CHAPTER 4

CONDITIONED TASTE AVERSION[1]

4.1 Introduction

The success of rodent baiting campaigns is often hampered by the gradual decline in the number of animals in the target population willing to consume bait. Some individuals will consume enough bait to constitute a lethal dose and die, but those that consume a sub-lethal dose will recover (Prakash 1988). Many of these survivors will suffer malaise and gastro-intestinal illness may, by associating consumption of the bait with this illness may become bait-shy (Chitty 1954). These rats will refuse to consume further bait, or reduce the amount of bait consumed when subsequently encountered. This phenomenon was originally termed 'bait shyness' or 'poison shyness', but now these terms are used to describe different phenomena. 'Bait shyness' is aversion associated with the bait substrate, whereas 'poison shyness' is aversion is associated with the active poison in the bait (Barnett and Cowan 1976). Both phenomena are forms of an acquired aversion and they have probably evolved as a defence against dietary poisoning (Reidinger and Mason 1987) by guarding against re-consumption of harmful food. When the aversion is associated with the taste of the food, as distinct from visual, odorous or other cues (Garcia *et al.* 1955), it is known as conditioned taste aversion (CTA). Therefore, CTA occurs when an animal associates the taste of a food with illness and avoids consuming that food in subsequent encounters (Garcia *et al.* 1974).

CTA could be used to influence feeding behaviour to help meet wildlife management objectives (Cowan *et al.* 2000). Potential uses include reducing predation on domestic livestock and endangered species or reducing damage to agricultural products (Nicolaus *et al.* 1989a). If used to generate an aversion to consuming particular prey, animals consuming prey treated with a CTA agent should subsequently avoid consuming that prey (Cowan *et al.* 2000). CTA offers a unique advantage to lethal control because individual and population-level effects are usually persistent. In contrast, if management focuses on lethal control, a rapid reinvasion of new, non-averse individuals will simply negate the effect of removing

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predators (Nicolaus *et al.* 1989a). CTA was first proposed to reduce sheep predation by coyotes (*Canis latrans*) (Gustavson *et al.* 1974) by invoking a two-stage learning process. Phase one involves inducing an aversion to the flavour of the food; this aversion will prevent consumption of the food but not attack of live prey. The second phase occurs when the cues associated with live prey (e.g. auditory, visual, olfactory) are associated with the flavour, thus preventing attack (Gustavson *et al.* 1974). Some studies suggest that CTA was successful in reducing predation on sheep by coyotes (Gustavson *et al.* 1976; Ellins *et al.* 1977; Gustavson *et al.* 1982), whereas others have suggested that CTA towards bait did not transfer to the killing of live animals (Conover *et al.* 1979; Bourne and Dorrance 1982; Burns 1983).

In other research, reduction of predation using only the initial pairing of illness with consumption of the food appears to have been more successful. Egg predation has been successfully reduced by CTA in species including free-ranging crows (*Corvus brachyrhynchus*) and ravens (*Corvus corax*) (Nicolaus *et al.* 1983; Nicolaus *et al.* 1989b; Dimmick and Nicolaus 1990; Avery *et al.* 1995), mongooses (*Herpestes auropunctatus*) (Nicolaus and Nellis 1987) and rats (*Rattus norvegicus*) (Massei *et al.* 2002). Dingoes (*Canis familiaris dingo*) (Gustavson *et al.* 1983) and foxes (Massei *et al.* 2003) have refused to consume untreated meat following consumption of treated meat. These successes with 'stationary' prey suggest additional potential uses of CTA in wildlife management rather than just reducing predation on live prey. Following developments in research on orally administered vaccines and contraceptives, baits are being increasingly used in wildlife management (e.g. Bradley *et al.* 1999; Masson *et al.* 1999). Many studies on carnivores, and in particular foxes, have used baits to deliver rabies vaccines, regulate fertility and control population size (e.g. Marks *et al.* 1996; Farry *et al.* 1998; Selhorst *et al.* 2001). Most of these studies have also stressed the need to improve the cost-effectiveness of baiting campaigns by reaching the maximum proportion of animals with the minimum number of baits. For foxes, multiple bait uptake, bait caching and monopolisation of bait by individuals (Chapter 3), are amongst those factors that may reduce the success of a baiting campaign (Trewhella *et al.* 1991; Saunders *et al.* 1999). A method that could deter individual foxes from eating or removing excessive baits, or denying others access to bait could improve the cost-effectiveness of baiting campaigns (see Chapter 7).

CTA has been experimentally induced in animals by administering an illness-inducing chemical post-food ingestion. Animals tend to associate adverse internal events with cues related to food (Galef and Osbourne 1978). Oral ingestion of the CTA agent is not essential, but the agent must be administered within a short enough period to allow the onset of symptoms to be associated with the conditioned stimulus being consumed. As a result a range of administration methods has been tried; CTA agents have been added into food (Gustavson *et al.* 1974; Gustavson *et al.* 1976; Conover *et al.* 1977; Bourne and Dorrance 1982), injected intraperitoneally (Gustavson *et al.* 1974) and via oral intubation (Gill *et al.* 2000; Massei *et al.* 2002). However, for practical purposes of reducing predation in wildlife management, it will probably be necessary to add the CTA agents into the food to target the predator (Cowan *et al.* 2000). If the agent is to be orally administered then it should ideally be tasteless and odourless to prevent detection. Few chemicals can be successfully incorporated into baits that completely retain the taste and smell of target food (Nicolaus *et al.* 1989b).

The ability of CTA to modify feeding behaviour is reliant upon finding suitable CTA-inducing chemicals (Gill *et al.* 2000). Issues of safety and detectability severely limit the number of compounds that may be used for practical applications of CTA in wildlife management. In addition to the ability to induce a robust CTA with a single dose, agents for field application should 1) be undetectable at doses likely to induce CTA; 2) be safe in terms of LD_{50} to potential target and non-target species likely to consume the bait; 3) be physically stable so as to allow its incorporation into baits for field use; and 4) have sufficient delayed activity to allow its full ingestion without illness (Nicolaus *et al.* 1989b; Cowan *et al.* 2000; Gill *et al.* 2000; Massei *et al.* 2002). Many chemicals have been tested for their potential to induce CTA in carnivores, such as lithium chloride, carbachol, and thiabendazole (e.g. Burns and Connolly 1980; Burns 1980; Bourne and Dorrance 1982; Gustavson *et al.* 1983; Nicolaus and Nellis 1987; Conover 1989). However, carbachol can be lethal to some mammals (Nicolaus and Nellis 1987) while both lithium chloride and thiabendazole are likely to be detectable at doses causing CTA (Conover *et al.* 1977; Burns and Connolly 1980; Ziegler *et al.* 1982).

Levamisole hydrochloride has successfully induced CTA to various food types in laboratory rats (Gill *et al.* 2000; Massei and Cowan 2002). Levamisole is an anthelmintic used in oral drenches for domestic stock, poultry and cats and dogs (Arundel 1985) and as an immodulator in anti-cancer therapy (Budavari 1996). It is rapidly absorbed in dogs with peak levels attained in 12 minutes, and causes up to ten percent of test subjects to vomit (Arundel 1985). Levamisole is tasteless and odourless (Remington 1975) and therefore has potential to be added directly to food for field use as a CTA agent. When tested with captive foxes, levamisole induced complete avoidance of the experimental food for periods from seven weeks (beef-flavoured minced turkey) (Massei *et al.* 2003) to greater that 12 months (pheasant meat) (G. Massei unpublished data). Further trials are required to determine the suitability of levamisole as a CTA agent for field use.

Understanding the factors that influence the strength and persistence of CTA is necessary for any practical application (Cowan *et al.* 2000). Previous studies have identified influences including type and severity of illness (Prakash 1988), length of time between consumption and onset of illness (Garcia *et al.* 1974), strength and intensity of the conditioned taste stimulus (Nowlis 1974), cue saliency and familiarisation (e.g. Nachman *et al.* 1977). Previous exposure to a flavour will decrease the ability of an animal to induce CTA to that flavour (Nachman *et al.* 1977). However, prior experience with a variety of novel flavours different to that used during conditioning may influence the acquisition and persistence of CTA. Capretta *et al.* (1975) found that immature rats that were pre-exposed to several distinctive flavours would be more likely to accept a novel flavour than those with less varied gustatory experiences. Observations from field studies (Boice 1971; Quy *et al.* 1992) suggest that wild rats living in dynamic environments with high food diversity are less neophobic than rats living under more constant conditions. If pre-exposing animals to a diversity of foods reduces neophobia to novel food, then the ability to acquire and retain an aversion to a novel food may extinguish with previous exposure to novel tastes. Most laboratory studies have raised animals on a single food before measuring CTA (e.g. Massei *et al.* 2002).

The amount of the food eaten during conditioning may also influence CTA. Barker (1976) and Bond and Westbrook (1982) found that the amount of food eaten during the initial presentation at conditioning was positively correlated to the strength of the aversion. However, Massei and Cowan (2002) found that persistence of the aversion was negatively correlated with the amount eaten during conditioning. Further investigations are needed to clarify the nature of this relationship.

CTA has often been studied using laboratory rats (*Rattus norvegicus*) as a model species to predict the CTA behaviour for other mammalian species because CTA has been assumed to have a common underlying physiological and neuroanatomical basis amongst mammals (Bures *et al.* 1998). However, the dietary habits of the model species must be considered (Daly *et al.* 1982; Ratcliffe *et al.* 2003). Dietary generalists have the ability to modify their feeding behaviour after an illness-inducing food encounter; monophagous specialists, such as the common vampire bat that feed exclusively on blood, lack the neurophysical pathways required for taste aversion learning (Ratcliffe *et al.* 2003). Dietary generalists such as *R. norvegicus* are omnivores (Lund 1994) and therefore neuroanatomically more suitable for CTA studies. In most cases, predictions based on CTA studies of *R. norvegicus* have been highly accurate when applied to other species, even in determining appropriate dose rates (Nicolaus *et al.* 1989a).

In this chapter, I report on two experiments on CTA. Given the problem of excessive bait uptake, caching and monopolisation by foxes, and the recent success in inducing CTA in pen trials, the ability of levamisole to induce CTA to bait in free-ranging foxes is examined. The second aims to determine whether CTA in rats can be influenced by pre-exposure to a varied diet; as noted above, this is an important consideration when developing strategies for field use of CTA. Foxes were unavailable for this trial, therefore laboratory rats (*R. norvegicus*) were used as a model species, given their generalist feeding habits (Lund 1994) and successful use as a model species for CTA (e.g. Nicolaus *et al.* 1989a; Massei and Cowan 2002).

4.2 Methods

4.2.1 Field studies of bait aversion in foxes

4.2.1.1 Study sites

The study was conducted on two agricultural properties, "Gundabooka" and "Larras Lake North", situated near the towns of Cumnock and Molong respectively in the Central Tablelands of New South Wales study area (see Figure 1.1). The treatment site ("Gundabooka") and control site ("Larras Lake North") are mixed grazing and cropping properties approximately 800 ha in area. Both properties consist of native and improved pasture grasses intersected by patches of open *Eucalyptus* woodland and cultivated areas of cereal and pasture crops. They were chosen for their similarity in habitat, management practices and location. The properties are spaced over 5 km apart. Based on the average home range size for foxes in this area (Saunders *et al.* 2002a) these sites were adequately distanced to ensure independence from the effects of foxes from each other.

4.2.1.2 Baiting

The basic protocol for the experiment on the treatment site follows the pre-treatment, treatment and post-treatment design (Reynolds 1999). Pre-treatment consists of presenting untreated food to examine whether food consumption would normally occur. When untreated baits are consumed, treated food (containing the aversive chemical) is presented. Following consumption of treated food, untreated food is re-presented to determine if an aversion to the food has been induced. Any changes in food consumption following treatment (i.e. during post-treatment) are likely to be due to foxes developing CTA to this food.

At the control site, each station was baited with untreated day-old chickens for the entire trial period and any consumed were replaced. This allowed the response of foxes to untreated day-old chickens to be determined. Day-old chickens (hereafter called bait) were used since they are highly attractive and palatable to foxes all year (Chapter 3). On the treatment site, pre-treatment consisted of placing untreated bait at each station until a fox removed it. A microtransmitter (Sirtrack, Havelock North, New Zealand) was then sewn into the abdominal

cavity of a fresh chicken bait and reburied in the station. Incorporating a transmitter allowed the fate of the bait to be determined (eaten, cached, moved etc.) by radio-telemetry, as described by Saunders *et al.* (1999). At each station on the treatment site, the treatment stage started following the consumption of the first bait with a transmitter. Treatment consisted of presenting a further day-old chicken injected with levamisole hydrochloride in the abdominal cavity. Levamisole hydrochloride was obtained from Sigma-Aldrich, Castle Hill, Australia. Because levamisole can generate CTA to meat at 70 mg kg^{-1} of fox body weight (Massei *et al.* 2003) and as the mean body weight of foxes in this area is 5 kg (Winstanley *et al.* 1998) a dose of 350 mg of levamisole was injected into each treated bait. Post-treatment consisted of presenting untreated bait with transmitter to determine if an aversion to the bait has occurred.

Between 23rd January and 8th February 2002, 30 and 27 bait stations were monitored every 1-2 days at the control and treatment sites respectively. All stations were placed adjacent to farm tracks, fences, and other prominent positions in open woodland, woodland and grassland habitats or along ecotones between habitats. No stations were placed in cultivated paddocks. Bait stations consisted of a circle of sifted soil 1 m in diameter with a single day-old chicken buried 7 cm below the soil in the centre. Tracks left on each bait station were used to ensure that foxes were responsible for bait uptake. Bait stations were positioned at least 400 m apart from each other to maximise the number of individual foxes visiting stations.

4.2.1.3 Bait uptake and consumption

Treated baits at the treatment site were monitored every 1-2 days for a seven-day period (i.e. after they were initially laid) and each bait consumed was replaced with another treated bait. At the end of the treatment period, an untreated bait, equipped with a transmitter, was placed at each bait station. These baits were monitored daily for 5 days and replaced when consumed. Since not all baits were initially found and taken on the same day, these periods were 'staggered' for each bait station, encompassing 12 and 10 days for the treatment and post-treatment stages respectively. Bait stations on the control site were monitored on identical days to those on the treatment site and any baits removed were replaced. Fox activity for each station was recorded as visit (station discovered but bait not unearthed or removed),

uncovered (bait exposed but not removed) or taken (bait removed). No microtransmitters were inserted into baits at the control site as previous trials had shown that >96.2%±0.42SE of untreated day-old chickens are eaten following discovery by foxes (Gentle, unpublished data). Given that levamisole-treated food reduced palatability of subsequent presented food (Massei *et al.* 2003), transmitters were incorporated into baits on the treatment site to ensure that any changes in palatability due to levamisole could be determined.

4.2.1.4 Fox abundance

Spotlight counts were undertaken on both sites at the beginning of the pre-treatment stage to determine relative fox density. Foxes were counted for three successive nights with the aid of a 100 Watt spotlight from a four-wheel drive vehicle. Counts were undertaken along a pre-defined transect, which was representative of the main habitats (open grassland, open woodland, woodland, and cultivation) within each site. The transects were 9.9 km and 11.9 km in length on the control and treatment sites respectively.

4.2.1.5 Analyses

Each site was analysed separately using logistic regression with random station effects. Lack of replication at the site level means that formal testing of site differences was not possible. Station/day records were included in the analyses once the initial untreated bait was consumed. A second indicator variable identified occasions when previous treated bait had been eaten at that station. Logistic regression was used to model the probability over time that a bait is taken on the logit scale log (p/(100-p)) where p is the percentage of baits eaten. Generalised linear mixed models were fitted in ASREML (Gilmour *et al.* 1999). Analysis of deviance (McCullagh and Nelder 1983) was carried out to test the hypotheses 1) that rate of bait uptake changed during the trial period and 2) that rate of bait uptake from a station was affected by consumption of treated bait at that station.

4.2.2 Laboratory study of diet diversity and CTA in Norway rats

The second experiment was undertaken to determine if exposure to a diverse diet affects the foxes ability to acquire and retain CTA. This is important for potential field applications of CTA since foxes are omnivores and are exposed to a wide diversity of food items in field situations (Saunders *et al.* 1995). However, given the logistical difficulties in keeping and handling caged foxes, this trial was undertaken using laboratory rats (*R. norvegicus*). The aim of this experiment was to test whether 1) the strength and persistence of CTA in immature laboratory rats is influenced by exposure to a varied diet and 2) the amount of food eaten at conditioning will influence the strength and persistence of CTA. The strength of CTA can be determined by the willingness of animals to eat the food following conditioning (using aversive chemicals) against that food. This may be determined experimentally by observing whether animals will eat the food following conditioning and if so, the amount they consume. The persistence of CTA, or the period that animals are reluctant to eat the food following conditioning, is determined by the number of post-conditioning encounters that it takes to resample the conditioned food and the amount of conditioned food eaten. Additionally, the amount of food eaten immediately before conditioning may be an important cue that assists in CTA acquisition, therefore influencing the strength and persistence of CTA.

4.2.2.1 Study animals

Thirty-five TAS strain rats (Tuck and Son, Battlebridge United Kingdom) were used during this experiment. Freshly weaned rats (21 days old) were chosen to ensure that prior feeding experiences did not influence their behaviour. Each rat was individually housed in a wire mesh cage in temperature (19-23^{o}C) and humidity (40-70%) controlled rooms on a 12 h light / 12 h dark cycle. Each cage contained a food pot and a food hopper. Water was provided *ad libitum*. Shredded paper bedding and a cardboard container were supplied for environmental enrichment. Cages were cleaned and bedding replaced at least twice per week.

The basic experimental design is shown in Figure 2.1. Rats were randomly allocated by sex and bodyweight into two groups. Both groups were continuously given SDS pellets (SDS Diet Services, Witham, United Kingdom) in the food hopper. Group 1 (varied diet) animals

were additionally given one food item per day selected from a total of 7 food items. All 7 foods were presented to group 1 animals before a specific food was presented a second time. Specific foods were raisins, unsalted raw peanuts, ground maize, quail eggs, white maggots, porridge oats and icing sugar (40:1), and ground SDS pellets. Group 2 (single diet) were fed on only ground SDS meal. Individuals were given 40 g of their respective group food in their food pot each day for 17 days before conditioning.

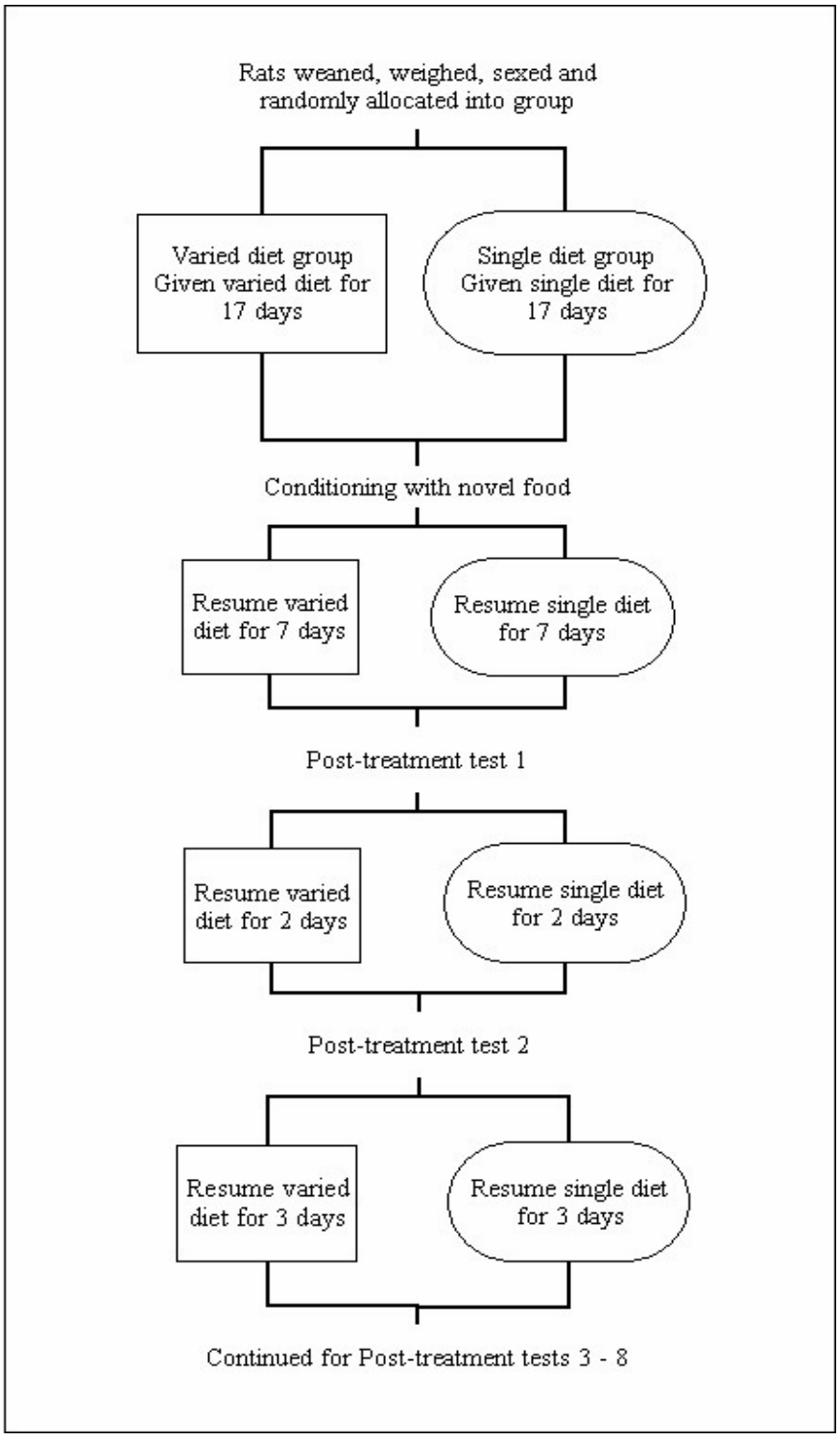

Figure 4.1: The basic experimental design for the diet diversity and CTA experiment, indicating the order of procedures undertaken on the varied and single diet groups.

4.2.2.2 Conditioning

All rats were deprived of food for 16 h before undergoing conditioning. I used a novel food of crushed digestive biscuits and cinnamon (40:1) for conditioning since it is highly palatable to rats (Gill *et al.* 2000; Massei and Cowan 2002). Each rat was presented with ca. 40 g of the novel food in a different container to their normal food pot. Individuals were observed during the 30 minute presentation of the novel food, after which the residual food was removed and re-weighed.

Rats that consumed less than 0.5 g in the initial 30 minute period were presented with the novel food for another 30 minutes. Only rats that consumed greater than 0.5 g of the novel food after this time were used for conditioning. I used thiabendazole suspended in polyethylene glycol (Sigma-Aldrich, Poole U.K) (200 molecular weight) to condition rats. Thiabendazole has been shown to induce strong and persistent CTA in laboratory rats (Massei and Cowan 2002). Body weights from the day before conditioning were used to calculate dose (200 mg/kg body weight) and intubation volume (2 ml/kg). The solution was given to each rat via oral intubation (gavage) to ensure that the aversion was associated with the novel food and not the taste of the thiabendazole (Gill *et al.* 2000; Massei and Cowan 2002). Rats were monitored for ill effects and returned to their normal diet only when they showed signs of recovery (not less than 2 h after conditioning).

4.2.2.3 Post-treatment testing

Rats' food consumption was monitored following conditioning; post-treatment testing began 7 days after conditioning when all rats had resumed normal feeding habits. Eight post-treatment tests were performed at two to three day intervals three times per week. Rats were deprived of food for 16 h before each test. Each test followed a two-choice protocol (e.g. Dragoin 1971) with approximately 40 g of crushed digestive biscuits and ground cinnamon (40:1) and ground wheat and pulegon (Sigma-Aldrich, Poole, U.K.) (600 g: 1 ml) presented in identical containers to those used during conditioning. Pulegon is a mint-flavoured deterrent that reduces food palatability. Ground wheat and pulegone (hereafter known as wheat) was used as the alternative choice for post-treatment testing since previous trials had shown it to

be significantly less palatable than crushed digestives and cinnamon (hereafter known as biscuits) (Gentle, unpublished data). Offering a less palatable food as an alternative choice during post-treatment tests gives added incentive to re-consume the biscuits and cinnamon. Animals were observed during this period and the weight of food eaten (g) recorded following completion of the 15 minute test period.

4.2.2.4 Analyses

The first post-treatment test was analysed separately to establish differences in aversion strength. This test was the first occasion that the rats were presented with the biscuits following conditioning. Therefore, the willingness of each rat to eat the biscuits and the amount of biscuits eaten are a measure of the aversion strength. Rats were classified as having eaten the food when >0.2 g was consumed during each test period. The proportion of rats consuming biscuits in the initial post-treatment test and averaged across all post-treatment tests, and the amount of biscuits consumed averaged for each group were compared to determine if the strength of the aversion differed between groups. Differences in the persistence of the aversion were tested by comparing the proportion of rats in each group (varied or single diet) consuming the biscuits in the final post-treatment test and across all post-treatment tests. Nonparametric statistics were used for hypotheses testing since the data were not normally distributed. Data were analysed using a two-group randomisation test (Manly 1991), using 500 randomisations.

4.3 Results

4.3.1 Field studies of bait aversion in foxes

4.3.1.1 Bait uptake and consumption

The transmitters indicated the proportion of baits consumed from those removed on the treatment site. Totals of 115 and 255 baits were consumed during the trial period representing 25.7% and 50% of all baits available on the treatment and control sites, respectively. The probability of a fox revisiting a station once the initial bait was consumed is shown in Figure 4.2. At the treatment site the overall rate of fox revisitation to a station (63.6% ± 6SE) remained constant during the trial period, regardless of whether untreated or treated bait was

consumed from that station. The probability that treated bait would be eaten from a station where previous treated bait was consumed was significantly less ($F_{1, 215}$= 9.7, P<0.01) than the probability of untreated bait consumption at stations where only untreated bait was consumed. There was no interaction ($F_{1, 128}$ = 0.04, P>0.05) between consumption of treated bait and subsequent avoidance of untreated bait at a station. The rate of bait uptake for treated and untreated bait did not change significantly at the treatment site for the trial period ($F_{1, 208}$=0.05, P>0.05) despite some evidence of a gradual decline (Figure 4.2). At the control site, fox revisitation after an initial bait was consumed was initially low (37.2% ± 7.5SE) but increased to peak at 86.4% (± 4.5SE). At this site foxes removed all baits when stations were visited; therefore the rate of bait uptake was identical to the visitation rate. There was a significant ($F_{1, 300}$=31.3, P<0.05) increase in the rate at which baits were taken with a linear response on the underlying logit scale.

The cumulative totals of all baits eaten on the treatment and control sites are presented in Figure 4.3. The rate of bait consumption on the treatment site was similar to that on the control until late into the treatment stage when it slowed considerably. This remained low until the post-treatment stage when the consumption rate increased.

4.3.1.2 Fox abundance

Between 10 and 12 foxes were seen per night on the control site, and between 14 and 18 on the treatment site. When corrected for the transect distance, fox abundance at treatment (1.3 foxes km^{-1} ± 0.08SE) and control sites (1.1 foxes km^{-1} ± 0.05SE) was similar.

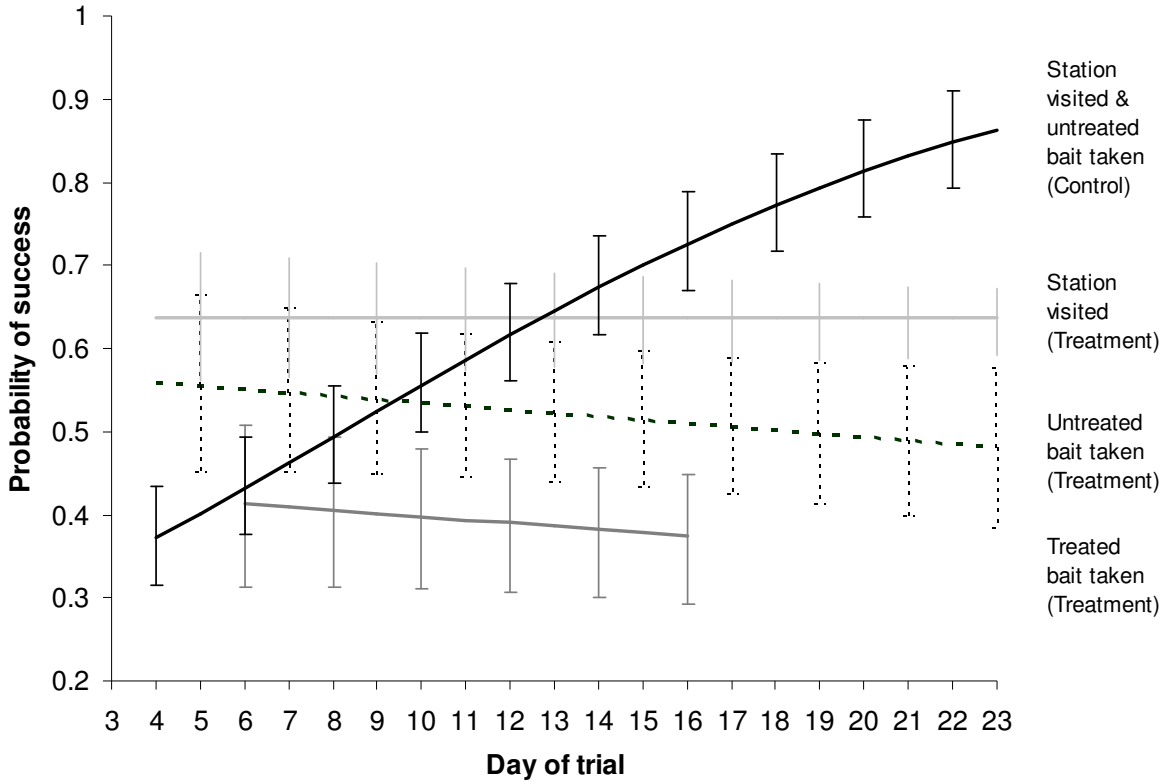

Figure 4.2: Logistic functions showing the probability of a fox visiting a station on each site (Treatment or Control) and the probability of a fox consuming a bait from each group (treated and untreated bait) on each site (Treatment or Control) during the trial period. Sites (Treatment or Control) are shown in parentheses. Error bars show standard error estimates. The probability is calculated for bait stations given prior discovery, i.e. once bait has been taken from that station.

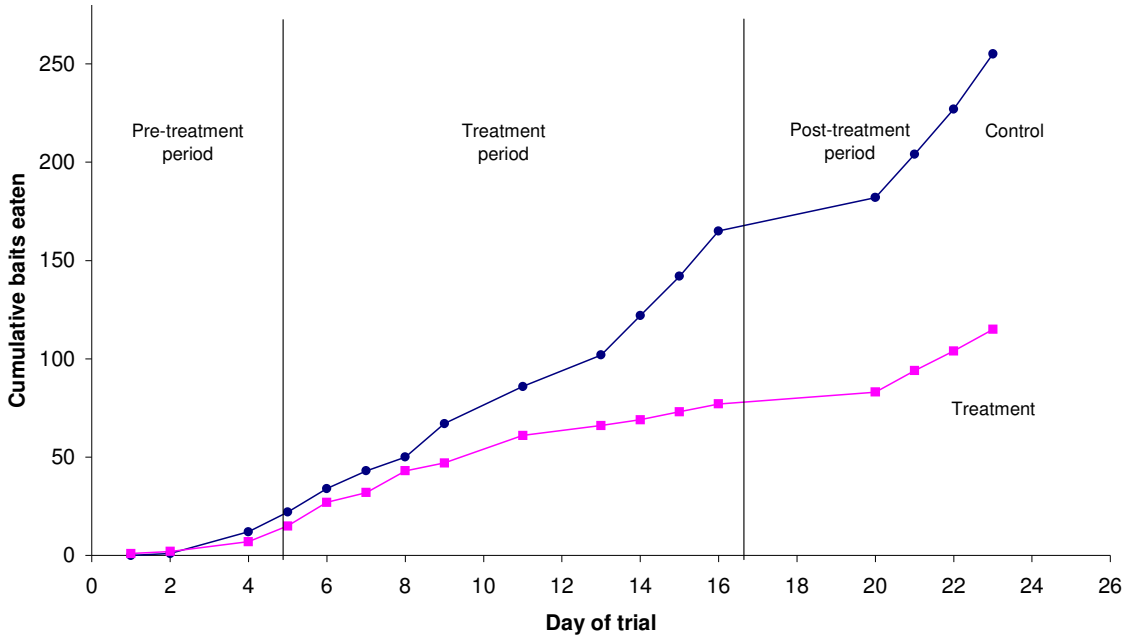

Figure 4.3: The cumulative number of baits eaten on the treatment and control sites during the pre-treatment, treatment and post-treatment trial periods.

4.3.2 Laboratory study of diet diversity and CTA in Norway rats

4.3.2.1 Study animals

Only 28 of the original 35 rats were used in this experiment. Three rats failed to consume greater than 0.5 g of biscuit during conditioning and four were unsuccessfully conditioned (orally intubated) with thiabendazole and were therefore excluded from the experiment.

4.3.2.2 Conditioning

The amount of biscuit eaten during conditioning was significantly different between groups (Randomisation test: <1/500, P<0.001) with the single diet group (mean = 1.27 g, SE =0.13, n=13) consuming more than the varied diet group (mean = 0.95 g, SE = 0.10, n=15). The amount of biscuit eaten during conditioning was compared with the number of post-treatment tests before rats consumed greater than 0.2 g of biscuits. There was no correlation (Figure 4.4) between the amount eaten during conditioning and the number of presentations before extinction of CTA for the single diet (Pearson's r = 0.22, P = 0.463) and varied diet (Pearson's r = 0.15, P = 0.598) groups.

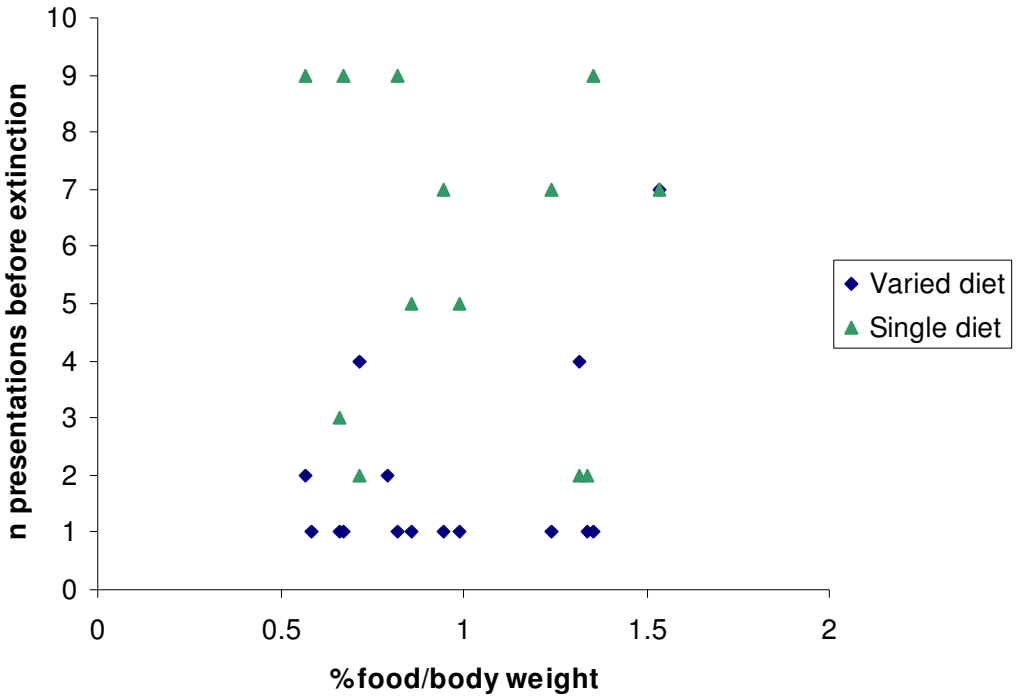

Figure 4.4: The mean consumption of biscuits expressed as a percentage of rat body weight at the initial presentation versus the number of post-conditioning tests before rats consumed >0.2 g of the biscuits.

4.3.2.3 Post-treatment testing

In the initial post-treatment test, no rats from either group consumed the biscouts and cinnamon so comparisons were based on the second post-treatments (Figure 4.5). A significantly greater (16/500, P=0.032) proportion of rats in the varied diet group consumed biscuit (0.667) than the rats in the single diet group (0.231). There was no difference however, in the amount of biscuits eaten expressed as percentage of total food eaten by the varied and single diet groups (84/500, P=0.168).

The mean amount of biscuit eaten expressed as percentage of total food eaten (see Figure 4.6) was not significantly different between the varied and single diet groups for the second post-treatment test (84/500, P=0.168), the final test (185/500, P=0.370) and across all tests (70/500, P=0.140). However, from the rats that were eating the biscuits within each group, the single diet rats ate significantly more than varied diet rats across all post-treatment tests (8/500, P=0.016).

Both groups readily consumed the alternative food during these tests, with no difference in the proportion of rats consuming wheat in the initial test (500/500, P =>0.999), second test (500/500, P=1.000) and across all post-treatment tests (460/500, P=0.920).

The single diet group showed an attenuated aversion, with a significantly lower proportion of rats consuming biscuit across all post-treatment tests (25/500, P= 0.05). This trend remained until the final test (see Figure 4.5), with a significantly (1/500, P=0.002) lower proportion of the single diet group (0.154) consuming biscuit than the varied diet group overall (0.600). There was no significant difference in the amount of biscuit eaten per group across all post-treatment tests (70/500, P=0.140) and in the final test (185/500, P=0.370). Given that there was no difference in the amount of biscuit eaten between the two groups, the average amount of biscuits eaten for rats consuming biscuits in each group was analysed. Rats in the single group that consumed biscuits consumed significantly more than those in the varied diet group across all tests (8/500, P = 0.016) and in the final test (5/500, P=0.010).

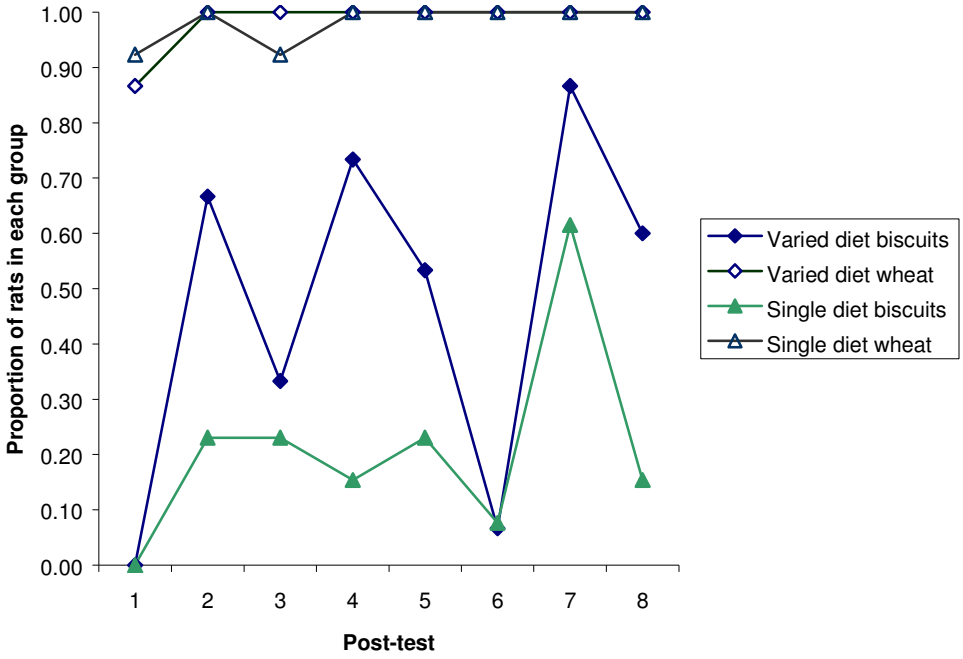

Figure 4.5: The proportion of rats in the varied and single diet groups consuming >0.2 g of biscuit and cinnamon (biscuits) or wheat and pulygon (wheat) in each post- test.

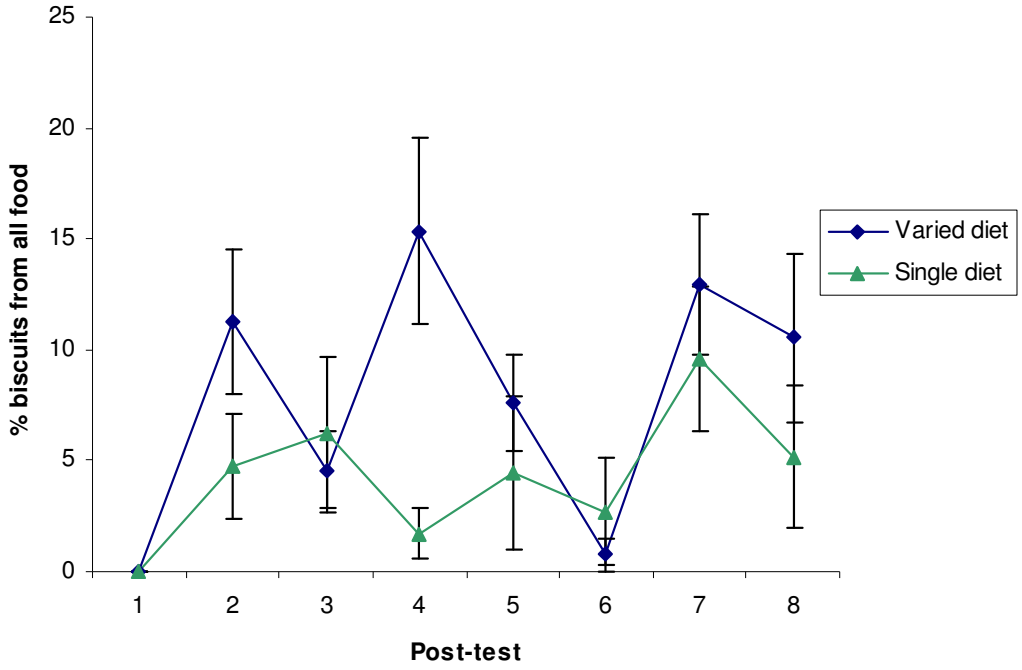

Figure 4.6: The mean percentage (± SE) of biscuit and cinnamon (biscuit) eaten by rats from total food consumed for those individuals that consumed biscuit in the varied and single diet groups in each post-treatments.

4.4 Discussion

4.4.1 Bait aversion

The lack of replication at the site level means that formal testing of site differences is not possible. Despite this, there were strong indications of differences between the rate of bait visitation and consumption at the control and treatment sites. The results show that foxes were less likely to consume treated bait from stations where a treated bait had previously been consumed than untreated bait from stations where untreated bait had been consumed. Given that levamisole induced a strong, lasting CTA after ingesting a single dose (Massei *et al.* 2003), this decrease is probably due to foxes forming an aversion to consuming treated baits. Bourne and Dorrance (1982) made a similar assumption in a field test of lithium chloride baits in targeting coyotes; they suggested that the number of baits consumed should decline markedly if resident coyotes formed an aversion. However, when untreated baits were presented at stations post-treatment, the trend of these baits being consumed returned to higher levels (see Figure 4.1). This indicates that after consumption of treated bait, foxes differentiated between treated and untreated baits, and discriminated against treated bait. Although there was evidence of a decline in consumption of untreated bait on the treatment site, the continued high rate of consumption post-treatment suggests that the aversion was associated to the presence of levamisole rather than the bait substrate.

CTA is successful when initial full consumption is followed by sudden and dramatic suppression of consumption of referent food, whether treated or not (Dimmick and Nicolaus 1990). On the treatment site, treated bait was avoided following treatment but there was no significant decline in untreated bait consumption following treatment. The avoidance of treated food only is a typical symptom for a repellent (Conover 1989) rather than a CTA-inducing agent. The ability to differentiate between treated and untreated bait is an obstruction to successfully inducing CTA. The chemical used to generate CTA must be undetectable by taste when mixed with the bait for consumption (Cowan *et al.* 2000). Detectability is problematic in the use of lithium chloride (LiCl) as an aversive conditioning chemical (e.g. Conover *et al.* 1977) since aversions will be formed to the distinctive salty flavour (Gustavson *et al.* 1976). Microencapsulation can reduce the odour and flavour cues of LiCl, and enhance

the potential for bait aversion (Burns 1983). Levamisole is odourless and tasteless to humans (Remington 1975) and unlikely to be detected in moderate doses in foxes. Massei *et al.* (2003) reported that caged foxes failed to discriminate between levamisole-treated and untreated beef-flavoured turkey meat, and presented an identical dose and similar concentration (11.7g kg^{-1}) of levamisole to this study (9 g kg^{-1}). However, bland-flavoured food may have inferior masking ability than stronger flavoured meats (Nicolaus *et al.* 1989a), making levamisole more detectable in day-old chicken than in flavoured turkey meat. Wild foxes may also be more sensitive in detecting novel flavours than caged foxes, which are habituated to consuming a variety of processed food items (such as dog biscuits). Alternatively, there may be other cues that wild foxes use to distinguish levamisole-treated bait. More work is needed to identify the cues responsible to determine whether the bait substrate (bland vs. strong flavour) or levamisole formulation (liquid vs. microencapsulated) can be altered to reduce levamisole detectability to wild foxes.

Additionally, the results highlight deficiencies in establishing CTA and quantifying the response in wild fox populations. In a study inducing CTA in wild bird populations, Nicolaus *et al.* (1989a) concluded that the spatial and temporal bait distribution should match the distribution of birds in a way that most efficiently affects the population. Bait stations that are spaced at 400 m intervals would allow individual foxes access to several stations during the experimental period. However, since not all stations had treated bait during the treatment period it is possible that some individuals did not consume treated bait during this period. Any strong CTA effect on individual foxes may have been difficult to quantify since other foxes may have been responsible for consumption of baits presented following treatment. This temporal distribution problem may be somewhat overcome by presenting treated baits for sufficient duration to ensure that all foxes within the area have the opportunity to visit and consume treated bait. Typical poison baiting operations continue for between 14 and 21 days, or until bait take is depleted (Saunders *et al.* 1995). Given the lack of independence of stations, the spatial distribution of the treatment would be improved by each station undergoing pre-treatment, treatment and post-treatment during the same periods regardless of prior consumption. This would ensure that few individuals within the population escape treatment, improving the likelihood of obtaining a population response.

Despite these shortcomings, levamisole was successful in reducing the percentage of baits consumed on the treatment site to almost half that of the control site. One practical use of these findings may be to reduce the uptake of rabies vaccine baits by individual foxes. Trewhella *et al.* (1991) suggest that dominant foxes may monopolise rabies vaccine baits, denying other individuals access. CTA could be used to reduce monopolisation and allow other foxes exposure to bait, thus improving the efficacy and efficiency of the technique. However, the use of CTA for such a purpose would not be straightforward. The successful oral vaccination of a fox depends on it consuming sufficient bait material (and enclosed vaccine) to gain the appropriate immune response. Therefore, utilising CTA to reduce bait monopolisation, without reducing the efficacy of the immunisation campaign, would be successful only where sufficient bait is consumed to achieve such an immune response *prior* to the development of a CTA. Inducing CTA to bait before achieving the desired level of bait consumption would act to *reduce* the efficacy of oral vaccination campaigns. Additionally, oral vaccination of foxes against rabies may need to be repeated in subsequent years, and individuals may have to be supplemented with the vaccine to gain the appropriate immune response. Given that the persistence of the aversion is related to the strength of illness (Garcia *et al.* 1974), the formulation or concentration of the CTA agent could be changed to influence the effective period of bait aversion. Reducing the persistence of aversion would allow others to access bait in the immediate baiting campaign, but not influence the effectiveness between campaigns. Clearly, further refinement of CTA would be necessary before applying the technique to vaccination campaigns to account for the potentially detrimental effects on bait uptake.

Similarly, the technique may assist in simulating poisoning trials. Adding levamisole to bait may act to effectively remove the individual from the susceptible population, simulating the death of a poisoned animal. If added to non-toxic bait with a biomarker such as Rhodamine-B (Fisher 1999) to measure the proportion of the population that is susceptible to consuming bait, the results will more accurately represent the results of a poisoning campaign. The technique would not completely simulate the effect of poisoning, since residents that have been made averse to consuming bait will remain able to exclude others from their territories.

The results are promising enough to warrant further investigation of CTA as a strategy to reduce multiple bait uptake and simulate poisoning trials; furthermore, the technique may be an important means of non-lethal predation management. Egg predation by foxes can significantly reduce recruitment and threaten the viability of ground-nesting birds (e.g. Tapper *et al.* 1996), and reptiles such as the Murray River turtle (*Emydura macquarrii*) (Spencer 2000) and loggerhead turtle (*Caretta caretta*) (Yerli *et al.* 1997). Given the results of this trial, and the recent success in reducing egg predation by rats using CTA (Massei *et al.* 2002), there is potential for CTA to reduce egg predation by foxes. However, further replicated field trials are required to refine this technique.

4.4.2 Diet diversity and CTA

A larger proportion of rats in the varied diet group ate biscuits than the single diet group at the second post-treatment test and across all post-treatment tests, although there was no difference between the mean amount of biscuits eaten per group during these tests. Both groups readily consumed wheat, the alternative food present during test sessions. This shows that the aversion shown by both groups is a true CTA and not simply an aversion against novel foods or novelty *per se*. Secondly, it indicates that CTA was stronger and more persistent in the single-diet group since a lower proportion of rats in this group consumed biscuits when initially presented post-conditioning and again across all subsequent post-treatments. Previous studies have reported inconsistent results; Braveman and Jarvis (1978) and Miller and Holzman (1981) found that prior exposure to different flavours did not influence the acquisition or persistence of CTA in adult rats, whereas Tarpy and McIntosh (1977) found a reduction in the attenuation of CTA in adult rats that had been exposed to multiple flavours. These inconsistencies may be explained by differences in methodology between experiments. Braveman and Jarvis (1978) and Miller and Holzman (1981) used fewer flavours (three to five) during pre-exposure than Tarpy and McIntosh (1977) and this study (nine and eight respectively). This suggests that the number of foods exposed to before conditioning may be important in influencing the strength and persistence of CTA. CTA may only be weakened by exposure to diverse foods when the level of exposure exceeds a threshold.

In addition, when testing the ability of rats to accept novel flavours, Capretta *et al.* (1975) found that juveniles were influenced by prior exposure to a varied diet (three to four foods) but adults were not. Capretta *et al.* (1975) suggested that an individual's previous experience may influence its behaviour to unfamiliar stimuli only if the experiences are during a (probably age-dependent) learning period. These findings, together with those from this study and others (Tarpy and McIntosh 1977; Braveman and Jarvis 1978; Miller and Holzman 1981) suggest that the threshold level of exposure to food diversity required to influence CTA may be age-dependent. However, Braveman and Jarvis (1978) and Miller and Holzman (1981) also used one-choice tests, which are less sensitive in measuring the strength and persistence of CTA than two choice-tests (Batsell and Best 1993) employed by Tarpy and McIntosh (1977) and this study. Further experiments are required using two-choice tests to determine whether the level of exposure to diverse foods influences CTA, and if there is an age-dependent relationship between CTA behaviour and prior exposure to novel foods.

During conditioning, the single diet group ate significantly more biscuits than the varied diet group. Laboratory rats are generally neophilic, showing a strong attraction towards new objects (Barnett and Cowan 1976). The single diet group was maintained exclusively on SDS until the initial presentation of biscuits. SDS appeared to be less preferred relative to some foods given to the varied diet group (Gentle, unpublished data). This continuous, exclusive availability of SDS may have prompted 'boredom', making the novel food presented more attractive to the single diet group. This deprivation probably increased their consumption of biscuits relative to the varied diet group that were accustomed to diversity. A heightened response towards novel foods has been noted during palatability trials with SDS versus different bait formulations (R. Quy, Central Science Laboratory, pers. comm. 2003).

These results are in contrast to those of Capretta *et al.* (1975) who found that immature rats pre-exposed to several distinctive flavours would be more likely to accept a novel flavour than those with a less varied diet. This may again be due to differences in methodology, specifically the length of test sessions used to measure consumption. Capretta *et al.* (1975) measured novel food consumption after 24 hours compared to 15 minutes in this study. Longer test sessions would disguise any initial haste to consume the novel food as shown by

the single-diet group. Any comparison between studies should therefore consider the length of the test session.

Previous studies have found a positive relationship between the amount of food eaten during conditioning and the strength of the aversion (Barker 1976; Bond and Westbrook 1982). In the present study there was no evidence of a relationship between the amount of biscuits eaten during conditioning and the number of presentations before biscuits were eaten. These results, like the weak negative relationship reported by Massei and Cowan (2002), appear to be influenced by marked individual variation in behaviour. Some rats that consumed large amounts of biscuits during conditioning continued to consume biscuits throughout subsequent presentations, albeit in smaller quantities. Other individuals consumed small amounts of biscuits during conditioning and did not resume eating during the remainder of the trial. Additionally, eight post-conditioning presentations used in this experiment may have been insufficient to determine any relationship.

This study has found that immature rats raised on a single diet will have a lower probability of consuming the food conditioned against in their second encounter, and in subsequent encounters, relative to rats raised on a varied diet. Applying this to field situations, juvenile wild rats exposed to a variety of novel foods should show a weaker and less persistent CTA than those exposed to low diversity. If CTA is to be used as a means to reduce predation then the level of protection will be reduced in conditions where the predators have been exposed to diverse foods as juveniles. Maximising the benefit of a CTA strategy may thus rely on targeting environments with low diversity, or modifying environments to decrease diversity.

These results have important implications for the use of rodenticides, particularly in baits, which is the mainstay of control programs in urban and agricultural environments (Caughley *et al.* 1998). Bait shyness is a common problem associated with sustained baiting programs. Bait shyness occurs when individuals that survive poisoning through ingesting sub-lethal doses of bait may become averse to consuming bait in subsequent presentations (Prakash 1988). Such aversion can last for long periods, significantly reducing the effectiveness of control programs. Bait shyness and CTA are forms of learned aversion and are induced by

similar behavioural mechanisms (Prakash 1988), therefore, bait shyness should be reduced in populations exposed to diverse foods. Additionally, surviving animals from populations in highly diverse environments will resume eating bait earlier than animals in relatively constant environments. A reduction in bait shyness will increase the effectiveness of baiting campaigns by improving the likelihood of individuals consuming bait.

There is some evidence from baiting programs that support these findings. Quy *et al.* (1992) found that baiting wild *R. novegicus* was generally less successful on arable farms where there was stored cereal present. Such farms offer an environment with a continuous and stable food supply, but with low diversity (Quy *et al.* 1992). In contrast, farms rearing livestock exclusively are a dynamic environment where rats are exposed to a diverse range of seasonal foods. This suggests that rodenticide applications may be improved by either targeting environments where food diversity is high, or manipulating the environment to increase food diversity. These findings in turn support Quy *et al.* (1994) who recommended that reducing habitat predictability by means such as increasing the turnover of feeds or changing distribution of food supply, may be sufficient to turn a predicted poor treatment into a successful one. The success of baiting campaigns could be improved by accounting for the diversity of food within environments.

Despite this experiment being designed to simulate food diversity that is likely to be encountered in the field, caution must be heeded in extrapolating the results from one species tested (rats) to another (fox). Other factors, such as social interaction and learning (e.g. (Schrijver *et al.* 2002; Fernandez-Vidal and Molina 2004) may interact with the results and alter them completely. Further research on foxes in field situations is required to examine the potential of dietary diversity and CTA, and the ability of other factors to impact upon them.

A decline in the strength and persistence of CTA through exposure to a varied diet suggests caution when extrapolating the results from laboratory CTA studies to field applications. If laboratory studies measure CTA with animals raised on a monotonous diet (e.g. Massei and Cowan 2002) and the results are applied to field situations (diverse food environments), the strength and persistence of CTA will be significantly overestimated. Overestimation will

decrease the expected effectiveness of CTA both in terms of the absolute level of protection offered and the period of protection. The effect of dietary diversity on CTA must be considered in the design and/or interpretation of laboratory studies for field applications.

4.5 Conclusion

The strength and persistence of CTA in laboratory *R. norvegicus* was attenuated by previous exposure to a diverse diet. This suggests that other dietary generalists, such as foxes, will also show a reduced CTA response when they are previously exposed to a diverse range of foods. Furthermore, it suggests that the results from laboratory studies where animals have been raised on single food diet will overestimate the effectiveness of CTA. However, these findings have only been tested on laboratory rats, and may be influenced by other factors not measured in this study (e.g. social, environmental).

The results from the field experiment indicated that levamisole failed to induce CTA in wild foxes most probably due to the ability of foxes to detect levamisole, but did suggest that consumption of illness-inducing bait reduced multiple bait uptake by individuals. If consumption of illness-inducing bait reduces the probability that subsequent presented bait will be eaten, then bait aversion may occur as a result of consumption of sub-lethal doses from bait in current poisoning campaigns. However, the results do not indicate the likelihood for bait aversion to occur as a result of 1080 poisoning practices. To determine this, the time until baits degrade to sub-lethal levels (Chapter 2) and the probability that foxes will find and consume baits within this time (Chapter 3) need to be investigated. This will be examined later in the thesis (Chapter 8.)