were wrapped in pieces of kangaroo hide large enough to completely cover the baits then secured with piece of cotton thread. An example can be seen in Figure 2.3. However, in this image the kangaroo hide is secured with a grass tree leaf rather than cotton. This approach was similar to methods employed in past studies that wrapped coyote baits in sheep hide (Linhart *et al.* 1968; Gustavson *et al.* 1976).

Figure 2.3: Wrapped bait.

Baits were deployed in riparian vegetation approximately 20-30m from unsealed roads where highest animal activity was likely and where baits are usually deployed in current ground-baiting operations (Department of Conservation and Land Management 1996; Hayward 2002; DPaW 2013; Dundas *et al.* 2014). The order of the four bait presentations was alternated and followed current regulations of required distance, being placed a minimum of 200m from any other bait (Department of Conservation and Land Management 1996; Dundas *et al.* 2014). The position of each bait station was marked on the side of the road with marker tape and the GPS coordinates were also taken for ease of recovery.

Animal activity was monitored for each bait using IR remote cameras (Reconyx HC500 HyperFire™ Semi-Covert; Reconyx Inc. Wisconsin USA) set to take five photos per trigger and attached to adjacent trees. At all bait stations vegetation was cleared or trimmed so as not to obscure the camera view. The sites were checked four times over a period of three months to monitor survival of baits. If the bait had already been taken, the camera was removed and redeployed at a new bait station. Each bait was monitored for a maximum of 30 days.

2.2.3 Photo analysis

Over the duration of Study 1, 9 982 images were produced and manually viewed as jpeg files using Windows Live™ Photo Gallery (Version 2009).

On analysis, recorded were: the species, date and time of camera trigger, activity around bait (walking past camera, investigating bait, consuming part of bait, consuming entire bait, carrying bait away), whether the bait was present or absent at time of visit and the total time spent at bait station. Separate individual records were defined if members of the same species that were sighted at least 30 minutes apart.

2.2.4 Statistical analysis

Statistical analyses were conducted using SPSS Statistics (2014) and Statistica 9 (2012).

A Kaplan-Meier survival analysis was conducted to compare the persistence of four different bait presentations (buried, wrapped, surface-laid and suspended). Following this, a log rank test was performed to determine if the differences in the survival distribution for the four different types of bait presentation were statistically significant.

χ^2 goodness-of-fit tests were run across all four presentation types (buried, wrapped, surface-laid and suspended) to find if species were taking baits relative to their abundance. They were conducted for the raven (*Corvus coronoides)*, quokka, mardo (Yellow-footed antechinus; *Antechinus flavipes*), Western grey kangaroo (*Macropus fuliginosus*) and Southern brown bandicoot. However, the χ^2 goodness-of-fit test could not be done for other species sighted (the western brush wallaby (*Macropus irma*), common ringtail possum (*Pseudocheirus peregrinus*), feral cat, emu (*Dromaius novaehollandiae*), echidna (*Tachyglossidae*) and common brushtail possum (*Trichosurus vulpecula*)), as their expected frequencies were less than five.

As data were non-normally distributed and not robust with a different number of baits presented per site, a non-parametric Kruskal-Wallis *H* test was conducted to determine if there was a significant difference in bait persistence between the sites. To determine where the differences occurred, Mann-Whitney *U* tests were conducted, and in recognition of multiple analyses, it was calculated that Bonferroni adjusted alpha levels of .0125 per test should be employed.

2.3 Results

2.3.1 Bait survival in different presentations

The cumulative survival for baits of each presentation type (buried, wrapped, surface-laid and suspended) over time (in days) is shown in Figure 2.4. Buried baits appeared to have a small advantage on survival (i.e. not being taken by non-target species) than other bait presentations. Following this, wrapped baits appeared to have a general survival advantage over surface-laid and suspended baits, and suspended over surface-laid. However, due to substantial crossovers in the survival curves this interpretation cannot be strongly supported. The survival distributions for the four bait presentations were not statistically significantly different (log rank test: $\chi^2{}_3 = 3.428$, $p = .330$).

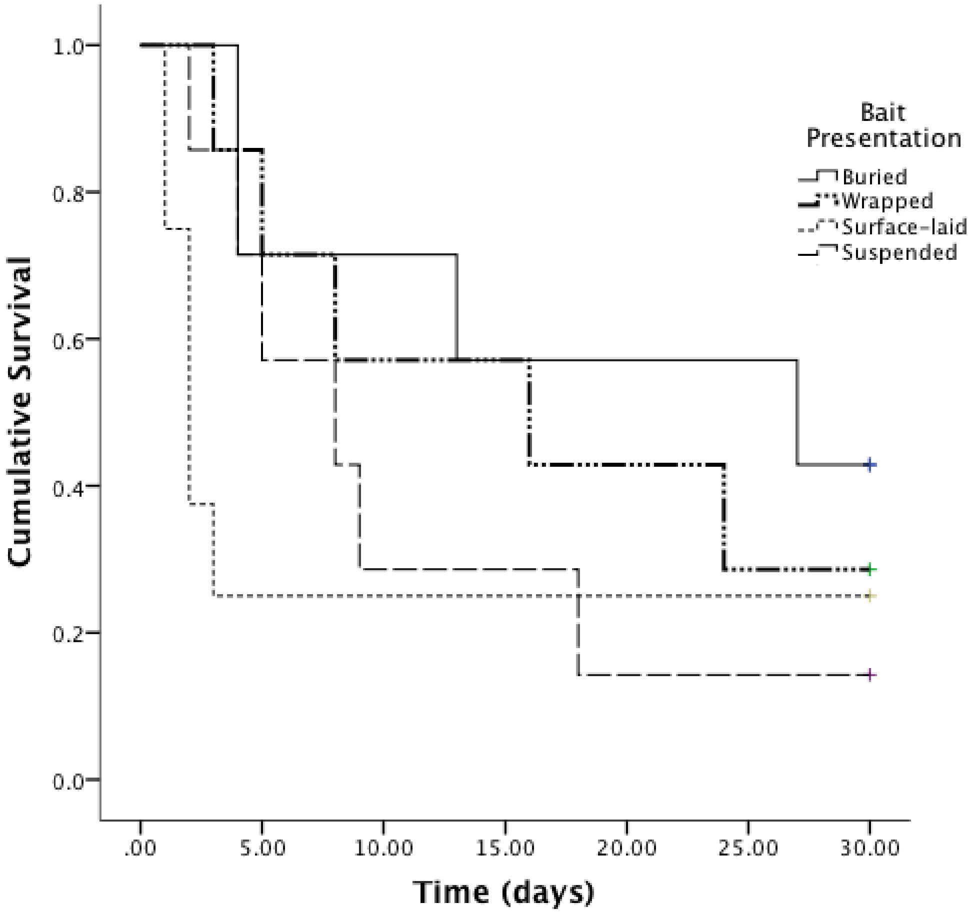

Figure 2.4: Kaplan-Meier survival curve for baits of each presentation type.

2.3.2 Identification of species taking baits

Ravens were the species that took the highest proportion of baits overall (Figure 2.5). They also took the highest proportion of suspended, wrapped and buried baits compared to other species, but took less surface-laid baits. Bandicoots and kangaroos were the second highest species to take baits, with bandicoots taking all baits except suspended, and kangaroos taking all baits except wrapped. Quokkas only took surface-laid and wrapped baits. The only other species to take baits were the common brushtail possum and western brush wallaby, which each only took one bait (a suspended and surface-laid bait respectively).

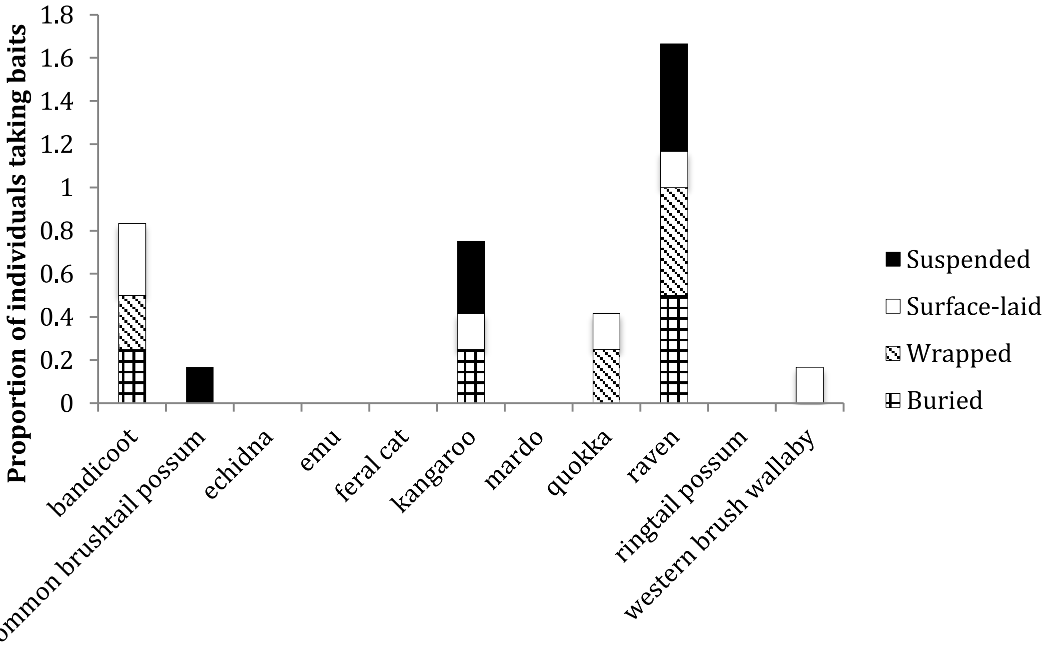

Figure 2.5: Proportion of individual animals taking baits as a function of species and presentation type.

For all the species, which had large enough sample sizes to be tested, significant differences were found between the proportion of species taking baits and their relative abundance (estimated by the number of individuals of each species sighted on camera when bait stations were empty) (Figure 2.6). Ravens, kangaroos and bandicoots were taking baits more than expected, while quokkas and mardos were taking baits less than expected.

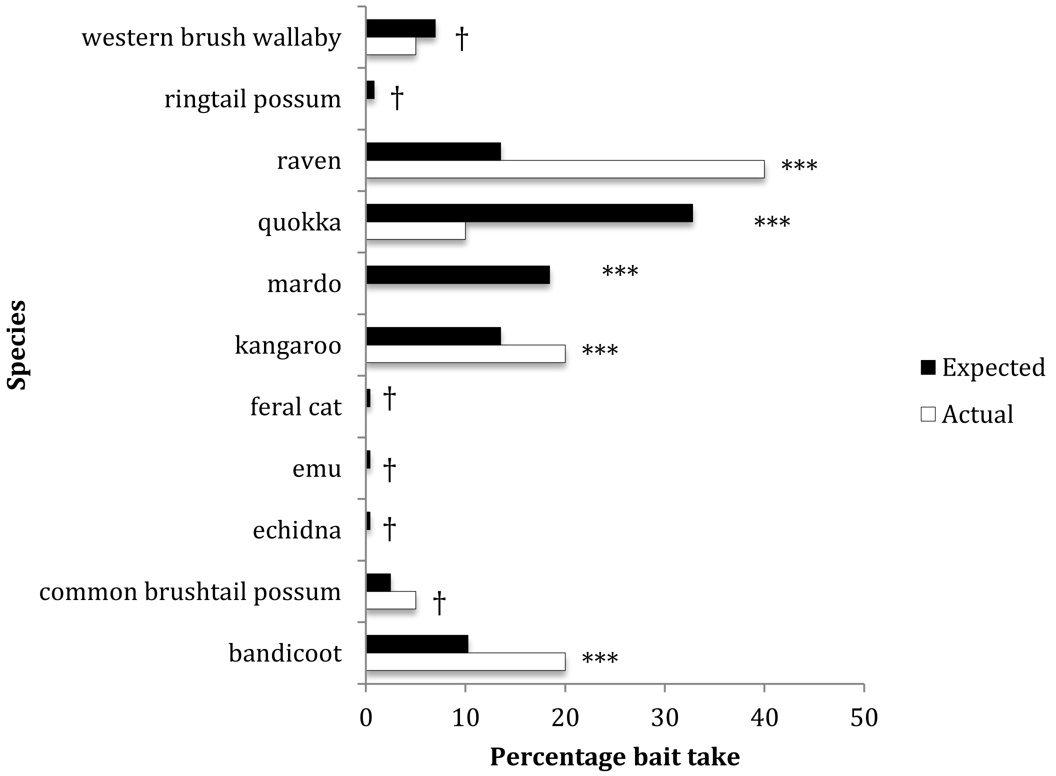

Figure 2.6: Percentage of bait uptake events compared to the relative abundance of each species.

Asterisk indicates a significant difference (χ^2 analysis) between the percentage of expected and actual bait take of each species sighted at bait stations at the four sites, Hadfield, Victor, Gervasse and Hamilton († not tested due to low sample size, *** *p*<0.001).

2.3.3 Effect of site

Figure 2.7 illustrates the results of bait presentation trials (Study 1) showing the median number of days till baits were taken at the four sites (Hadfield, Victor, Gervasse and Hamilton). Boxplots show the median values (solid horizontal lines), 50th percentile values (box outlines), 90th percentile values (box whiskers), outliers (data points more than 1.5 box-lengths from the edge of their box) (open circles), and extreme outliers (data points more than 3 box-lengths away from the edge of their box) (asterisks). Difference in letters between sites indicates significant difference (Mann-Whitney *U* test) between the median number of days till baits were taken at each site.

The median number of days till baits were taken was not significantly different for Hadfield, Victor or Gervasse. However, the number of days until bait uptake at Hadfield and Victor were significantly different to Hamilton, while Hamilton and Gervasse were not significantly different. Overall bait persistence was lowest for Hadfield and baits persisted for longest at Hamilton.

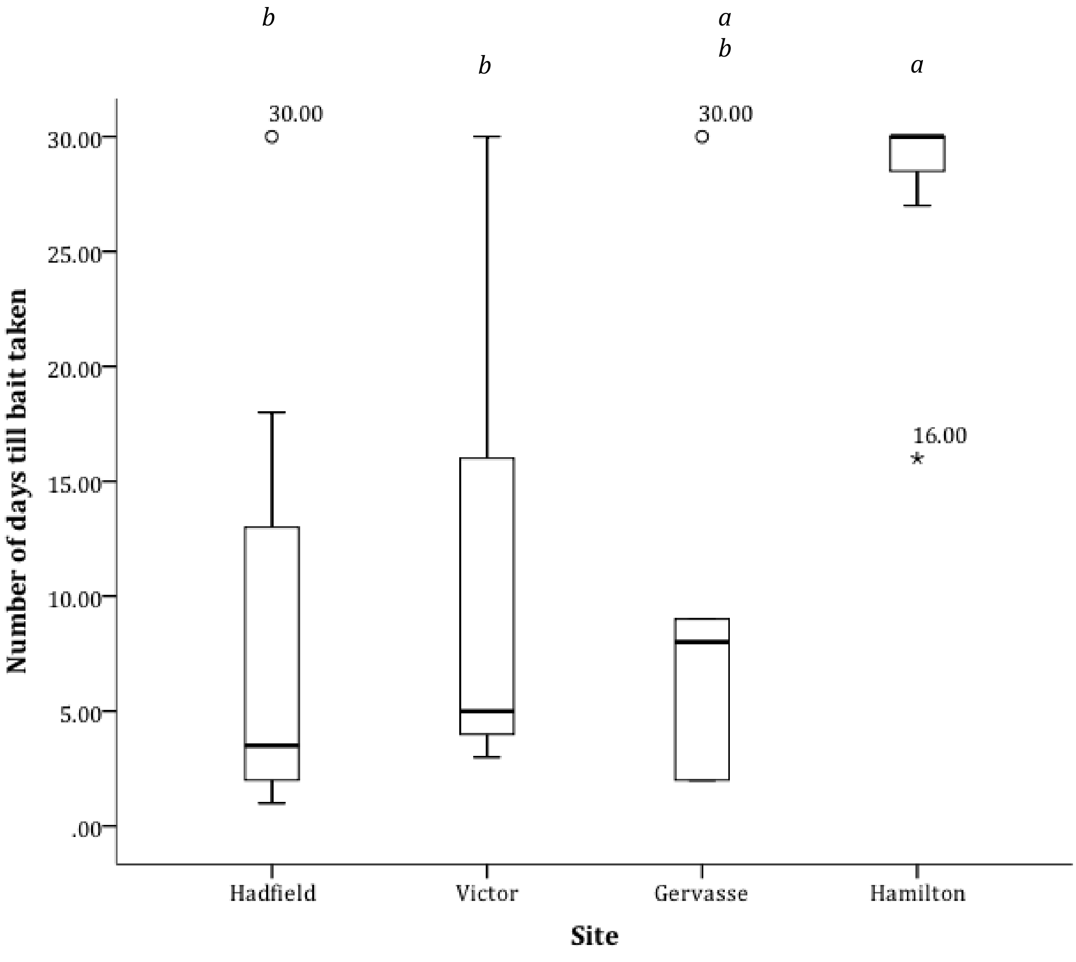

Figure 2.7: Effect of site on bait uptake (Study 1).

2.4 Discussion

2.4.1 Bait persistence

Findings of Study 1 suggested that there was no statistically significant difference in bait longevity between the four bait presentations (surface-laid, buried, suspended and wrapped). However, visually, differences were identified. Buried baits had the lowest non-target uptake, followed by wrapped, suspended and surface-laid baits. Limited statistical power may have obscured any association, with only n = 7 buried, n = 7 suspended, n = 7 wrapped baits, and n = 8 surface-laid baits. To overcome this limitation, future studies should have many more replicates and ensure they are of equal numbers for each presentation. While the finding that buried baits had marginally lower non-target uptake, should be treated with caution, it was consistent with previous research (Allen *et al.* 1989; Thomson and Kok 2002; Moseby *et al.* 2011).

2.4.2 Target (fox) uptake

Bait uptake by the target species (foxes) also needs to be considered. One reason for this is that while the current study suggested buried baits to be the optimum bait presentation to reduce non-target uptake, in previous studies burying of baits increased fox caching behaviour and decreased fox bait uptake (e.g. Thomson and Kok 2002). However, since no foxes took any baits, or were even sighted passing bait stations in the present study, no conclusions can be made about the optimum bait presentation for the target species or the presentation that makes the best compromise for increasing target and minimising non-target uptake.

2.4.3 Non-target uptake

Although surface-laid baits had the highest non-target uptake in this study, it is usually suggested that such presentation maximises fox bait uptake (e.g. Armstrong and Batini 1998; Thomson and Kok 2002). One reason for this could be that surface-laid baits produce a more intense olfactory and visual cue, making them more likely to be encountered (Henry 1986; Thomson and Kok 2002). Wrapping baits may provide a solution to this issue and warrant future investigation, still offering the benefits of surface-laid baits but in a more target-specific way. Wrapping baits in kangaroo hide, while still accessible, may not be as attractive or palatable to non-target species (e.g. Gustavson *et al.* 1976), many of which are usually herbivorous (e.g. Dundas *et al.* 2014). This may have contributed to the difference in expected versus actual bait-take for some species. For example, kangaroos took baits from all presentation types except wrapped (e.g. Figure 2.8), and over all species, wrapped baits persisted the second longest time in the environment. To futher investigate the findings of this study it should be repeated, but also measuring target bait uptake by foxes, perhaps in areas where they are more abundant.

Figure 2.8: Image of Western grey kangaroos investigating a wrapped bait.

While wrapped baits were accessible to kangaroos, none were taken. This is likely to be because they were not attractive or palatable in this presentation.

2.4.4 Which species took the baits?

The study sites were chosen based on non-target abundance (Hayward 2002; Dundas *et al.* 2014). However, in this study, no non-target species were taking baits relative to their estimated abundance (estimated from the number of camera sightings in empty bait stations). This was inconsistent with the results of Dundas *et al.* (2014) in which all species, except for feral pigs (*Sus scrofa*), were taking baits in proportion to their activity index. Baits in the Dundas *et al.* (2014) study, however, were all surface-laid, perhaps optimising their availability to non-target species (Allen *et al.* 1989; Thomson and Kok 2002; Moseby *et al.* 2011). On the other hand, in the current study suspension and burying of baits may have limited bait accessibility (e.g. Figure 2.9) (Allen *et al.* 1989; Thomson and Kok 2002; Algar and Brazell 2008; Moseby *et al.* 2011). For example, quokkas only took 10% of baits (none of which were suspended or buried) in the present study but made up 33% of sighting records on camera. Dundas *et al.* (2014) found that quokkas took 48% of baits, which was not significantly different from their calculated activity index (50% of visits to bait stations). Overall, the difference in non-target uptake between the current and the Dundas *et al.* (2014) study could support a conclusion that bait presentation does have an impact on non-target uptake.

Figure 2.9: Image of a quokka unable to reach a suspended bait.

While quokkas were recorded at suspended bait stations, none were taken. This is likely to be due to bait suspension limiting accessibility.

In this study, ravens accounted for most bait uptake, removing 40% of baits taken. This is consistent with some previous studies, which found birds to be the most significant non-target bait consumers (Allen *et al.* 1989; Thomson and Kok 2002). High bait take by birds may have various implications. Firstly, as with all non-target uptake, it reduces the number of baits available to foxes and therefore reduces the overall efficiency and cost-effectiveness of control programs.

Secondly, birds have been observed to drop more than half of the baits they take (55%) up to 400m away from baiting stations, either whole or partially eaten (Thomson and Kok 2002). While the fate of baits after they were taken was not monitored in the current study, there are significant potential consequences. Similar to the risks of fox caching of baits, baits moved by birds are a risk to non-target species such as domestic dogs and vulnerable native fauna (Thomson and Kok 2002). It also increases the chance of sub-lethal poisoning if baits are partially eaten when they are dropped, which has potential to lead to 1080 aversion in foxes (Kay *et al.* 1999; Kinnear *et al.* 2002; Thomson and Kok 2002). The increased risks that may come with bait take by birds, specifically, highlight the need to consider bait uptake at a species level, as well as just target versus non-target.

Identification of species can allow for specific precautions, such as bait presentation, to be implemented to reduce their uptake. In the present study, bait presentation did not seem to have an effect on the raven bait uptake as they took a relatively similar number from each presentation (n = 2 buried and wrapped baits, n = 1 surface-laid bait and n = 3 suspended baits). This result could be explained by overall limited sample size. Nonetheless, the findings were not consistent with previous studies that suggested bait presentation to significantly impact on the uptake of birds. For example, Thomson and Kok (2002) found that birds mostly took untethered baits (88%), followed by tethered (33%), then buried (17%). However, they were also least likely to consume untethered baits and most likely to consume tethered baits. One suggested reason for this is that the tether decreases the difficultly of shredding the tough, dried meat baits as they are held in place. Tethered baits are therefore recommended by the authors to reduce the likelihood of bait movement by birds and risks to domestic and vulnerable non-target fauna (Thomson and Kok 2002). Thus, as previous studies have shown that the optimum bait presentation is also species-specific (and in the case of birds coming with other potential associative risks), further compromises may have to be made to find the presentation that reduces non-target uptake overall.

2.4.5 Alternative explanation of findings

Overall, it is important to remember that this study did not produce statistically significant results. Another potential interpretation of the non-significant findings could be that bait presentation simply does not have an effect on non-target uptake, although findings from previous studies (e.g. Thomson and Kok 2002) suggest this is unlikely and differences are more likely due to limited statistical power. This study therefore sets a good foundation to investigate the theory that bait presentation has a significant effect on non-target uptake if followed with a larger study perhaps over multiple seasons and with additional sites and replicates.

2.4.6 Conclusion

Study 1 revealed non-target bait uptake across all presentations, with buried baits persisting longest in the environment. These findings, and their consistency with other reports, support the theory that burying baits reduces their accessibility to many species (Allen *et al.* 1989; Thomson and Kok 2002; Moseby *et al.* 2011). Future research needs to focus on identifying which species are taking the baits to better target bait presentation, which in the case of birds, could also contribute to minimising some associated risks that come with their bait uptake. Reducing non-target uptake could assist in minimising the number of accidental poisonings of non-target species, and also in maximising the number of baits available to the target species.

It was not possible to determine bait availability to target species due to limited abundance of foxes in the target sites. Future research investigating the impact of different bait presentations on target uptake is required, so that the appropriate compromise can be made between minimising non-target and maximising target uptake. This would optimise both the efficiency and cost-effectiveness of 1080 fox control baiting operations.

3 CHAPTER 3: STUDY 2: IMMEDIATE AND ADDITIVE EFFECT OF DETERRENTS ON AVERSION

3.1 Introduction

The high level of non-target uptake (e.g. Dundas *et al.* 2014) is reducing the cost-effectiveness of 1080 fox baiting operations (Kay *et al.* 1999). Bait type (e.g. Calver *et al.* 1989) and presentation (e.g. Thomson and Kok 2002) and their effect on non-target uptake have been explored in previous research. However, no studies have investigated the use of aversive tastant agents to deter non-target species from taking fox baits. Aversive agents have been shown to deter unwanted browsing activities (e.g. Andelt *et al.* 1994a; Baker *et al.* 2005). They are usually successful at higher concentrations, as has been shown with two deterrents, hot sauce (Andelt *et al.* 1994a), and the bitter compound, denatonium benzoate (El Hani *et al.* 1998b). As a result, it was hypothesised that aversive tastant agents of the highest concentration would deter non-target species.

3.2 Methods and materials

3.2.1 Study sites

Study 2 investigated potential deterrents and their ability to cause aversive behaviour. This study was conducted over a total of 19 days from August 8 to 27 2014 in seven sites near Jarrahdale (Table 3.1, Figure 3.1 and Figure 3.2). Study 2a tested for immediate aversion of nine different deterrents over 19 days. Study 2b tested for accumulative aversion to chilli for a total of nine days from August 10 to 19 and to Bitrex for a total of nine days from August 18 to 27. Three replicates were set up within each site with each feeding station being placed a minimum of 20 to 30m apart.

Table 3.1: Study 2 site descriptions

		Site	Access	Latitude and Longitude
Study 2	a. Immediate aversion	Midgegooroo	Off Canning Dam Road	S32° 10.955' E116° 06.585’
		Mile	Close to Canning Dam Road off Albany Highway	S32° 13.002' E116° 08.029’
		Thirty-One Mile	Off Albany Highway	S32° 15.843' E116° 10.435’
	b. Additive aversion	Balmoral	Off Balmoral Road	S32° 20.419' E116° 04.897’
		Chandler	Off Jarrahdale Road	S32° 17.871' E116° 08.012’
		Bee Farm	Off Bee Farm Road accessed from Chandler Road	S32° 16.435' E116° 08.659’
		Rosella	Off Nettleton Road	S32° 16.028' E116° 04.809’

Figure 3.1: Study 2a map of study sites taken from Google Earth (2014).

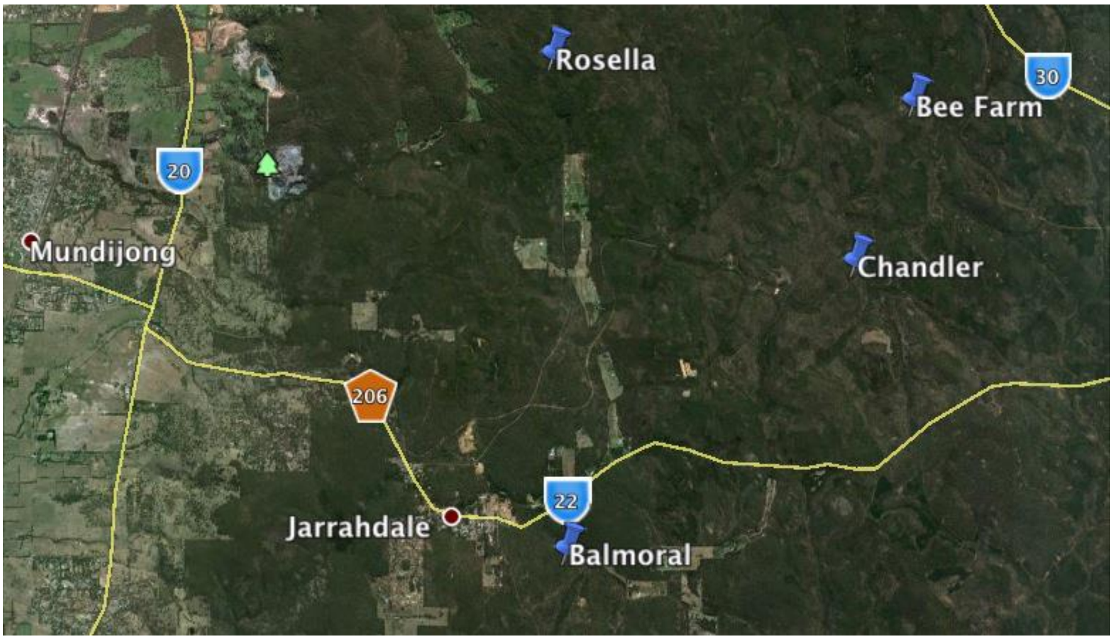

Figure 3.2 Study 2b map of study sites taken from Google Earth (2014).

3.2.2 Experimental design for Study 2a: Immediate deterrent aversion

To test the effectiveness of nine potential deterrents (washing-up liquid, citric acid, wasabi, baking powder, bicarbonate soda, salt, sodium saccharin, Bitrex and chilli) in causing aversion in non-target species, apples of three treatment groups differing in deterrent concentrations were set up in a cafeteria-style presentation (e.g. Baker *et al.* 2005) (Figure 3.3). Apples were cut into eight pieces and eight pieces were used for each of three treatments (control, low and high concentrations of deterrents). From this point on each apple piece will be referred to as an apple, as each piece was accounted separately as bait. For each experimental group the apples for each of the three treatments were dyed a different colour (red, green or blue) using natural food dye to allow monitoring of the fate of apples for each treatment. The treatment-colour combinations were kept constant for the three replicates within each site (Table 3.2), but were varied between the sites to ensure that there was no natural attraction/aversion to a particular food colour. On the 'untreated' days when no compound was added to the apples, they were still dyed using these three colours.

Table 3.2: Treatment-colour combinations for each site (Study 2a).

Site	Control	Low concentration	High concentration
Midgegooroo	Blue	Green	Red
Mile	Green	Red	Blue
Thirty-One Mile	Red	Blue	Green

At each site, the apples were placed in three piles approximately 20 cm apart, skin facing up to reduce the chance of the deterrent getting washed off in the event of rain (Figure 3.3). For consistency, low deterrent concentration apples were placed on the left of the camera, control apples in the middle and high deterrent concentration apples on the right. IR remote cameras were attached to trees approximately 0.75m from the ground, with a field of view that included each feeding station and its surrounds. The apples were placed approximately 1.5m away from the camera, within its detection zone. At all feeding stations vegetation was cleared or trimmed so as not to obscure the camera view.

Figure 3.3: Cafeteria-style presentation of apple baits.

For all the high condition apples on treatment days all the different deterrents were painted on (if liquid or paste) to cover every surface of the apple. However, if they were in powder form they were put in a zip lock bag and with one apple at a time the bag was shaken until the powder covered all surfaces of the apple. All the low condition compounds were diluted with water so that they were 87.5% deterrent. All then were either liquid or paste form and were painted onto apples. This excluded wasabi, chilli and Bitrex. 'Mild' wasabi paste was used for the low concentration (27.1% wasabi) and 'hot' wasabi paste (58% wasabi) was used for the high concentration. For high concentration chilli an 80% reaper chilli hot sauce was used and the same hot sauce was diluted to 40% for the low concentration. Bitrex (98%) was made up to 0.05% for low and 0.1% for high concentration, based on concentrations use by El Hani *et al.* (1998b). Control apples were painted with water.

Study 2a began with all untreated apples (day 1) followed by alternating treatment and untreated days until all nine deterrents had been tested (finishing with an untreated day), making a total of 19 days for Study 2a. The trial order of compounds is indicated in Table 3.3. Feeding stations were checked daily and the number of apples taken from each group recorded. All uneaten apples were collected after each day and replaced with fresh apples until the end of the study period. Camera batteries were also checked and replaced as required.

Table 3.3: List of potential deterrents tested for Study 2.

Trial order	Compound	Brand name	Preparation	Low concentration	High concentration
1	Washing-up liquid	Original Fresh		87.5%	100%
2	Citric acid			87.5%	100%
3	Wasabi			27.1%	58%
4	Baking powder			87.5%	100%
5	Bicarbonate soda			87.5%	100%
6	Salt			87.5%	100%
7	Sodium saccharin			87.5%	100%
8	*Denatonium benzoate	Bitrex	98%	0.05%	0.1%
9	*Chilli		80% Reaper chillies	40%	80%

* compounds also used for Study 2b – to test accumulative effects of the compound on bait take by native species.

3.2.3 Experimental design for Study 2b: Additive deterrent aversion

Study 2b tested whether aversion was an additive effect for chilli and Bitrex. The study focused on chilli and Bitrex as they had shown some successful deterrence in previous studies (e.g. Kaukeinen and Buckle 1992; Andelt *et al.* 1994b; El Hani *et al.* 1998a). The feeding stations were set up in the same cafeteria-style presentation as for Study 2a, with control, low and high concentration treatments. Chilli was tested at two sites and Bitrex at two sites, with three replicates at each site. These feeding stations were set up for a total of nine days, with untreated apples presented on day 1, treated apples presented over days 2-8, and untreated apples again presented on day 9.

Table 3.4: Treatment-colour combinations for each site (Study 2b).

Site	Control	Low concentration	High concentration
Balmoral	Green	Red	Blue
Chandler	Blue	Green	Red
Bee Farm	Red	Blue	Green
Rosella	Blue	Green	Red

3.2.4 Photo Analysis

For Study 2a and 2b a total of 143 845 images were produced. These were manually viewed as jpeg files using Windows Live™ Photo Gallery (Version 2009).

On analysis, recorded were: the species, date and time of camera trigger, order the three conditions were visited, and the interaction with each condition [no interaction (walking past camera), sniff/lick, carry away, bite, consume bait], total time spent at each condition, the total time spent at the camera station in total and the number of apples of each condition eaten. Separate individual records were defined if members of the same species that were sighted at least 30 minutes apart.

3.2.5 Statistical programs

Statistical analyses were conducted using SPSS Statistics (2014) and Statistica 9 (2012).

3.2.6 Study 2a: statistical analysis

To determine if the treatment type (control, low or high) animals first approached was random, χ^2 goodness-of-fit tests were performed for each potential deterrent and the untreated days. However, for washing-up liquid this test could not be performed, as the expected frequency was less than five. χ^2 goodness-of-fit tests were also conducted just for quokkas and mardos individually. However, tests could not be performed for washing-up liquid and wasabi in quokkas, and only on chilli and untreated days in mardos, as all other expected frequencies were less than five. Expected frequencies were also less than five for all other species sighted: the common brushtail possum, echidna, Western grey kangaroo, scarlet robin (*Petroica boodang*), shingleback lizard (*Tiliqua rugosa*), skink (*Scincidae*) and western silvereye (*Zosterops lateralus choronotus*), and therefore no other χ^2 goodness-of-fit tests could be conducted.

χ^2 goodness-of-fit tests were also used across all species, in quokkas and mardos to determine if the total number of apples taken was evenly distributed across control, low and high on each experimental day. However, in the mardo tests could not be conducted on days 1-3 or day 5 due to no apple uptake, and also on days 6, 8, 10-12 and 16 as expected frequencies were less than five.

One-way multivariate analyses of variance (MANOVAs) were conducted to determine the effect of day: pre-treatment (untreated), treatment (aversive agent) and post-treatment (untreated) on the number of control, low and high condition apples taken. They were conducted on three days at a time (i.e. days 1-3, 3-5, 5-7, 7-9, 9-11, 11-13, 13-15, 15-17 and 17-19) to see if there were any carry-over effects of aversion from potential deterrents. Arcsine square root values were used for the dependent variable calculated from proportions of control, low and high apples taken. These tests were run on quokkas only, rather than all species to find species-specific responses to taste and aversion. No other species sighted had a large enough sample size on each day. Preliminary assumption testing revealed that data were normally distributed (assessed by Shapiro-Wilk test, $p > .05$), there were no outliers (assessed by inspection of boxplot and Mahalanobis distance, $p > .001$), no multicollinearity (assessed by Pearson correlation, $p > .05$), linear relationships (assessed by scatterplot) and homogeneity of variance-covariance matrices (assessed by Box's test of equality of covariance matrixes, $p > .001$). Tukey post-hoc tests were also conducted on any significant results.

A Kruskal-Wallis *H* test was conducted to determine if there were differences in the number of apples taken between the three sites (Midgegooroo, Mile and Thirty-One Mile). This non-parametric test was selected because data were not normally distributed, even when transformed. To determine where the differences occurred Mann-Whitney *U* tests were conducted. Tests were conducted using Bonferroni adjusted alpha levels of .017 per test.

3.2.7 Study 2b: statistical analysis

To determine if the treatment (control, low or high) that animals first approached was random at Barmoral and Chandler, the sites testing if there was an additive effect of chilli on aversion, a χ^2 goodness-of-fit test was performed for all species. This test was also performed on just quokkas, however it could not be conducted on the other species sighted (common brushtail possum, Western grey kangaroo, mardo, raven, shingleback lizard and western silvereye), as the expected frequencies were less than five.

χ^2 goodness-of-fit tests were also performed on all species together, and also just quokkas and ravens at Bee Farm and Rosella, the sites testing for aversion after exposure to seven days of Bitrex, to see if the treatment animals first approached was evenly distributed. However, the χ^2 goodness-of-fit test could not be conducted on the other species sighted (echidna, Western grey kangaroo and shingleback lizard), as the expected frequencies were less than five.

To determine if chilli or Bitrex could have an additive effect and act as deterrents creating aversion towards apples various tests were conducted. A paired *t*-test was performed to find if there was a statistically significant difference between the total number of apples eaten at each replicate at untreated day one and untreated day two, after exposure to the chilli for seven days. Before this test was performed assumptions were tested and there were no outliers in the data, as assessed by inspection of a boxplot and the difference scores for untreated day one and two were normally distributed, as assessed by Shapiro-Wilk's test ($p = .148$). The same test was conducted for Bitrex. As data were not normally distributed even when transformed and the assumption for Wilcoxon signed-rank tests of a symmetrically shaped distribution was also violated, a Sign test was conducted.

Paired-sample *t*-tests were also used for the chilli trials to determine whether there were statistically significant differences between the number of apples eaten for each replicate from untreated day one to day two for control, low and high treatments separately. Assumptions were tested for each test and all three had no outliers and differences were normally distributed according to Shapiro-Wilk's test for control and high conditions ($p = .571$, .646 respectively). The data were not normally distributed for the low condition, however the test was conducted nonetheless due to the robustness of the test and because the non-parametric equivalent test (Wilcoxon signed-rank test) produced the same result. This was also conducted for Bitrex for each the control, low and high conditions. There were no outliers in the data and the difference scores for untreated day one and untreated day two were normally distributed according to Shapiro-Wilk's test for the control, low and high conditions (p = .186, .427 and .091 respectively).

Finally, Friedman tests were run to determine if there were differences in the experimental groups (control, low and high) in the distributions of number of apples taken on untreated day one, before the chilli or Bitrex treatment had been applied. This non-parametric test was used for both the Chilli and Bitrex because the experimental groups were not normally distributed for control, low and high as assessed by Shapiro-Wilks test ($p > .05$), even when the data were transformed. Friedman tests were also run for untreated day two, after exposure to seven days of chilli or Bitrex treatment, again due to non-normally distributed data.

Friedman tests were also performed on just quokkas in the chilli trials due to outliers, just quokkas in the Bitrex trials due to non-normally distributed data and just ravens in the Bitrex trials due to non-normally distributed data.

For any Friedman tests that showed a statistically significant result, pairwise comparisons were conducted (SPSS, 2014) using Bonferroni adjusted alpha levels of .017 per test, to determine where the differences occurred.

To determine if there was a statistically significant effect of site on the number of apples taken a Mann-Whitney *U* test was conducted between Balmoral and Chandler, the two chilli sites, and between Bee Farm and Rosella, the two Bitrex sites. This non-parametric test was chosen as in both cases there were outliers in the data, as determined by visual inspection of a boxplot and the data were also not normally distributed (Shapiro-Wilks $p < .05$). The assumption that distributions of the number of apples taken were similar for both the chilli sites was met, however, not for the Bitrex sites. Therefore, for the Bitrex sites only differences in mean ranks rather than medians could be inspected.

3.3 Results

Study 2a: Immediate deterrent aversion

3.3.1 Treatment type first approached

Table 3.5 shows the results for immediate deterrent trials (Study 2a) for (a) all species, (b) for quokkas only, and (c) for mardos only. There was no significant difference in the treatment type (control, low or high), and therefore in the three apple colours, that the animals first approached across the 274 visits for all the untreated days analyzed together for all species ($\chi^2_2 = 0.26$, $p > .05$), quokkas ($\chi^2_2 = .03$, $p > .05$), and mardos ($\chi^2_2 = 1.80$, $p > .05$). There was also no difference in the treatment first approached for all trials except Trial 9: chilli (Table 3.5). For this trial, there were significantly more of the control treatments visited first. However, this only applied when all species were analysed together, but not when quokkas or mardos were looked at individually.

Table 3.5: Results of immediate deterrent trials (Study 2a).

			Treatment effect on:		
Trial order	Compound	Number of visits	First treatment visited	Apples taken on trial day	Apples taken on following day
a. All species					
1	Washing-up liquid	3	†	***	**
2	Citric acid	26	$\chi^2_2 = 0.43$ *ns*	*ns*	*ns*
3	Wasabi	16	$\chi^2 = 1.56$ *ns*	***	***
4	Baking powder	23	$\chi^2_2= 0.36$ *ns*	***	*ns*
5	Bicarbonate soda	30	$\chi^2 = 1.89$ *ns*	**	*ns*
6	Salt	29	$\chi^2_2 = 0.75$ *ns*	***	*ns*
7	Sodium saccharin	44	$\chi^2_2 = 4.24$ *ns*	*ns*	*ns*
8	Bitrex	34	$\chi^2_2 = 1.91$ *ns*	*ns*	*ns*
9	Chilli	58	$\chi^2_2= 8.05$ *	***	***
b. Quokkas only					
1	Washing-up liquid	3	†	***	**
2	Citric acid	17	$\chi^2_2 = 0.38$ *ns*	*ns*	*ns*
3	Wasabi	11	†	***	**
4	Baking powder	19	$\chi^2_2= 0.36$ *ns*	**	*ns*
5	Bicarbonate soda	26	$\chi^2 = 1.89$ *ns*	*ns*	*ns*
6	Salt	26	$\chi^2_2 = 0.45$ *ns*	***	*ns*
7	Sodium saccharin	37	$\chi^2_2 = 5.94$ *ns*	*ns*	*ns*
8	Bitrex	25	$\chi^2_2 = 0.99$ *ns*	*ns*	*ns*
9	Chilli	26	$\chi^2_2= 2.90$ *ns*	***	***
c. Mardo only					
1	Washing-up liquid	0	†	†	†
2	Citric acid	3	†	†	†
3	Wasabi	3	†	†	†
4	Baking powder	3	†	†	*
5	Bicarbonate soda	4	†	†	†
6	Salt	4	†	†	†
7	Sodium saccharin	5	†	**	†
8	Bitrex	8	†	†	**
9	Chilli	26	$\chi^2_2= 5.04$ *ns*	*	†

† not tested due to low sample size
ns not statistically significant at $p<0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

3.3.2 Effect of deterrents

Figure 3.4 illustrates the results of immediate deterrent trials showing the total number of apples taken from each experimental group (control, low high) over each treatment day for (a) all species, (b) for quokkas only, and (c) for mardos only. The rainfall (mm) in 24-hour intervals to 9am recorded for Jarrahdale by Weatherzone (BOM 2014) is also shown.

When all species were analyzed together and also in quokkas in isolation, there was a significant difference in the number of apples taken from control, low and high concentration treatments when washing-up liquid, wasabi, baking powder, bicarbonate soda, salt and chilli were applied (Figure 3.4). However, only in washing-up liquid, wasabi and chilli conditions was there a significant difference (aversion) on the following untreated day. There was also a general increase in the number of apples consumed up until day 11. However, with mardos only when sodium saccharin and chilli were used was there a significant difference between the number of apples taken from each treatment, and it never carried over to the following day. Overall, it is hard to make any firm conclusions on the response of the mardo, due to overall limited sample size. It is also suggested that any effect of rainfall was minimal as even on the two days with the highest rainfall (day 14 and 19) there were not any obvious differences in the total number of apples taken or the experimental groups they were taken from (for all species, quokkas only and mardos only).

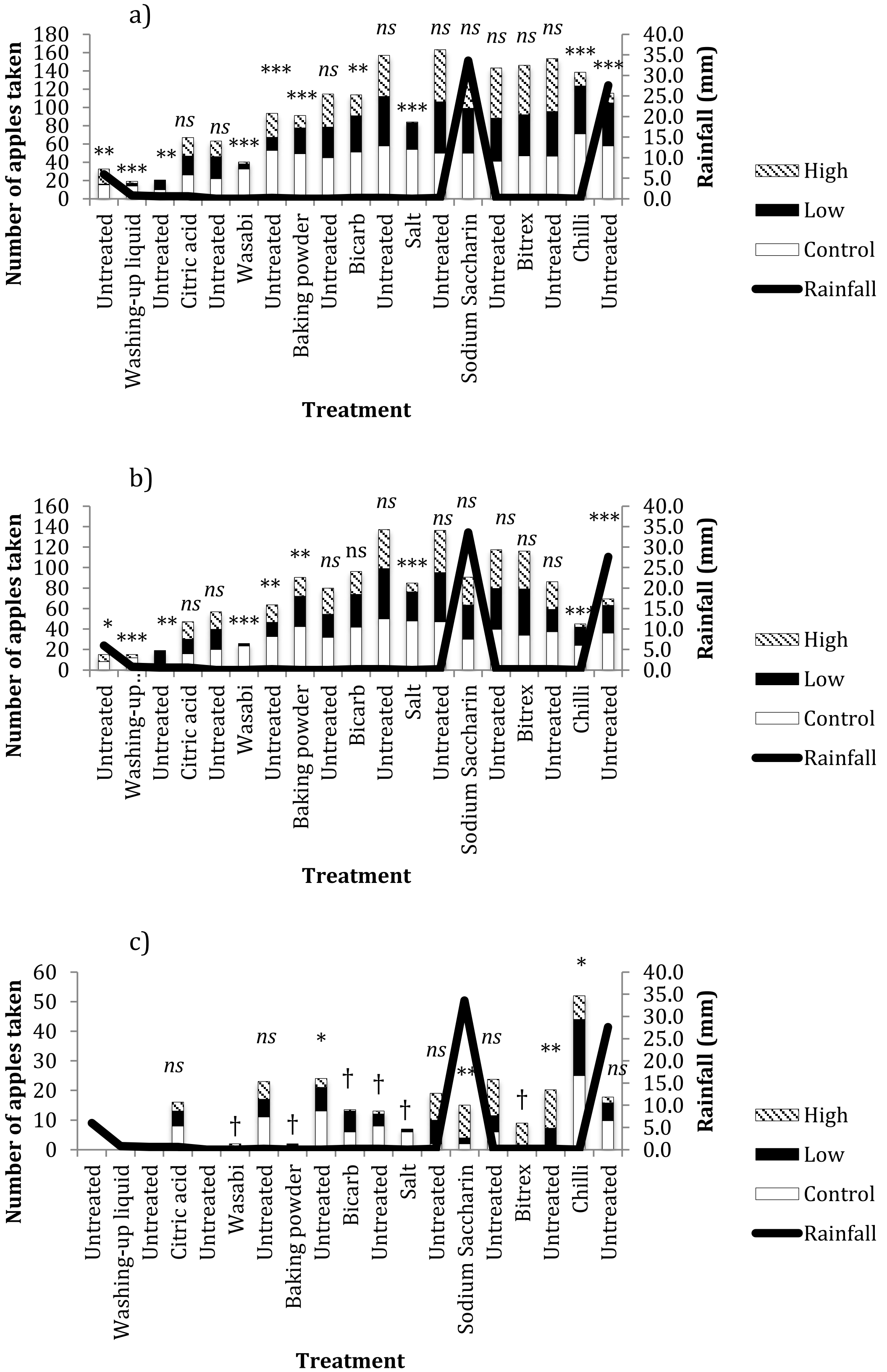

Figure 3.4: Results of immediate deterrent trials (Study 2a).

Asterisk indicates significant difference (χ^2 analysis) between the number of control, low and high apples taken at the three sites, Midgegooroo, Mile and Thirty-One Mile († not tested due to low sample size, *ns* not statistically significant at $p<0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

3.3.3 Carry-over effects

Table 3.6 shows MANOVA results testing the proportion of control, low and high treatment apples eaten (all added as dependent variables) with day as the categorical factor. Wasabi was significantly associated with reduced apple consumption by quokkas, especially at the high concentration, and there was a mild carry-over effect (aversion) the next day. Salt was also associated with reduced apple consumption, but there was no apparent aversion on the following untreated day. No other compounds were also associated with a significant aversive effect in quokkas. This also revealed that the consecutive presentation of different potential deterrents did not have a significant effect as no deterrents had carry-over effects onto days testing for the next deterrent.

Table 3.6: Summary of MANOVA results for immediate deterrent trials.

									Day (Tukey's post-hoc analysis‡)				
Compound tested	Days	MANOVA results †							Pre-untreated		Treatment		Post-untreated
Washing up liquid	1-3	$F_{2,4}$	=	2.12	p	=	0.236		a		a		a
Citric acid	3-5	$F_{6,12}$	=	1.20	p	=	0.372		a		a		a
Wasabi	5-7	$F_{6,24}$	=	1.52	p	=	0.215		a	←*→	b		a, b
Baking powder	7-9	$F_{6,22}$	=	0.99	p	=	0.451		a		a		a
Bicarb	9-11	$F_{6,28}$	=	1.41	p	=	0.246		a		a		a
Salt	11-13	$F_{6,30}$	=	**2.79**	p	=	**0.028**		a	←**→	b	←**→	a
Sodium saccharin	13-15	$F_{6,26}$	=	2.07	p	=	0.092		a		a		a
Bitrex	15-17	$F_{6,26}$	=	1.68	p	=	0.167		a		a		a
Chilli	17-19	$F_{6,26}$	=	0.76	p	=	0.604		a		a		a

† The degrees of freedom vary between trials since preference could not be determined for days when no apples were eaten.

‡ letters link days that did not have a different profile of apple treatments eaten.

*$p<0.05$, ** $p<0.01$

3.3.4 Effect of site

Figure 3.5 illustrates the median total number of apples taken from feeding stations at the three sites (Midgegooroo, Mile and Thirty-One Mile) for immediate deterrent trials. Boxplots show the median values (solid horizontal lines), 50th percentile values (box outlines), 90th percentile values (box whiskers) and outliers (open circles). Difference in letters between sites indicates significant difference (Mann-Whitney *U* test) between the median total number of apples taken at each site. The median total number of apples taken at Thirty-One Mile feeding stations was significantly less than at Midgegooroo and Mile.

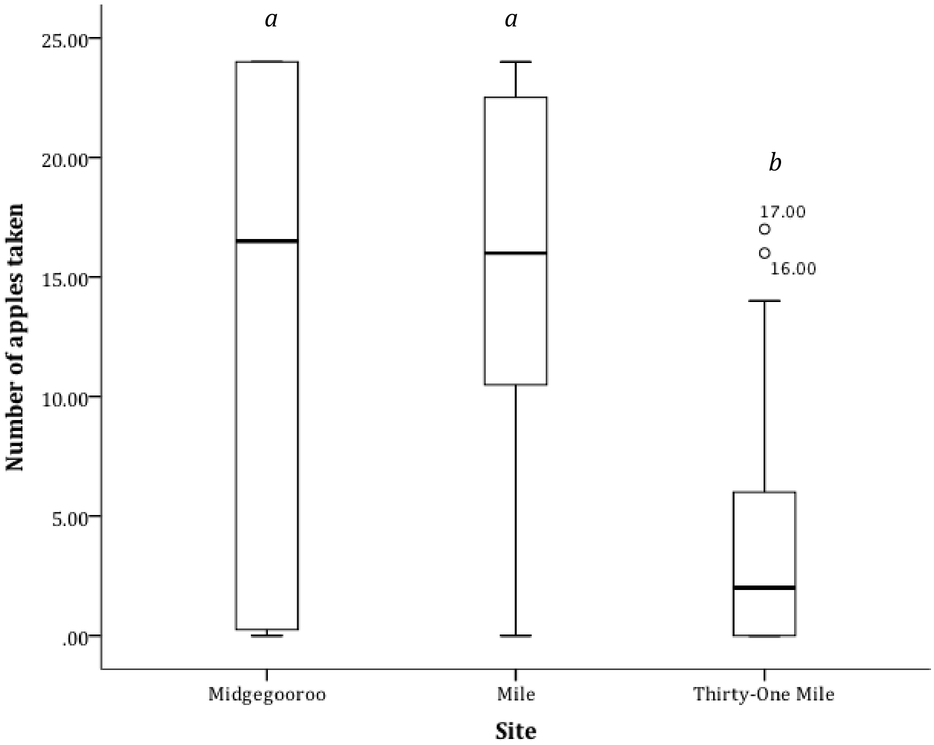

Figure 3.5: Effect of site (Study 2a).

3.4 Study 2b: Additive effect of deterrent on aversion

3.4.1 Treatment type first approached

Table 3.7 shows the results for cumulative deterrent trials (Study 2b) for the chilli treatment for (a) all species, (b) for quokkas only, and for the Bitrex treatment for (c) all species, (d) for quokkas only, and e. for ravens only. There were no statistically significant differences in the treatment type (control, low or high) that animals first approached before, during and after chilli or Bitrex treated apples were presented.

Table 3.7: Results of additive deterrent trials (Study 2b).

			Treatment effect on:		
Trial order	Compound	Number of visits	First treatment visited	Apples taken on untreated day 1	Apples taken on untreated day 2
a. Chilli all species					
1 & 9	Untreated	62	$\chi^2_2 = 1.58$ *ns*	*ns*	**
2-8	Chilli	220	$\chi^2_2 = 0.88$ *ns*		
b. Chilli quokkas only					
1 & 9	Untreated	47	$\chi^2_2 = 0.30$ *ns*	*ns*	**
2-8	Chilli	157	$\chi^2_2 = 2.84$ *ns*		
c. Bitrex all species					
1 & 9	Untreated	51	$\chi^2_2 = 0.47$ *ns*	*ns*	*ns*
2-8	Bitrex	147	$\chi^2_2 = 0.78$ *ns*		
d. Bitrex quokkas only					
1 & 9	Untreated	36	$\chi^2_2 = 0.17$ *ns*	*ns*	*ns*
2-8	Bitrex	108	$\chi^2_2 = 0.50$ *ns*		
e. Bitrex ravens only					
1 & 9	Untreated	15	$\chi^2_2 = 3.60$ *ns*	*ns*	ns
2-8	Bitrex	38	$\chi^2_2 = 0.68$ *ns*		

† not tested due to low sample size
ns not statistically significant at $p<0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

3.4.2 Effect of deterrents

Figure 3.6 shows the total number of apples taken for additive chilli trials from each experimental group (control, low high) over each treatment day for (a) all species, and (b) for quokkas only. The rainfall (mm) in 24-hour intervals to 9am recorded for Jarrahdale by Weatherzone (BOM 2014) is also shown.

While there was no statistically significant difference in the number of apples taken from control, low and high conditions on untreated day 1, as soon as chilli was applied there was a significant difference for all species (Figure 3.6a), but also for quokkas alone (Figure 3.6b). This response remained significant after exposure to seven days of chilli, on day 9, the second untreated day. Therefore, chilli was significantly associated with bait consumption for all species, and also specifically quokkas, which overall had a very similar response (Figure 3.6).

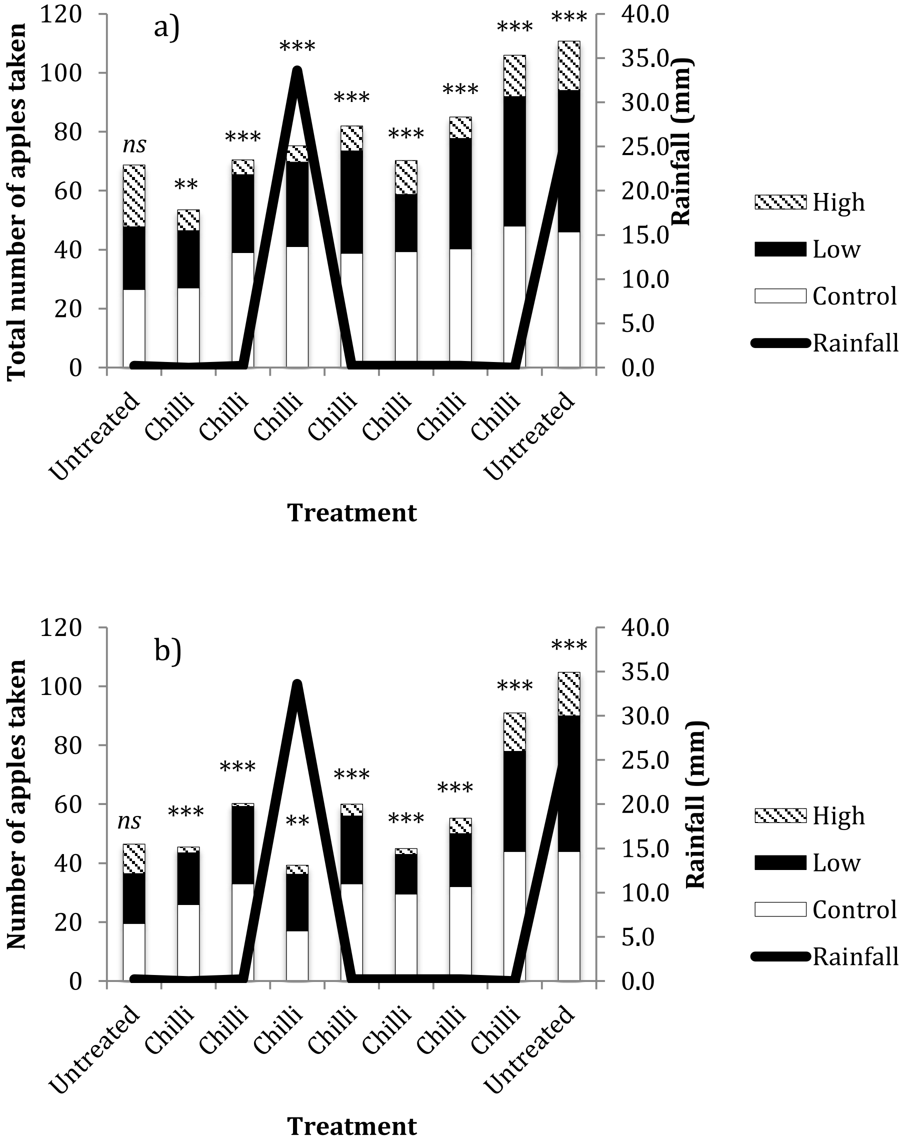

Figure 3.6: Results of additive chilli deterrent trials (Study 2b).

Asterisk indicates significant difference (χ^2 analysis) between the number of control, low and high apples taken at the two sites, Balmoral and Chandler (*ns* not statistically significant at $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Figure 3.7 shows the total number of apples taken for additive Bitrex trials from each experimental group (control, low high) over each treatment day for (a) all species, (b) for quokkas only, and (c) for ravens only. The rainfall (mm) in 24-hour intervals to 9am recorded for Jarrahdale by Weatherzone (BOM 2014) is also shown. Unlike in the additive chilli trials, even when Bitrex was applied there was no statistically significant difference in the number of apples taken from control, low and high conditions for all species (Figure 3.7a). This is also the case with quokkas, excluding the difference on day 8 (Figure 3.7b). However, as this was the only day with a significant difference between the number of control, low and high apples taken it is more likely that it occurred by chance, rather than being a response to Bitrex. Overall Bitrex had no effect on apple consumption in all species, and specifically in the quokka. However, no firm conclusions can be about the effect on ravens due to limited sample size (Figure 3.7c).

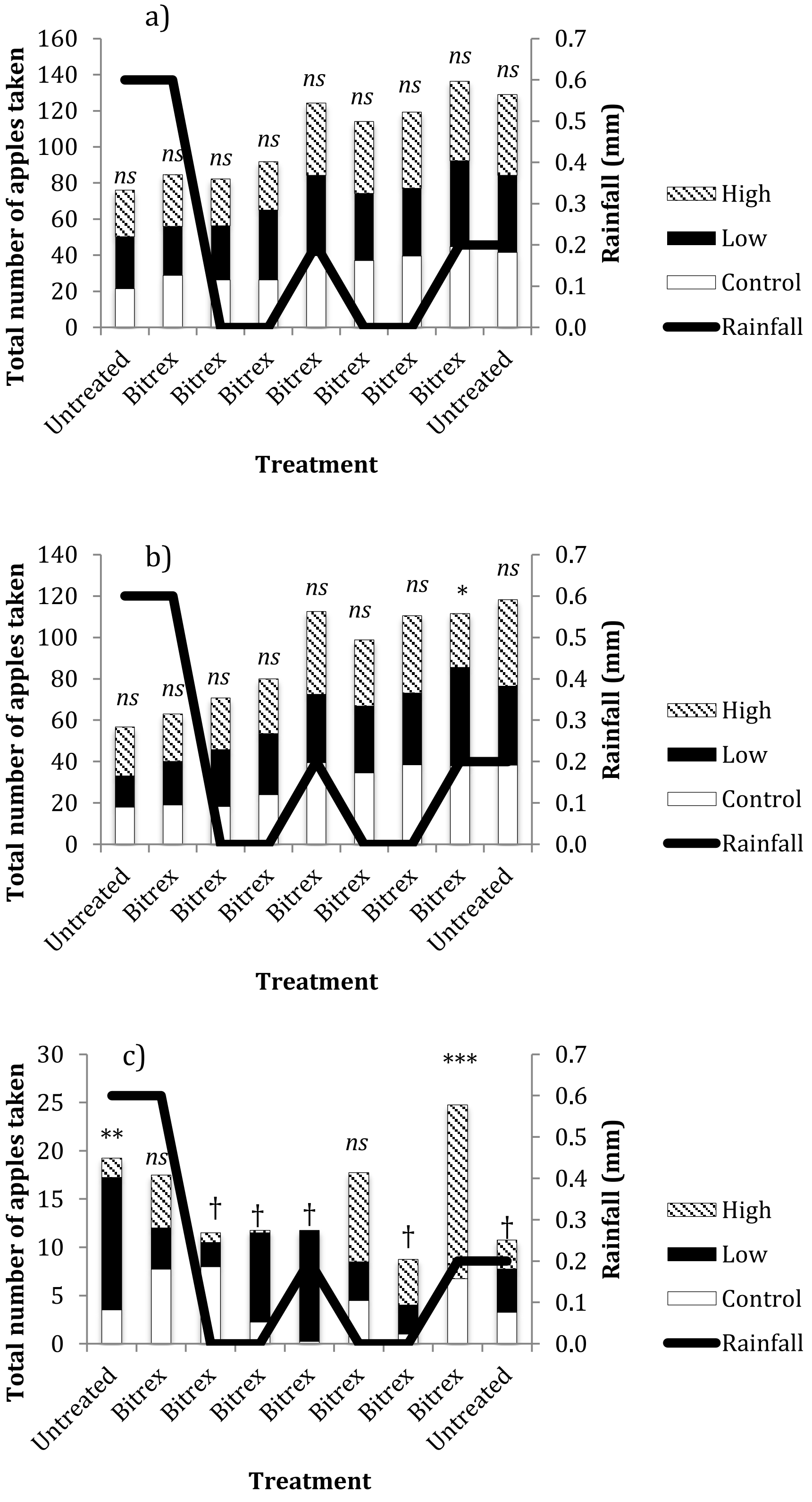

Figure 3.7: Results of additive Bitrex deterrent trials (Study 2b).

Asterisk indicates significant difference (χ^2 analysis) between the number of control, low and high apples taken at the two sites, Bee Farm and Rosella (*ns* not statistically significant at $p<0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and † not tested due to low sample size).

Table 3.8 illustrates results of *t*-tests (or the non-parametric equivalent: Wilcoxon ranked-sign test) for accumulative chilli trials showing the difference in the mean number of apples taken on untreated day 1 (before exposure to chilli) and untreated day 2 (after exposure to chilli) for (a) all species, and (b) quokkas only.

There was no significant difference in the total number of apples eaten for all species after exposure to seven days of chilli, (Table 3.8a) a similar finding for quokkas alone (Table 3.8b). However, there was a significant increase in the number of control and low condition apples eaten, but not in the number of high condition apples.

Table 3.8: Difference in number of apples taken for untreated day 1 and 2 in additive chilli trials.

Number of apples eaten	Untreated 1		Untreated 2				
	M	*SE*	*M*	*SE*	*T*	*df*	*p*
a. All species							
Total	11.46	2.94	18.46	.91	-2.104*ns*	5	.089
Control	4.42	1.08	7.67	.33	-2.93*	5	.03
Low	2.54	.95	8.00	.00	-4.67**	5	.005
High	3.50	1.41	2.79	.62	.418*ns*	5	.694
b. Quokka only							
Total	7.75	3.50	17.29	.84	-2.534*ns*	5	.052
Control	3.08	1.14	7.33	.33	-3.364*	5	.020
Low (Wilcoxon signed-rank test)	*Mdn* = 2.5		*Mdn* = 8.0		*Z* = -2.060*	5	.039
High	1.67	1.28	2.46	.55	-.514*ns*	5	.629

ns not statistically significant at *p*<0.05, * *p*<0.05, ** *p*<0.01.

Table 3.9 illustrates the results of *t*-tests (or non-parametric equivalents: Wilcoxon ranked-sign test or sign test) for accumulative Bitrex trials showing the difference in the mean number of apples taken on untreated day 1 (before exposure to Bitrex) and untreated day 2 (after exposure to Bitrex) for (a) all species, (b) quokkas only, and (c) ravens only.

There was no significant difference in the total number of apples eaten for all species (Table 3.9a), and for quokkas alone, after exposure to seven days of Bitrex (Table 3.9b), but not for ravens (Table 3.9c). Quokkas also consumed a significantly higher amount of low condition apples on the second untreated day. However, there no other significant differences between untreated day one and two.

Table 3.9: Difference in number of apples taken for untreated day 1 and 2 in additive Bitrex trials.

Number of apples eaten	Untreated 1		Untreated 2				
	M	*SE*	*M*	*SE*	*t*	*df*	*p*
a. All species							
Total (Sign test)	*Mdn* = 4.75		*Mdn* = 8.00		**	5	.003
Control	3.58	1.65	6.92	.90	-2.380*ns*	5	.06
Low	4.79	1.50	7.13	.69	-1.708*ns*	5	.148
High	4.29	1.63	7.46	.54	-2.12*ns*	5	.088
b. Quokka only							
Total	9.46	4.38	19.71	2.76	-3.154*	5	.025
Control	3.00	1.61	6.38	1.11	-2.460*ns*	5	.057
Low	2.50	1.45	6.38	1.09	-2.995*	5	.030
High	3.96	1.77	6.96	0.72	-2.119*ns*	5	.088
c. Raven only							
Total (Wilcoxon signed-rank test)	*Mdn* = 0.00		*Mdn* = 1.00		*Z* = 0.00*ns*	5	1.00
Control (Sign test)	*Mdn* = 0.00		*Mdn* = 0.00		*ns*	5	1.00
Low	2.29	1.23	.75	.48	1.567*ns*	5	.178
High (Wilcoxon signed-rank test)	*Mdn* = 0.00		*Mdn* = 0.00		*Z* = -.447*ns*	5	.655

ns not statistically significant at $p<0.05$, * $p<0.05$, ** $p<0.01$.

For untreated day one, before any chilli was applied, the number of apples taken decreased from control (*Mdn* = 4.5) to low (*Mdn* = 3.0) and high (*Mdn* = 3.0) treatments for all species, but these differences were not statistically significant, $\chi^2_2 = 1.44$, $p = .49$. However, on untreated day two, after the chilli, there was a significant effect of the experimental group on the number of apples taken at the $p < .05$ level for the three conditions, $\chi^2_2 = 11.47$, $p = .003$. There were significant differences in the number of apples taken for high (*Mdn* = 3.00) and control conditions (*Mdn* = 8.00) ($p = .014$) and for high and low conditions (*Mdn* = 8.00) ($p = .006$) at the adjusted $p < .017$ level. However, there was no significant difference between control and low conditions ($p = .773$).

Similarly, there was not a significant difference in the number of apples taken from each treatment on untreated day one, before chilli, in quokkas, $\chi^2_2 = 2.235$, $p = .327$, despite the median amounts eaten decreasing from control (*Mdn* = 3.25) to low (*Mdn* = 2.50) and high treatments (*Mdn* = 0.50). Also on untreated day two, after exposure to seven days of chilli, there was a statistically significant difference in the median number of apples taken from each treatment, $\chi^2_2 = 10.182$, $p = .006$. While there was not a significant difference in the median number of control (*Mdn* = 7.50) and low condition apples (*Mdn* = 8.00) taken ($Z = 6.50$, $p = .577$), there was a significant difference between the median number of control and high conditions (*Mdn* = 2.50) ($p = .011$) and the median number of low and high conditions ($p = .011$) at the adjusted $p < .017$ level.

The number of apples taken on untreated day one before exposure to Bitrex, for all species decreased from low (*Mdn* = 5.88) to high (*Mdn* = 4.38) to control treatments (*Mdn* = 2.75), but these differences were not statistically significant, $\chi^2(2) = 1.733$, $p = .420$. On untreated day two, after exposure to seven days of

Bitrex, the number of apples taken from control, low and high conditions were also not significantly different, χ^2_2 = 1.44, *p* = .49, all with a median of 8.00.

When just looking at quokkas, there was also no significant difference between the number of apples taken from control (*Mdn* = 1.00), low (*Mdn* = 3.88) and high (*Mdn* = 0.50) treatments on untreated day one (before Bitrex), χ^2_2 = 1.00, *p* = .607, or on untreated day two (after seven days of Bitrex), χ^2_2 = .545, *p* = .761, where the median number of apples taken from all treatments was 8.00.

This again was the case when looking at ravens individually. On untreated day one (before Bitrex) there was no significant difference between the number of control (*Mdn* = 0.00), low (*Mdn* = 0.00) and high (*Mdn* = 1.38) apples taken, χ^2_2 = 2.714, *p* = .257. There was also no significant difference between these treatments on untreated day two (after seven days of Bitrex), χ^2_2 = 1.400, *p* = .497, where the median number of apples taken from all treatments was 0.

3.4.3 Effect of site

While there was no significant difference between the number of apples taken at Balmoral (*Mdn* = 14.5) and Chandler (*Mdn* = 14.25), *U* = 311.50, *z* = -.918, *p* = .359, the two chilli sites, there was a statistically significant difference between Rosella (mean rank = 39.48) and Bee Farm (mean rank = 15.52), the two Bitrex sites, *U* = 41.00, *z* = -5.98, *p* < .001.

3.5 Discussion

In Study 2 nine potential deterrents were tested for immediate aversion and chilli and Bitrex were tested for accumulative aversion. Discussion of findings focuses on the response of quokkas as taste is suggested to be species-specific (e.g. Chandrashekar *et al.* 2006), and quokkas were the only species that had a large enough sample size to conduct all statistical analyses. Findings suggest that high concentrations of wasabi and salt had an immediately aversive effect on apple consumption by quokkas, and only in wasabi did the effect carry-over to untreated baits. Results also suggested that chilli was aversive in high concentrations in the test for accumulative aversion for all non-target species. However, the other six deterrents tested for immediate aversion (washing-up liquid, citric acid, baking powder, bicarbonate soda, sodium saccharin and Bitrex), and also Bitrex for accumulative aversion, did not demonstrate any significant effect, even at high concentrations.

3.5.1 Immediate aversion to wasabi

While previous studies have not explored the use of wasabi as an aversive agent, the plant has known defence mechanisms to deter consumption (Yu *et al.* 2001; Al-Anzi *et al.* 2006; Heinricher *et al.* 2014). As a member of the cruciferous (Brussicaceae) plant family, wasabi (*Wasabia japonica*) (Yu *et al.* 2001; Heinricher *et al.* 2014), contains glucosinolates, which have been recognised to play a role in deterring herbivores (as well as fungi and nematodes) (Yu *et al.* 2001). Wasabi also produces isothiocyanates, which are released when cells are disrupted or damaged (such as when consumed). One of these is allyl isothiocyanide, which is the primary pungent ingredient in wasabi, causing burning sensations and stimulation of the nasal passages (Yu *et al.* 2001; Al-Anzi *et al.* 2006; Heinricher *et al.* 2014). This could explain why quokkas, as a browsing herbivore (Hayward 2005; Department of the Environment 2014), reduced their consumption at high wasabi concentrations and were still deterred when presented with untreated apple baits.

3.5.2 Immediate aversion to salt

Unlike wasabi, findings suggested that high concentrations of salt only deterred quokkas on the day of application. They did not show a significant carry-over effect onto the following untreated day. One explanation for this difference, is that avoidance of wasabi developed as a mechanism, known as conditioned taste aversion (Garcia and Hankins 1977; Cowan *et al.* 2000; Macdonald and Baker 2004), while salt avoidance developed as repellency (Atkinson and Macdonald 1994; Macdonald and Baker 2004). Conditioned taste aversion develops when an animal learns to avoid a food item after associating it with an illness (Garcia and Hankins 1977). It produces an association between the food taste (or odour) and the illness, that creates future avoidance of both conditioned and unconditioned food items (Cowan *et al.* 2000; Macdonald and Baker 2004), such as was observed in wasabi. On the other hand, repellency develops when a noxious taste (or odour) repels an animal from consumption (e.g. Atkinson and Macdonald 1994). The repellent is detected and avoided at each new encounter, rather than rejected even in untreated food items (Macdonald and Baker 2004). This means that salt would not be useful for deterring non-target species, unless it was applied to every bait. This would particularly be a problem if it also repelled the target species (foxes). Therefore, compounds that are able to create aversion to untreated baits (conditioned taste aversion) are preferable.

3.5.3 Immediate aversion to chilli

In the trials testing for immediate aversion there was a significant difference in the number of high chilli concentration apples that quokkas consumed, compared to control and low condition apples. However, tests for carry-over effects suggested that chilli did not have a significant effect between the days (treated or untreated). This finding is inconsistent with the expected result based on past research. Capsaicin in chilli peppers induces sensations such as a burning or tingling in the mouth as they irritate the trigeminal nerve fibres (Dearing *et al.* 2005; Moore *et al.* 2005). If the sensation is too intense, such as at high concentrations, food consumption is usually reduced (Pass and Foley 2000; Dearing *et al.* 2005; Moore *et al.* 2005). This aversion at high concentrations is thought to be general across mammals (Mason *et al.* 1991; Jordt and Julius 2002; Levey *et al.* 2006). This is because the capsaicin in chilli is regarded to have no physiological role in plants that produce it (Bernays and Chapman 1994; Coley and Barone 1996; Adler *et al.* 2001; Levey *et al.* 2006). The spiciness of chilli is instead suggested to have evolved to deter consumption by granivores and herbivores (Levey *et al.* 2006). For example, captive mule deer have been demonstrated to be deterred from browsing on apple twigs by application of high concentrations of hot sauce (with the active ingredient capsaicin) (Andelt *et al.* 1994a). While quokkas are also a browsing herbivore (Hayward 2005;

Department of the Environment 2014), the current study did not show a similar result.

The different findings between the current and previous research could be explained by limited statistical power. There may be too much variation in the available sample of quokka responses to adequately access statistically significant differences. While not appearing to have a significant effect on total apple consumption, the lack of statistical significance could also have been influenced by rainfall, as on the day following the chilli treatment, there was 27.6mm of rain, the second highest rainfall event in the study. Whatever the reason, the inconsistency in results reinforces the need for replication to determine if chilli does in fact cause aversion in non-target species, such as quokkas.

3.5.4 Immediate aversion to Bitrex

The current study also found that Bitrex had no significant effect on quokka consumption of apple baits. It is suggested that they may be indifferent or tolerant to Bitrex, as it does not cause immediate aversion (or accumulative aversion in Study 2b) at high concentrations. This finding differs from the results of other studies that suggested Bitrex to be repulsive to many mammals (Kaukeinen and Buckle 1992; Andelt *et al.* 1994a; Kleinkauf *et al.* 1999) and produce aversion at higher concentrations (e.g. El Hani *et al.* 1998b; Macdonald and Baker 2004). However, it is also consistent with some studies. For example, Ani-spray, a commercial bitterent agent containing denatonium benzoate, had no effect on deer browsing when applied to apple twigs, even at high concentrations that would be unbearably bitter to humans (Andelt *et al.* 1994a). One reason for this response in some mammals and not in others is suggested to be differences in diet, as will be briefly discussed below (Glendinning 1994). Glendinning (1994) suggested that there is a trade-off between the ecological costs and benefits of a bitter rejection response, which in mammals can be generalised for carnivores, omnivores and herbivores (browsing and grazing).

Aversion to bitterness is suggested to have evolved as the majority of naturally occurring poisons are bitter (at least to humans) and it is assumed to depend on the potential for these compounds in the animal's diet (Glendinning 1994). For example, browsing herbivores generally have a higher threshold for bitterness and tolerance for dietary poisons. Glendinning (1994) suggests that it would be a great survival disadvantage for herbivores to avoid bitter (often potentially poisonous) foods. It would significantly reduce the range of their diet since many bitter secondary chemicals (e.g. tannin) are widespread in the diet of browsers (e.g. forbs, shrubs, trees), although uncommon in the diet of grazers (e.g. grasses). As a result browsers produce many tannin-binding salivary proteins, while grazers often produce none, making browsers (in most cases) more tolerant to bitter chemicals than grazers (Glendinning 1994).

Quokkas being a browsing herbivore (Hayward 2005; Department of the Environment 2014) are therefore predicted to have evolved a higher bitter taste threshold, tolerance to dietary poisons and production of tannin-binding salivary proteins (Glendinning 1994). This theory might be informative to 1080 baiting operations. Bitrex is not predicted to be useful, as even in high concentrations non-target species, such as quokkas, are indifferent to it. As well as this, because the target species (foxes) are carnivores, rarely encountering these bitter compounds in their diet, it suggests that Bitrex would repel foxes (Glendinning 1994). This is supported by a study on captive foxes that was able to successfully condition them to avoid untreated milk after exposure to milk containing Bitrex (Macdonald and Baker 2004). However, this finding also supports a conclusion that species have different sensitivities and tastes and therefore, there may be a aversive agent that deters some non-target species, but not foxes.

3.5.5 Accumulative effect of chilli on aversion

The test for accumulative aversion (Study 2b) provided another investigation of the effectiveness of chilli and Bitrex as non-target deterrents. While Bitrex also produced no significant effect in Study 2b, findings suggested that additive chilli had a significant effect. However, these results should be investigated more closely. In this test for accumulative aversion, application of high concentration chilli significantly reduced consumption by the first treatment day. Accumulative aversion towards baits treated with the deterrent Ziram was seen in badgers (*Meles meles*) (Baker *et al.* 2005), where badgers ate all the Ziram-treated baits on treatment nights one and two, with uptake declining to zero from night three to night nine, and continuing to be avoided over the following 20 treatment nights (Baker *et al.* 2005). In the present study, high concentration chilli-treated apples were avoided from the first treatment day, indicating that chilli is immediately aversive to non-target species. This is not consistent with the results of Study 2a, which found no significant carry-over effect of chilli, showing the need for replication in future studies.

3.5.6 Impact on target species

While salt, wasabi (Study 2a) and chilli (Study 2b) have demonstrated deterrence of non-target (quokka) uptake of a highly palatable bait (Thomas *et al.* 2003), to be effective in application to 1080 baiting operations they would have to either not deter foxes or treated baits treated would have to be only exposed to non-target species. While the effectiveness of salt, wasabi or chilli in repelling the red fox has not been explored, as mammals some assumptions can be made about their potential avoidance response to hot peppers at high concentrations (Mason *et al.* 1991; Pass and Foley 2000; Jordt and Julius 2002; Dearing *et al.* 2005; Moore *et al.* 2005; Levey *et al.* 2006). Previous research has investigated coyotes (*Canis latrans, Canidae*), and their response to capsaicin in chilli (Lehner 1987; Wilbanks 1995). Capsaicin has been investigated for use in non-lethal coyote control by application to collars on sheep, to reduce livestock predation (Lehner 1987). While it was suggested to be an ineffective control technique due to coyotes changing their method of attack, capsaicin did repel coyotes from the collars (Lehner 1987), and in another study capsaicin was successful in repelling coyotes from inanimate objects such as irrigation hosing (Wilbanks 1995; Mason 1997). There is a possibility that foxes (also from the *Canidae* family) may show a similar response. While there is an argument that it should be tested, the likely impact on foxes suggests that baits containing these aversive agents be selectively exposed to only non-target species.

3.5.7 Limitations of this study

There are several limitations of the current study, and their consideration is important to give direction to future researchers:

Firstly, as can be seen from the results (Figure 3.4) the total number of apples eaten increased over time at the sites where the nine different deterrents for immediate aversion were tested. This overall increase came with a rise of animal activity as more individual animals found the feeding stations. Therefore, the first treatments, in particular washing-up liquid could not be as effectively tested for aversion as other tastant agents tested later, with some feeding stations not even found. For example, while washing-up liquid showed a significant difference in the number of apples taken from each treatment (control, low and high) its potential effect was not reported due to lack of sample size. Non-target animals only found the feeding stations at one site and took approximately 8.80% of all apples put out on that day. This could be overcome with a period of pre-feeding with untreated baits to attract animals and allow them to find feeding stations before any tastant agents are applied. Pre-feeding has shown to be successful in increasing bait take in many studies (e.g. Coleman *et al.* 2007).

Secondly, there could have been a positional bias, especially as the control, low and high apples were placed in the same position each time. In general, animals tend to eat baits in the order they are encountered (Baker *et al.* 2005); however, it should be tested whether this positional preference has a significant effect on bait uptake. When tested over all species, chilli was the only aversive agent that the treatment type (control, low or high) first approached was not random, and the control treatment was more likely to be first visited. One reason for this could be that chilli may have a detectable odour that deterred the initial approach of some non-target animals (Macdonald and Baker 2004). Another possibility is that there was a carry-over effect from previously presented wasabi (however this is unlikely as a difference was only observed on the chilli treatment day). However, this finding that chilli was detectable before its initial approach was not found in quokkas in isolation or in the accumulative chilli aversion tests (for all species or quokkas). Due to these inconsistencies, deterrence arising from chilli odour should be investigated, as it would prevent any development of aversion once untreated baits were presented as non-target species would have learned the difference (Macdonald and Baker 2004).

Finally, it could not accurately be determined whether there was a significant effect of colour on bait uptake. As colour also changed between sites, its effect could not be separated. The effect of colour could be investigated on the first untreated day of each experiment before any treatments had been applied. However, due to many of the feeding stations not being found by species, particularly on this first day, effect of colour could not be accurately investigated. Therefore, a period of pre-feeding before any treatments are applied would also be useful to examine if there was an effect of colour on bait uptake.

3.5.8 Conclusion

This experiment showed that salt, wasabi (Study 2a) and chilli (Study 2b) could potentially be used to create aversion in non-target species towards highly palatable baits. However, aversion to untreated baits (conditioned taste aversion) was only seen in wasabi and chilli, and was only measured in the short-term (one day of untreated baits), despite longer-term aversion needed to be applicable for use in 1080 baits. Future research would be beneficial as the use of aversive tastant agents has the potential to decrease non-target uptake and increase target specificity of 1080 baiting programs improving their overall efficiency.

4 CHAPTER 4: GENERAL DISCUSSION, CONCLUSION AND SUGGESTIONS FOR FUTURE RESEARCH

This study demonstrated that bait presentation is likely to impact on the persistence of baits and that the tastant agents salt, wasabi and chilli have the potential to deter non-target uptake. If non-target uptake of 1080 fox baits is not controlled, the number of baits available to foxes will decrease, along with the chance of their encounter. Not controlling foxes will threaten the survival of many vulnerable and endangered native fauna species, as well as livestock (Saunders *et al.* 1995; 2010). To produce the adequate amount of control, the number of baits deployed may have to be increased, which in turn would increase the risk of poisoning to domestic dogs and other vulnerable non-target species. It also would have economic consequences, as the cost-effectiveness of 1080 baiting programs would likely be reduced.

This chapter presents the hypotheses that were introduced at the beginning of chapters two and three and the important findings of the current study that indicate whether or not they are supported. Suggestions for future research are proposed and main conclusions of the study are discussed.

4.1 Overall aim of the current study

To improve the effectiveness of current 1080 fox baiting regimes by minimising native non-target uptake.

4.1.1 Hypothesis 1: Buried baits will be the optimum bait presentation minimising non-target uptake.

The results did not support the hypothesis as there was no statistically significant difference between the number of days till baits were taken for the four different bait presentations (surface-laid, wrapped, suspended and buried). However, when analysed visually, findings showed that buried baits had the lowest non-target uptake and the longest persistence in comparison to the other bait presentations.

4.1.2 Hypothesis 2: Aversive tastant agents of the highest concentration will deter non-target species.

High concentrations of wasabi (Study 2a) and chilli (Study 2b) were able to immediately deter uptake by non-target species leading to aversion even when untreated baits were presented. Since taste is often species-specific, many of the analyses focused only on quokkas, the species of highest abundance in this study. While in quokkas a high concentration of salt deterred uptake, this aversion did not follow through to untreated baits. None of the other deterrents tested produced statistically significant aversion in quokkas.

4.2 Suggestions for future research

This study provides a good stepping-stone for identifying techniques to reduce non-target uptake of 1080 fox baits and improving current baiting operations. It focused on the uptake of non-target species. However, it is also important to consider the effect of bait presentation and aversive tastant agents on the target species (the fox). Specifically, if bait presentation significantly reduced fox uptake it may contribute to more problems than benefits. Instead, the use of any technique needs to be a compromise between increasing target and reducing non-target uptake. Currently, burying baits is suggested when the priority is reducing non-target uptake and surface-laid baits are used when there is not a high risk of non-target poisoning (Armstrong and Batini 1998; Department of Agriculture 2007; DPaW 2013). Further research on the uptake of wrapped baits has been recommended in chapter two, as they may offer a trade-off between the two priorities.

In terms of aversive agents, it is recommended that future studies investigate how long aversion to untreated baits can last to see if a period of isolated non-target pre-feeding with treated baits prior to baiting season is a possible technique that could be implemented. If instead repellent compounds that do not produce aversion to untreated baits are found to be the best option, their impact on foxes should be investigated; as to be effective baits would always have to be treated.

Additionally, a longer-term study over multiple seasons and over a wider range of habitat types would be highly valuable. Currently in Western Australia, 1080 fox baits are aerially deployed four times per year (approximately every three months) and smaller areas are targeted with ground baiting roughly once every month (DPaW 2013). This covers all seasons and a large range of sites. A longer-term study would improve the assessment of the generalizability of any findings to current baiting programmes. More replicates and increased sample size would assist with any limitations from reduced statistical power, as was experienced in the current study. Therefore, more confidence could be put into determining the effectiveness of bait presentation and aversive agents in reducing non-target uptake, and in the potential for their application in current 1080 baiting operations.

4.3 General Conclusion

Even in Western Australia, where native fauna species have a relatively high 1080 tolerance (King *et al.* 1981; McIlroy 1986; King and Kinnear 1991), non-target uptake is still a concern. This is because it diminishes the efficiency of labour, time and costs in 1080 baiting operations as the number baits available to foxes is reduced (Allen *et al.* 1989; Algar *et al.* 2007; Moseby *et al.* 2009b; Moseby *et al.* 2011). Techniques to reduce non-target uptake, such as different bait presentations (e.g. Thomson and Kok 2002) and the use of aversive tastant agents (Hone and Mulligan 1982; McIlroy 1994; Glen *et al.* 2007; Dundas *et al.* 2014) have been suggested. This study demonstrated that non-target species have a preference for surface-laid baits and are least likely to take buried baits. This conclusion was made despite statistical significance, but was consistent with past studies (Allen *et al.* 1989; Thomson and Kok 2002; Moseby *et al.* 2011). It was also found that high concentrations of salt, wasabi and chilli have potential to create immediate aversion in non-target species. While the current study provides a good starting point to improving the species specificity and decreasing non-target uptake, more research is required before either technique can be implemented into current 1080 fox baiting regimes. If nothing is done to reduce non-target uptake, 1080 baiting may become ineffective in controlling fox numbers and the threats that foxes pose, especially on many vulnerable and endangered species (Saunders *et al.* 1995; 2010), may rise dramatically.

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