THE BEHAVIOURAL RESPONSE OF A SMALL PREY ANIMAL TO THE ODOUR CUES OF FAMILIAR PREDATORS IN A NEW ZEALAND MODEL SYSTEM.

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ABSTRACT

There is a rich ethological literature examining the effects of predators upon the behaviour of their prey. There are also apparent discrepancies in results reported particularly with respect to wild versus domesticated or captive subject species, and especially amongst Rodentia where predator avoidance behaviours predominate. Using New Zealand as a model system, the aims of this thesis were to determine how prey animals respond behaviourally to a familiar predator odour; to evaluate the role of predator exposure in prey behaviour; and to test whether captive-derived results are applicable in wild populations.

I undertook experiments under three different conditions: the standard laboratory Y-maze choice test, an enclosure test, and a field test. In each experimental scenario the prey (ship rats) were initially presented with odour treatments derived from a familiar predator (stoat), familiar non-predator (possum) and novel non-predator (chinchilla). The rats' behaviour was then video recorded for analysis of either avoidance or non-avoidance behaviour. The captive enclosure rats and the free-living rats exhibited clearly definable non-avoidance responses to stoat odour, but in the Y-maze the same captive rats did not exhibit a consistent response.

I hypothesized that the non-avoidance behaviours represented a form of predator inspection behaviour. Further captive enclosure and field experiments aimed to test whether the non-avoidance response to stoat odour was specific or generalized. Under both conditions the most marked non-avoidance behaviour was in response to male stoat and secondarily to the mixed sex cat odour. My research concluded with a snap trap experiment pairing stoat-odour lure and no-odour snap traps, yielding an overwhelming capture success in the stoat-odour lured traps.

Significant developments in the study of predator-prey behaviour resultant from this research include the development of a captive experimental protocol that generates comparable field results, the description of non-avoidance responses to predator odour, and the reporting of predator inspection a behaviour previously unreported in ship rats and rarely reported within Rodentia. Future studies should aim to verify the non-avoidance behaviours in other rat species as a first step to determining whether this is a ship rat specific behaviour, or if its lack of expression in other rodents is an experimental artefact.

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Chapter 1 GENERAL INTRODUCTION

1.1 PREDATOR – PREY ECOLOGY

Ecosystems represent an irreplaceable resource, the collapse of which will potentially have catastrophic consequences reaching beyond their immediate locale. Such losses may, and arguably already have, included species of great economic, medical, or aesthetic value. Ecosystems are never simple, and history has already demonstrated that the loss or introduction of a single species, or even single individual can have disastrous and irreversible consequences. Not only are ecological resources at risk, as exemplified by the infamous Stephens' Island cat (King 1984), that killed all surviving individuals of the Stephen's Island wren, economic resources can be equally as threatened by the introduction or removal of a single species – imagine a world without bees? The *Varroa* mite and other organisms make that a localised threat for many apiarists (Bernd 2010).

Predator-prey interactions are an integral component to the functioning of all ecosystems and have been the subject of considerable theorizing and experimental research. Despite this there exist disparities in the predator-prey research suggesting that while we know a great deal, much more remains to be learnt and understood. In order to make the best use of increasingly stringent financial resources globally, the onus is on current and emerging researchers to address these disparities and anomalies; thus demonstrably advancing our understanding of predator-prey ecology.

1.1.1 POPULATION ECOLOGY

Predator-prey interactions have a certain mathematical appeal, they seem to be intuitively dynamic: prey numbers increase, therefore predator numbers increase, ultimately leading to prey decline and resultant predator crash; and the cycle starts over. The Lotka-Volterra (continuous time) and Nicholson-Bailey (discrete time) models are examples of early, somewhat simplistic mathematical predator-prey models (see Bonsall & Hassell 2007 for review). Together these models have formed the basis for more advanced mathematical and computer modelling of predator-prey theories. Many of the later mathematical models are based on the assumption that population density of predators and prey is the driving force behind the patterns and cycles seen in nature. More complex models are able to integrate components such as resource availability or multi-predator systems.

1.1.2 PREDATOR – PREY SYSTEM MODELLING

Predator – prey models were initially developed as simple two-species models, whereby the predator population was often seen to oscillate along with that of its prey species e.g.

snowshoe hare and lynx. However, also seen in nature are cases where, for example: (1) prey seemed to reduce to low levels and stay there, (2) prey populations irrupted periodically, and (3) no effect on prey could be determined because the predator-prey population fluctuations appeared unrelated. Many of these early models did not take into account ecosystem complexity. The snowshoe hare and lynx, or lemming and arctic fox relationships typify advances in our understanding of the complexities of predator-prey interactions. Based upon 200 years of fur trapping records, the initial interpretation by Charles Elton (1924), and later Elton and Nicholson (1942) was one of predator-prey oscillation. More recently this relationship has been re-examined and while the lynx population may be regulated by a single process, food resource limitation (i.e. hare numbers); the snowshoe hare population is regulated by multiple ecological processes possibly involving both food limitation and predation (Stenseth et al. 1997).

Advances in both ecological understanding and computing power have led to the development of models that include more than one level of predator or prey; or other factors such as food resources e.g. Courchamp, Langlais & Sugihara 1999; Courchamp, Langlais & Sugihara 2000; Blackwell et al. 2003. While these newer models are an improvement because they do attempt to acknowledge some of the multiple interactions that occur within any ecosystem, they still fall short of satisfactory. All these models require assumptions about regulation to be made. While all predators are reliant upon bottom up processes, the supply of prey, the effects of predators can be variable. For example hyper-predation is a top-down process which may cause prolonged prey suppression (Letnic & Dickman 2006) and can in extreme cases drive prey into a predator pit, or even to local extinction (Dickman pers comm.); the meso-predator release model (Courchamp et al. 1999) assumes that top-order predator density drives middle-order predator density (top-down regulation); irruptive cycles assume that food resources drive prey-density (bottom – up regulation). Furthermore many of the models of predator-prey interactions assume a co-evolution of species (Begon, Mortimer & Thompson 1996). This is clearly no longer the case in many island nations, including New Zealand where for example all mammalian predators are introduced, and did not themselves co-evolve e.g. mustelids (three species, all from Europe), *Rattus* spp. (three species: Pacific, Asia & Europe), and possums (*Trichosurus vulpecula;* Australia).

1.2 EVOLUTIONARY ECOLOGY

What happens when a predator has eaten all the rabbits it can easily catch? Does it persevere with catching every last rabbit? Does it allow itself to starve? While an extreme specialist predator may be limited to those two options, most predators are not that specialised (Krebs & Davies 1993). When the rabbits become too scarce or difficult to catch the predator simply shifts its attention elsewhere; rodents, birds, even invertebrates will provide sufficient nutrition for the predator to survive until rabbit populations recover: i.e. predators are responsive at an individual as well as species level.

Mating and reproductive success for both predators and prey may depend on factors such as the size and quality of a territory held, mate selection, mate guarding, nest defence and food procurement for mate and offspring. Thus the evolutionary arms race between predator and prey drives each at both individual and species level to develop strategies that may in the long term be physiological, such as increased speed or strength; but is often initially behavioural e.g. to run away from an attacker or chase down prey, fight off an attacker or take on larger prey. Both predators and prey must meet needs other than to eat but not be eaten in order to ensure their lifetime reproductive success. How an individual behaves will largely determine their lifetime reproductive success, and if such behaviours are learned they will also affect the success of their offspring and subsequent generations (see Krebs & Davies 1993; Schmitz 2005 for discussion).

While the effects of factors such as population density and food resources can be successfully modelled and predictions made, how animals as both individuals and species behave can seriously impair the reliability of modelling in real world situations. This shortcoming of mathematical modelling was recognised in 1989 by Lima and Dill who strongly encouraged further research into the role of predation in decision making by prey animals: "..Few failures, however, are as unforgiving as the failure to avoid a predator: being killed greatly decreases future fitness..." (Lima & Dill 1989).

1.2.1 THE ROLE OF BEHAVIOUR IN PREDATOR – PREY INTERACTIONS

As living beings neither predators nor prey are governed by mathematical rules, their very existence is a direct result of their ability to adapt to new challenges including eating but not being eaten (Krebs & Davies 1993; Schmitz 2005). Much research has been carried out in the field of predator-prey interactions, particularly the 'fear' response of prey animals to a predator cue. Within the mammalian, and more specifically the Rodentia literature, there is a general consensus of interpretations that suggest prey species exhibit an avoidance or aversive behaviour when confronted with a predator threat. Whilst this response is well demonstrated in laboratory studies, field evidence of such behaviour is more difficult to identify (reviewed by Kats & Dill 1998; Apfelbach et al. 2005).

Not all prey animals exhibit an avoidance response towards a predator cue, in some species, particularly piscean and group living species a behaviour referred to as 'predator inspection' has been reported (FitzGibbon 1994; Godin & Davis 1995; Brosnan, Earley & Dugatkin 2003;

Caro et al. 2004; Dugatkin et al. 2005). This differs from more aggressive behaviours such as mobbing and maternal aggression primarily in that it does not culminate in an attack on the predator (e.g. Vieth, Curio & Ernst 1980; Hein 1996; Swaisgood, Owings & Rowe 1999; Owings et al. 2001). The exhibition of this seemingly highly risky approach behaviour has been variously attributed to mate selection, individual status within the group, co-operative reciprocity within the group, alerting the predator to its detection, information gathering and risk assessment amongst other causes. Surprisingly, despite the plethora of rodent predator-prey literature, and the group living propensity of many rodent species, there is no clear reporting of 'predator inspection' in rodent research.

1.3 RODENTS IN BEHAVIOURAL RESEARCH

Predator-prey interactions, particularly the avoidance response of prey species to both familiar and unfamiliar predators is a popular theme of research with a wealth of literature (Kats & Dill 1998; Apfelbach et al. 2005). In part this can be seen to stem from the importance of such studies for both human and non-human behavioural research. Laboratory rodents and their 'fear' responses feature highly within both the psychological and neuroscience literature (e.g. Adamec et al. 1998; Dielenberg & McGregor 1999; Adamec, Blundell & Collins 2001; Blanchard et al. 2001; McGregor et al. 2002; Endres, Apfelbach & Fendt 2005; Adamec, Blundell & Burton 2006; Guimaraes-Costa et al. 2007; Mitra, Adamec & Sapolsky 2009); and the ecological and ethological literature (e.g. Adamec & Shallow 1993; Naidenko et al. 2003; Voznessenskaya et al. 2003; Hubbard et al. 2004; Zhang, Sun & Novotny 2007). While the psychological and neuroscience research often seeks to elicit a very specific response: 'fear'; and measure this via physiological parameters and changes within the brain (e.g. Adamec et al. 1998; Adamec et al. 2001; Adamec et al. 2006; Mitra et al. 2009); the ecological and ethological fields focus more upon behavioural changes such as aversion or avoidance, albeit with some physiological manifestations such as breeding suppression and body weight (e.g. Adamec & Shallow 1993; Naidenko et al. 2003; Voznessenskaya et al. 2003).

The rodent literature is dominated with studies indicating avoidance responses towards a range of predator cues, however, other study subjects particularly piscean, avian and other mammals are often reported to exhibit non-avoidance responses towards predators and predator cues (e.g. Owings & Owings 1979; Vieth et al. 1980; Godin & Davis 1995; Randall, Hatch & Hekkala 1995; Swaisgood et al. 1999; Kleindorfer, Fessl & Hoi 2005; Ramp, Russell & Croft 2005; Pelligrini, Wisenden & Sorensen 2010). Similarly there appears to be a discrepancy between the results of laboratory research and that of field research. For example predator cues are often found to be effective at eliciting avoidance or foraging responses in laboratory studies (Heale & Vanderwolf 1994; Kiesecker, Chivers & Blaustein

1996; Ward, MacDonald & Doncaster 1997; Borowski 1998; Burwash et al. 1998a; McGregor et al. 2002; Wolff 2004; Ylonen et al. 2006), but not under field conditions (e.g. Ward, et al. 1997; Burwash et al. 1998b; Mappes, Koskela & Ylonen 1998; Bramley and Waas 2001; Orrock, Danielson & Brinkerhoff 2004; Wolff 2004; Baxter et al. 2006; Mella, Cooper & Davies 2010).

There is some suggestion that it may, at least in part, be the nature of the cue: i.e. a live predator, faeces, urine, scent gland extract, hair/fur, body rub, or synthetic odour; that is responsible for the variability in prey response under different experimental conditions (Blanchard, Griebel & Blanchard 2003). Unfortunately there are very few examples of studies in which the same experimental approaches in terms of subject species or odour are directly compared between laboratory and field environments. In the few examples that exist either the two environments produce differing results (e.g. Burwash et al. 1998a; Burwash et al. 1998b; Mappes et al. 1998); or one environment produced unclear results (e.g. Bramley & Waas 2001). The scale of the experimental environment has also been cited as a possible reason for apparent discrepancies between field and captive research, and it may be that studies with non-significant results remain unpublished (Jonsson, Koskela & Mappes 2000).

Along with the responses of prey animals to their predators, a second common theme within predator-prey research has been the mechanism by which such responses evolve: specifically the role of genetics (innate) versus experience (learned). Again there is much debate within the published literature (see Brown & Chivers 2005 for a detailed discussion). Some research has demonstrated that prey animals can be 'taught' to avoid predators (McLean, Lundie-Jenkins & Jarman 1996; McLean et al. 2000) via social or cultural learning (Brown & Chivers 2005) or aversive training. Some species have been taught to avoid 'exotic' predators (e.g. Maloney & McLean 1995) for which the prey can have no innate response.

Rodents, notably several rat and mouse species, are extremely successful colonisers and invaders as evidenced by their global spread concurrent with humans (MacDonald, Mathews & Berdoy 1999). To have achieved such global success as a species they must be both adaptable and capable of learning in order to survive in new habitats and when faced with unfamiliar predators. Indeed captive research demonstrates the ease with which young rats can be taught new foraging skills such as pine-comb stripping, by their 'mothers' (Zohar & Terkel 1996). The very fact that rodent species are so widely domesticated, both as laboratory research animals and pets, is testament to their adaptability. Thus it seems likely that a rodent population living in an environment in which predator exposure was limited might respond differently to a predator cue than populations living under 'normal' predator exposure, due to differences in their predator experience and opportunities to learn (Bramley, Waas & Henderson 2000).

Contrary to this suggestion, within the rodent predator response literature, there is considerable evidence for, and acceptance of, an innate predator avoidance or fear response (e.g. Adamec & Shallow 1993; Dielenberg & McGregor 1999; Adamec et al. 2001; Blanchard et al. 2001; McGregor et al. 2002; Hubbard et al. 2004; Endres et al. 2005; Guimaraes-Costa et al. 2007). This response is primarily reported in the widely used predator naive domestic laboratory variants of mouse and rat species.

Domestication is recognised as having both physical and behavioural effects on animals including a reduction in brain size comparative to body size; increased tendency for sociality; and a greater retention of juvenile, submissive behaviours (Clutton-Brock 1999). In laboratory rats specifically, behavioural differences including increased tameness and curiosity, as opposed to fear of novel objects, has long been recognised (Barnett 1975). However, the effects of domestication and particularly the behavioural traits that might have been deliberately or inadvertently selected for are not widely considered in the literature. This despite some researchers' cautioning even the suitability of particular strains (c.f. 'breed') of laboratory rodent for some research questions (Robinson 1983), and comparisons between wild and laboratory *Mus musculus* which show clear differences (Coulston, Stoddart & Crump 1993).

The heavy reliance upon both domesticated laboratory variants, and laboratory based studies in the field of predator-prey interactions in general, requires that attention be given to ensuring that our results and interpretations are indeed reliable outside of the context of any given study. This concern was raised in 1999 with respect to the Norway rat when MacDonald, Matthews and Berdoy expressed concern at the dearth of literature pertaining to wild-type Norway rats. What there was primarily comprised of toxicological studies for poisons, with "scarcely a handful" of an estimated 24,000 publications dealing with the behaviour and ecology of Norway rats in the wild (MacDonald et al. 1999). The situation with the ship rat (*Rattus rattus*) in New Zealand is similar, whilst there is literature relating to ship rat population demography, diet and control (e.g. Daniel 1973; Innes 1977; Daniel 1978; Hooker & Innes 1995; Innes et al. 1995; Brown et al. 1996; Alterio, Moller & Brown 1999; Innes et al. 2001; Blackwell et al. 2003; Shapiro 2005; Spurr et al. 2007; Wilson et al. 2007); there is very little research that investigates the behaviour of this species, much of which was framed to produce management-orientated outcomes (e.g. Cowan 1976; Burwash et al 1998a; Burwash et al. 1998b; Bramley & Waas 2001; Spurr et al. 2007). While management advances are a desireable outcome particularly in pest species research, a more holistic behavioural approach may ultimately prove more insightful and useful.

1.4 PREDATOR-PREY INTERACTIONS IN CONSERVATION

Predator-prey interactions are integral to understanding and preserving the increasingly fragile global ecology, whether it's the preservation of top order carnivores such as African lion (*Panthera leo*), or the predator naive, flightless little spotted kiwi (*Apteryx owenii*). Within continental or oceanic ecosystems, it is often the top order predators that are at the greatest risk from factors such as range contraction, prey scarcity and human predation, examples include Ethiopian wolf; African large cats such as lion and cheetah; and polar bear (IUCN 2011). It is acknowledged that in these large scale ecosystems it is the state of the higher order predators that might best reflect the state of the ecosystem as a whole (e.g. Kirk et al. 2010; Stevens et al. 2010). However, in island systems it is often prey species that are the focus of conservation efforts, particularly where exotic predators such as rats and mustelids have been introduced. New Zealand and the Hawai'ian archipelago offer numerous examples of faunal demise largely due to exotic predators (King 1984; Wilson 2004; see also Innes 2005 for discussion; Anon 2012). Such systems, while in many respects complex, often have an exotic predator suite that does not suffer from the complications of a co-evolution of predator and prey. Thus the system in New Zealand offers an opportunity to study in the real world a system much more akin to the original mathematical models such as Lotka-Volterra and Nicholson-Bailey, than the continental systems which inspired them.

1.5 NEW ZEALAND AS A TEST SYSTEM

The devastating effect of these exotic predators on the New Zealand indigenous ecosystem makes predator-prey research both economically and emotively important. New Zealand offers an opportunity to investigate the effect of predator exposure on a prey species both in captivity and in the wild. Two particularly widespread invasive pest species in the New Zealand forest ecosystem are the stoat (*Mustela erminea*) and the ship rat (*Rattus rattus*) (Innes 2005; King & Murphy 2005). While these two species are not co-evolved predator and prey in their natural habitat (Europe and the Indian sub-continent respectively), they have a recognised predator-prey relationship in New Zealand (Innes 2005; King & Murphy 2005).

Both ship rats and stoats are often targeted for lethal control for the protection of endemic avifauna in particular, but not always at the same time. In areas where the control is targeted for stoats (or possums) although ship rats are also killed (Innes 2005; King & Murphy 2005), their numbers are sometimes so high that the control has little impact, or the scale of control is such that they are able to rapidly re-invade post control operation. Thus the circumstance arises where populations of ship rats can be found living under conditions where stoat populations have been drastically reduced, i.e. under low predator exposure; and where stoat predators have not been controlled, i.e. 'normal' predator exposure. Similarly domestic cat (feral or house) populations are present in high or low density according to proximity to human settlement or forest edge. Thus populations of ship rats with different predator learning opportunities potentially exist.

1.6 THESIS RATIONALE

Much of the predator-prey research to date, particularly that investigating the avoidance responses of prey to predator cues, has been undertaken using a domesticated laboratory variant of a rodent species Norway rats (*Rattus norvegicus*). These animals are not merely predator naive, but have been selectively bred for laboratory research for hundreds of generations, thus whilst their predator naivety has been cited as evidence for the innate avoidance responses seen, little consideration has been given to their domesticated status and the potential influence of, for example, 'personality traits' such as boldness or timidity that may have deliberately or inadvertently been selected for.

While there is some acknowledgement of the role of genetics and breeding in laboratory rodent behavioural research (Sousa, Almeida & Wotjak 2006), this factor is rarely given much consideration in the interpretation of results. Where wild captured or free-living study animals are used there is less evidence for the avoidance behaviours commonly reported using laboratory Norway rats (as reviewed by Kats & Dill 1998; Apfelbach et al. 2005). Another potential problem in the translation of much predator-prey research into the 'real world' is the experimental methodology. The use of enclosed spaces and impositions of temporal limits i.e. experimental scale; whilst effective in standardising experimental procedure may not be conducive to the exhibition of 'natural' behaviours by the subject animals (Jonsson et al. 2000; Blanchard and Blanchard 2003).

Other potential shortcomings are the subjectivity of behaviour and its analyses; and the recognised possible confusion in interpretation of aversion versus avoidance behaviour e.g. (McGregor et al. 2002). Similarly the same behaviours might be viewed and interpreted differently by different researchers. For example, Ramp and colleagues (2005) compare their interpretation of Parma wallaby (*Macropus parma*) response to dog urine with that of Blumstein and colleagues (Blumstein et al. 2002) who suggested that as Parma wallabies spent an increased amount of time foraging in the presence of dog urine than herbivore urine they had lost the ability to respond to predator urine within only a few generations of captivity. Ramp and colleagues (2005) found a similar trend, but interpreted it as evidence of predator inspection as a deliberate anti-predator strategy. While behavioural research is often recognised for its subjectivity, and physiological data are necessary as supporting evidence (Beausoleil 2006), the procedures involved are not always practicable and particularly in wild individuals, the stresses associated with obtaining these measurements may mask genuine responses.

In order to move replicable predator-prey research out of the laboratory and into the 'real world' much more work needs to be undertaken: for example utilising species that have not been subjected to generations of domestication; utilising experimental environments and procedures that are more subject friendly and natural; and aiming for demonstrably replicable results between captive and field settings. Whilst these objectives have largely been met in the piscean, avian and large mammal predator-prey literature, the rodent predator-prey literature in particular remains largely dominated by domesticated subject species and laboratory based research. There are a few notable exceptions such as Bramley and Waas (2001), Bramley, Waas and Henderson (2000), and to some extent Harper and Vietch (2006) although this latter study was more concerned with interference competition between wild Norway and Pacific rats than predator- prey interactions *per se*. Thus in order to better understand the role of experience in rodent predator-prey interactions a less traditional approach needs to be taken.

1.7 AIMS OF THE STUDY

The aims of this thesis were to (1) determine how prey animals respond behaviourally to a familiar predator odour, (2) evaluate the role of predator experience in prey behaviour, and (3) test whether captive-derived results are applicable in wild populations.

Using ship rats (prey), and stoats and cats (predators) in New Zealand as a model system, in order to address these aims I had several research objectives:

A. To analyse prey (ship rat) behavioural responses to the odour of a co-existing and thus presumed familiar predator (stoat) in terms of avoidance behaviours as measured by approaches and foraging; and any non-avoidance behaviours exhibited.

B. To compare the responses elicited in wild caught ship rats under captive conditions with those elicited in free-living ship rats in field experiments.

C. To compare the behavioural responses of ship rats sourced from predator managed versus unmanaged locations i.e. areas of normal versus low potential predator exposure.

Accomplishment of these aims will add to the base of knowledge about predator-prey interactions, particularly the behavioural responses of wild versus domesticated subjects in New Zealand. As exotic predator management is integral to the preservation of many island ecologies, with invasive *Rattus* spp. frequently occurring as either a primary or secondary predatory threat to endemic fauna, a greater understanding of the behavioural responses of these species will add considerably to further developing management strategies.

1.8 MEASUREMENT OF RESPONSES AND ISSUES OF METHODOLOGY

Measuring the 'fear' or aversive and / or avoidance response of rodents in particular has taken two forms: the physiological which may for example include direct or indirect measure of corticosteroids or other indicators (Ribeiro-Barbosa et al. 2005; Ylonen et al. 2006); or monitoring of breeding success (Naidenko et al. 2003); and behavioural responses such as time spent in the presence of a cue, arm selection in a Y-maze, hiding, freezing, rearing, and other variously described 'cautious' approach behaviours (reviewed by Kats & Dill 1998; Apfelbach et al. 2005). Behavioural measurements are generally extensive and subjective in comparison to the physiological measures in which defined parameters are objectively measured and recorded.

The way in which an experiment is undertaken may affect the range of behaviours displayed. Many researchers cite freezing as a defensive response in rats (e.g. Blanchard et al. 2001; Hubbard et al. 2004; Endres et al. 2005; Guimaraes-Costa et al. 2007), and thus when this is exhibited in an experimental context the rat is classed as demonstrating 'fear' towards the stimuli. Under wild conditions a freeze response may well occur, but at some point the rat must move on: how and when that next action occurs may be a more accurate reflection of the true motivation of the animal. Thus in much laboratory research where the subject animals are both spatially and temporally restricted it may be that the behaviours reported are only the first in what would naturally be a series of responses (Blanchard & Blanchard 2003).

Where research is undertaken on domesticated variants of rodent species, often they are totally predator naive. If predator response is in part a learned behaviour, these animals have been deprived of this opportunity and cannot be expected to demonstrate behaviours that have not been developed within their behavioural repertoire. Similarly many domestic laboratory rodents are group living species, and are housed in small colonies within the laboratory environment, but are removed from the natural and secure group setting either permanently or temporarily and tested in isolation. Thus an animal that is in its natural state colony living is being assessed as an isolated individual in the inescapable presence of a presumably 'fear-inducing' stimulus, a predator cue; i.e. there is little regard for the ethology of the subject animal within the experimental design (Genaro & Schmidek 2000). That there are discrepancies in the literature, particularly between wild and captive predator-prey research is therefore unsurprising.

In order to address some of these issues an attempt was made to:

1) use a non-domesticated, wild captured rodent as a subject species;
2) naturalise the captive housing and experimental setting both spatially and temporally as much as possible, including the use of remote video recording of behaviour;
3) use a predator cue presentation that was representative of what might be encountered in the wild habitat;
4) use easily definable and quantifiable behaviours for preliminary analyses of avoidance behaviour;
5) utilise any unexpected but readily and frequently observed behaviours that might become apparent when the video was reviewed;
6) undertake a more traditional laboratory approach experiment as a comparison to a more natural captive experiment;
7) undertake a field version of the experiments to assess the replication of results in comparison with both the captive experimental approaches.

1.9 ISSUES OF NOMENCLATURE

The response of prey species to cues of a predation threat is a well researched subject and the evidence and examples used throughout this thesis are largely sourced from two different disciplines: that of psychological or neurological research; and that of ecological research, often with a methodology that is best described as 'behavioural' or 'ethological' research. In rodent research in particular this does introduce some potential confusion because the differing disciplines use different nomenclature. For example "The major behavioural mechanism facilitating predator detection is an activity pattern labelled 'vigilance' in the ethological literature and 'risk assessment' in the psychological literature." (Apfelbach et al. 2005). Lima and Steury (2005) in the ecological text 'Ecology of Predator-Prey Interactions' use the terms 'predation risk', 'risk perception' and 'risk assessment' when discussing the decision making strategies of prey species. Other authors refer to 'defensive behaviours' which include both 'primary' and 'secondary' defences, and behaviours including 'freezing', 'flight', 'defensive attack' and 'risk assessment' (Apfelbach et al. 2005; Blanchard & Blanchard 2005). Throughout this thesis I have used terms which I consider best describe what I interpret I am seeing or is occurring. To clarify my use of these terms I have compiled a discussion of commonly used terminology.

1.9.1 RESPONSE TERMINOLOGY

Avoidance / Aversion: both terms are regularly found in the literature. 'Avoidance' of a predator odour cue has often been reported, implying it is the odour of the predator that is causing the response, however there is some suggestion that the behaviour may be more appropriately termed 'aversion' as it is the noxiousness of the odour cue presented that causes the avoidance behaviour rather than what the odour cue represents (McGregor et al. 2002). This may be particularly true of highly concentrated odour cues presented in confined spaces. For example the concentration of the odour cue has also been reported to have an effect on how *Toxoplasma gondii* infected rats respond to cat urine (Vyas, Kim& Sapolsky 2007).

I used 'body odour' as the treatment cues, and whilst some donor odour was discernable to the human nose, particularly the male stoat and chinchilla odour, it was not to my senses 'noxious'; and in two of the three protocols the odour was presented in an open air environment. Therefore I have elected to use the term 'avoidance', understanding it to refer to any behaviour that the rats exhibit to avoid the odour source or its immediate surroundings, whilst acknowledging that there is a possibility that there may be an element of 'aversion' in motivating the responses.

Non-avoidance: any behaviour that does not involve avoiding the sample odour presentation regardless of motivation (noxiousness or what the odour represents).

Investigatory behaviour: a broad description of behaviours which involve the rats approaching to close quarters the sample odour presentation and includes rearing approach, sniffing, mouthing, and removal.

Fear: a term predominantly found in the psychology or neuroscience literature where the emotive state of 'fear' is induced via the presentation of 'fear-inducing' stimuli such as a predator cue. The 'fear' response is often measured via various physiological parameters such as blood or faecal corticosteroids or changes in brain chemistry. I have largely favoured the term avoidance in my writing, unless referring to specific examples were 'fear' is the terminology used, as it has less emotive connotations.

Rearing approach (RA): the rat approaches the treatment odour presentation (to within approximately 20cm) and rears onto its hindquarters with its attention apparently focused on the presentation (captive experiment).

Approach (A): the rat enters the foraging tray directly above which is located the test treatment odour.

Sniff approach (SA): the rat approaches the treatment odour presentation and reaches towards it either on all fours or in a rearing position to investigate at close quarters i.e. within 5cm but without physical contact the treatment presentation (captive and field experiments).

Mouthing (M): a descriptive term that I apply when the rat approaches the treatment odour presentation and reaches towards it either on all fours or in the rearing position and physically takes the odour presentation into its mouth, this sometimes escalates from a 'mouthing' to a vigorous tugging of the treatment presentation (captive and field experiments).

Chew or remove (CR): a descriptive term that I apply to the physical evidence of a mouthing interaction, that in some cases seems to escalate beyond mouthing (evidenced by tooth marks and small tears in the zip-lock bag containing the odour samples), into a removal behaviour where the entire presented cue, or the blanket into which the odour is permeated, is physically removed from its original presentation location.

1.9.2 EXPERIMENTAL TERMINOLOGY

Predator or competitor exposure: refers to the likelihood of the subject rats having encountered either specific predators (stoat and cat) or competitor (possum) in their natural habitat due to human intervention affecting their (predators or competitors) population density. I considered stoat and possum management had reduced the level of exposure, and therefore opportunities for the rats to learn from experience. Near proximity to human settlement and known cat populations I considered to similarly affect the rats learning opportunities with respect to a higher likelihood of cat exposure than sites deeper within the National Park where cats, although present, were more widely dispersed and thus a low encounter predator.

Managed / unmanaged: refers to the presence or absence of a human intervention affecting the population density of the predator (stoat) and competitor (possum) populations. Management does not equate to eradication, but direct and proxy evidence does support a significant reduction in the stoat and possum population density in the managed areas in comparison to the unmanaged areas. Thus the home habitat of the rat is either managed i.e. reduced exposure, or unmanaged i.e. normal levels of exposure to the predator or competitor species population.

Location and site: location refers to the area within a given management strategy from which the rats were sourced, usually a stretch of track or trap-line; site refers to specific points within a location where experimental apparatus were established for field research. Thus the captive rats were sourced from six locations; the fieldwork was undertaken at four of those six locations, and within each of the four field locations two experimental sites were established (see Chapter 2 General Methods; Figures 2.1 – 2.3).

Juveniles / sub-adults (J/SA): the 'cut-off' point between a juvenile rat and a sub-adult is somewhat arbitrary based on a weight of ca 30g, with weaning occurring at around 38g, whilst sexual maturity is estimated to be reached by age 3-4 months (Innes 2005). Thus the majority of rats categorized as J/SA in this research are probably more correctly termed sub-adult, but assessment was often visual only, based upon video footage, thus actual weights were not necessarily available. Captured rats that were borderline in size between sub-adult and adult were categorized via sexual maturity. If there was evidence of testicular descent or vaginal perforation then the rat was described as adult. For the purposes of this research a distinction only needed to be drawn between very young and potentially predator naive or very inexperienced individuals; and independent potentially predator experienced individuals thus the issue of distinguishing on video footage alone between older sub-adults and young adults was avoided.

1.10 THESIS METHODOLOGY AND STRUCTURE

Predator-prey research has benefitted immensely from the use of domestic rodent species in rigorously controlled experimental environments because this provides a manageable environment and malleable subject species through which to gain some insight into the possible responses of prey animals to predator cues. However, there is now a need to expand upon that approach. The concept that laboratory research has limited value in field applications, or is potentially unreliable due to the very controlled and contrived nature of subject, setting and experimental protocol, is not new (Ylonen & Wolff 1999; Genaro & Schmidek 2000; Jonsson et al. 2000; Blanchard & Blanchard 2003). Nor is the recognition of the bias in Norway rat biology and behaviour towards the domestic laboratory variants (MacDonald et al. 1999). Field research often lacks some experimental rigour and control, particularly over external factors and subject life-history, but if predator – prey theory is to be advanced it needs to be shown to apply in real-world scenarios.

If we accept that predator-prey responses such as breeding suppression and predator avoidance are generalizable concepts, rather than species or context specific traits then they must be observable in wild populations, not just in laboratory studies. Unfortunately there are very few examples of directly comparable field and laboratory predator – prey studies in the mainstream literature, and those there are do little to engender confidence. For example, ship rat studies have been undertaken with mixed laboratory results in terms of predator odour avoidance and no field evidence in either study for an avoidance response (Burwash et al. 1998a; Burwash et al. 1998b; Bramley & Waas 2001), and research into predator induced breeding suppression in voles is equally contentious (Mappes et al. 1998). Similarly the Bruce effect and parental investment hypotheses have been found lacking when laboratory and field studies are compared or a wider species pool investigated (see Ylonen & Wolff 1999 for a brief discussion). The experiments constituting this thesis utilise direct measures of behaviour in both captive and free-living subjects using comparable methodologies.

In this thesis the captive experiments take two forms, a naturalistic approach designed to allow the subject animals freedom of expression in behaviour, and a spatially and temporally restricted Y-maze experiment more representative of the traditional approach to predator cue response research. The free-living experiments were designed to assess whether the captive results could be replicated in a truly free-living population, thus assessing the validity of each captive experimental approach in eliciting natural behaviours. Overall these experiments had the aim of examining some behavioural responses of a rodent prey species to a familiar predator when exposure to the predator, and thus predator experience may vary between prey populations.

To avoid excessive repetition of detailed methodologies in each relevant chapter I have elected to present a General Methods chapter. This chapter (Chapter 2) details the phase 1 captive enclosure, Y-maze and field experiment methods in detail and where modifications or amendments were made to this methodology in phase 2, these are described.

Chapter 3 addresses objectives (A) and (C). I present two experiments: a captive enclosure experiment (naturalistic setting) and a captive Y-maze experiment; each testing the avoidance response of ship rats to the presentation of a stoat body odour cue. In the enclosure experiment the ship rats are also presented with an unfamiliar omnivore (domestic chinchilla; *Chinchilla lanigera*) odour, a familiar omnivore (possum; *Trichosurus vulpecula*) odour and a blank (control) odour on different nights for comparison. In the Y-maze the rats are presented with one of the three odours paired with the control cue each night. The Y-maze experiment was designed to largely follow the methodology adopted by Bramley et al. (2001) in their research also utilising wild-captured ship rats.

The rats themselves were sourced from different stoat and possum management backgrounds, thus were expected to have had differential exposure to, and thus learning experiences with a familiar predator, stoat; and familiar competitor, possum. I compared the willingness of the rats to approach and forage in the presence of the different odours to determine the presence of any evidence of avoidance responses exhibited by the rats in the enclosure. In the Y-maze, the same rats were assessed via frequency of entry into the treatment or control arm; and their first response upon release into the maze. The evidence of avoidance behaviour exhibited by the rats under the two experimental conditions is compared and discussed.

Chapter 4 further addresses objectives (A) and (C); and begins to address objective (B) via captive enclosure, Y-maze and field experiments. I describe and analyse a sequence of escalating behaviours which I interpret as non-avoidance (investigatory) responses to the odour presentations. The behaviours described range from a close approach to the odour source, sniffing of the odour source, a mouthing of the odour source and at its most extreme a removal of the odour source. I discuss the results in the light of competing explanatory hypotheses and examine the evidence for differential behaviours attributable to the learning opportunities of the subject rats according to management background.

Chapter 5 continues to advance on achieving objectives (A) through (C). I repeat, with modifications, both the captive enclosure and free-living experiments in the presence of three predator odour cues, and a foreign con-specific male odour cue. I discuss the results in support of my preferred explanatory hypothesis: ‘predator inspection’ and whether management background and proximity to known predator populations, i.e. opportunity to learn, affects the exhibition of the behaviours. Finally I introduce the possibility of either chemical or parasitological alternative explanations for the investigatory behaviour.

Motivators other than the predator odour may be driving the investigatory behaviours elicited: one such possibility is neophilia. In Chapter 6 I undertook a snap trapping experiment at one of my field behavioural sites to test the neophilia hypothesis against the predator inspection hypothesis. I presented the rats with paired traps, one ‘baited’ with stoat odour (treatment), the other with a ‘blank’ odour (control). The capture success of the treatment and control traps, along with the demographics of the rats kill-trapped was recorded and assessed for support of either a novel object attraction (neophilia), or a learnt predator inspection interpretation. This chapter continues to address objective (A).

There were two other major alternative hypotheses to explain the behaviour witnessed: chemical cue overlap or a behaviour modifying parasite infection. Neither of these could be fully tested with the resources available, however the possibility of toxoplasmosis affecting the rat behaviour was addressed both behaviourally and pathologically. The chemical cue overlap possibilities were in part addressed through the results of the preceding experiments. Chapter 7 presents the results of my research with respect to these two alternative possibilities and discusses their potential as behavioural motivators.

A general discussion of my results is presented in Chapter 8, where I draw together the results of all the experiments. I discuss the disparities in results between experimental approaches and suggest reasons for these. I discuss my results in terms of the management of ship rats and its New Zealand predators within the New Zealand forest ecosystem, and in a broader context in terms of the field of predator-prey research. Finally I present suggestions of future lines of research to test my interpretations of the behaviour as predator inspection and my hypotheses as to the role of learning in the development of the investigatory behaviours displayed.

I also present a series of appendices of supporting material that, whilst important in the development, application and interpretation of my experiments, was considered too detailed to be incorporated in the main body of work. Appendix 1 describes in detail the development and construction of the captive enclosure and the housing and experimental protocols. Appendix 2 describes the development and testing of the odour presentation. Appendix 3 contains the statistical reports prepared by Tian Mao and Leiyan Wang (supervised by Professor Steve Haslett, Department of Mathematics and Statistics, Massey University, NZ) relating to the mouthing response reported in Chapter 4. Appendix 4 is the laboratory report for the *T. gondii* PCR.

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Chapter 2 GENERAL METHODS

2.1 INTRODUCTION

The majority of the experiments undertaken in this research are variations on three methodologies: captive enclosure experiments, a Y-maze experiment and field experiments. In order to avoid significant repetition within chapters 2 – 5 I present here a description of the methodology used for the captive and field research, and the Y-maze experiment. Specific details such as dates and treatment cues vary with later experiments and the reader is referred to the methods section of each individual experiment for these specific details. Unless otherwise stated the experimental protocols follow that which I present below.

Predator cues: In avian and mammalian species there is evidence that acoustic cues, both con-specific and hetero-specific are utilised by prey species to detect predators e.g. (Blumstein et al. 2008; Muller and Manser 2008; Nocera and Taylor 2008). Physical cues such as faeces, urine and glandular seccretions contain a range of chemical components which may be longer or shorter lived, may communicate information about diet, and may provide some indication of 'deposition date' (Conover 2007; Parsons and Blumstein 2010). The prey animals' response may depend upon the information it is capable of gleaning from these deposits. Much odour based research has been undertaken on aquatic species, an environment in which odour transmission may be very effective and more reliable than visual cues to the prey species. I elected to use body odour as a cue because of its' perceived immediacy in terms of predator presence, and because of the evidence for reduced habituation, essential in an experiment where 'reinforcement' would not be occurring (Blanchard et al. 2001).

2.2 CAPTIVE EXPERIMENTS

2.2.1 GENERAL PROCEDURE

Subject animals: Ship rats were live captured from four separate locations within the Lake Waikaremoana (LW) area of Te Urewera National Park, New Zealand (Latitude: 38° 46' 0 S, Longitude: 177° 4' 60 E) and two separate locations within the Egmont National Park (ENP), New Zealand (Latitude: 39° 17' 60 S, Longitude: 174° 4' 0 E) between August 2009 and December 2010 (Figure 2.1). I incorporated capture sites that represented 'reduced predator exposure' i.e. active stoat management by trapping and proximity to known house cat populations; 'reduced possum competitor exposure' i.e. possum management by infrequent poisoning; and unmanaged predator / competitor exposure. Thus the ENP site consisted of a paired stoat-managed (SM) and stoat-unmanaged (SuM) location (Figure 2.2); and the LW sites consisted of paired stoat-managed and stoat-unmanaged locations, 'near' cat (NC) and 'far' cat (FC) locations; and paired possum-managed (PM) and possum-unmanaged (PuM) locations (Figure 2.3).

Support for the differences in stoat predator densities at the various sites were obtained as follows: at Lake Waikaremoana where a direct measure of stoat activity inside and outside the stoat trapped areas was not available, survivorship of threatened brown kiwi (*Apteryx mantelli*) chicks provided a proxy. Chick survival at the time of this research was estimated at 56% within the stoat-managed zone and 10% outside (J. McLennan personal communication, June 2010); at ENP stoat tracking tunnels deployed in June / July 2009 (in advance of a planned 1080 drop) recorded 14% tracking (1/7 tunnels) inside the stoat-managed zone and 63% (5/8 tunnels) tracking outside the stoat-managed zone (Kemp 2009). Cat presence was assumed based upon the proximity of observed tame and feral cat populations (near cat) and distance into the national park (far cat) (C. Gillies personal communication, May 18, 2012). The LW possum control had been undertaken within six months of the first capture session, with one capture / 1800 trap nights in post-control monitoring (O. Harris, personal communication, November 2, 2009), and within one year of the final possum odour field experiment. The return of possums post-control is usually three to five years, thus it was assumed that for the duration of this research phase possum-managed rats were living under lower possum density than normal. No possums were observedon any of the video recordings, nor was there any other evidence of possum activity such as unusually high frequency of empty sprung traps or actual possum captures at this location.

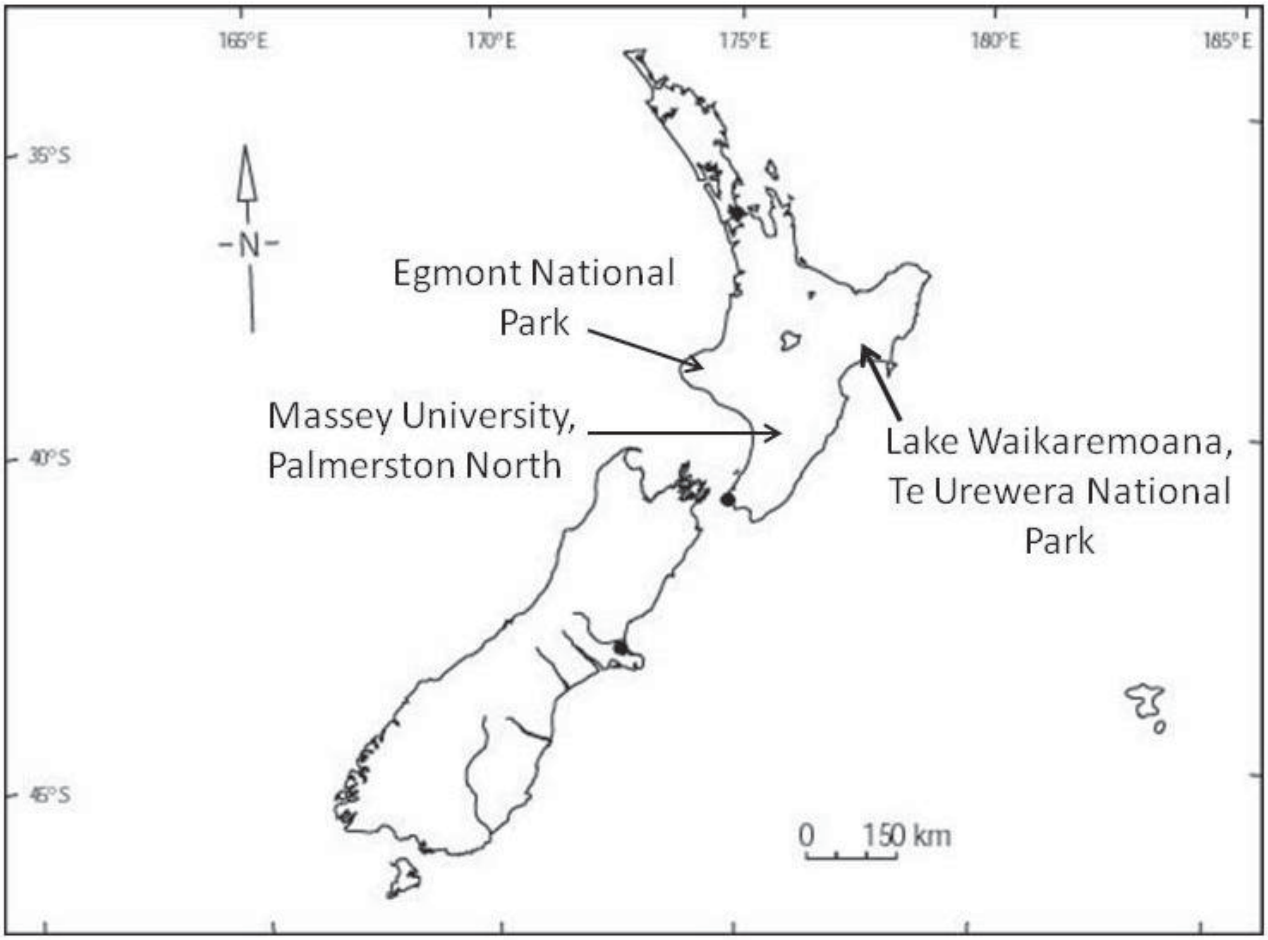


Figure 2.1 The general location of the New Zealand rat source sites: Egmont National Park and Lake Waikaremoana (Te Urewera National Park); and the location of the captive experiments: Massey University, Palmerston North (Source of map http://www.hi.com.au/hi-media/heinemannfiles/atlas/nz_001.jpg).

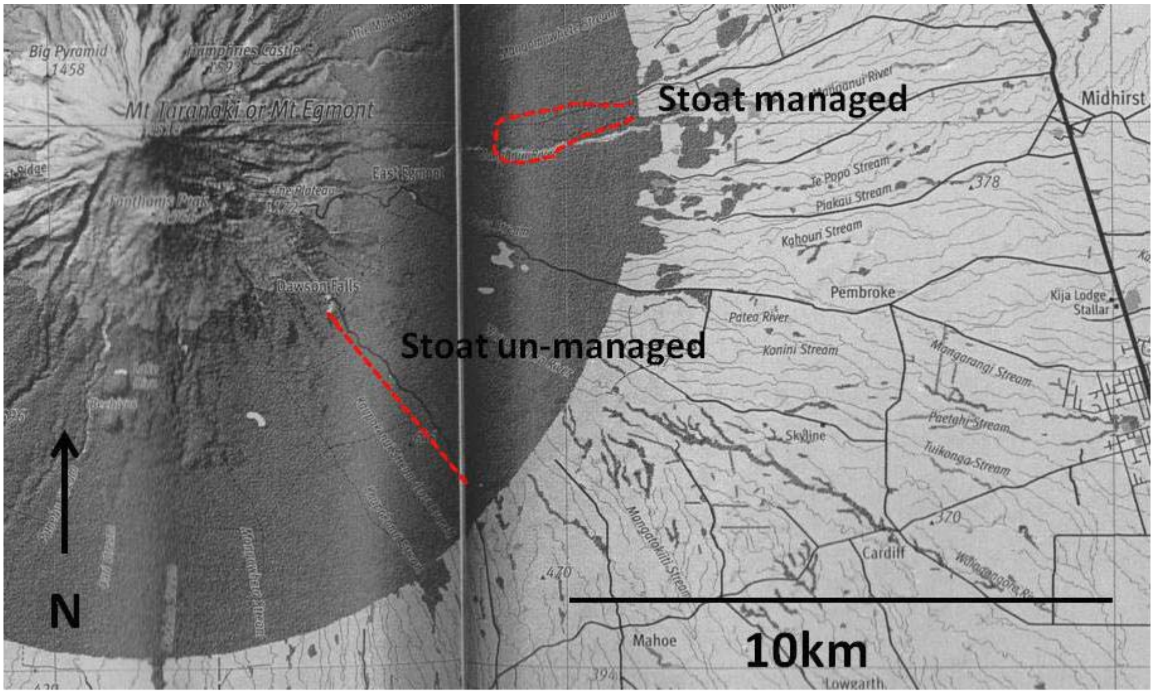


Figure 2.2 Locations of the stoat-managed (York Loop) source area and the stoat un-managed (Manaia Road) source area of Egmont National Park (Map sourced from The Geographic Atlas of NZ, 2005).

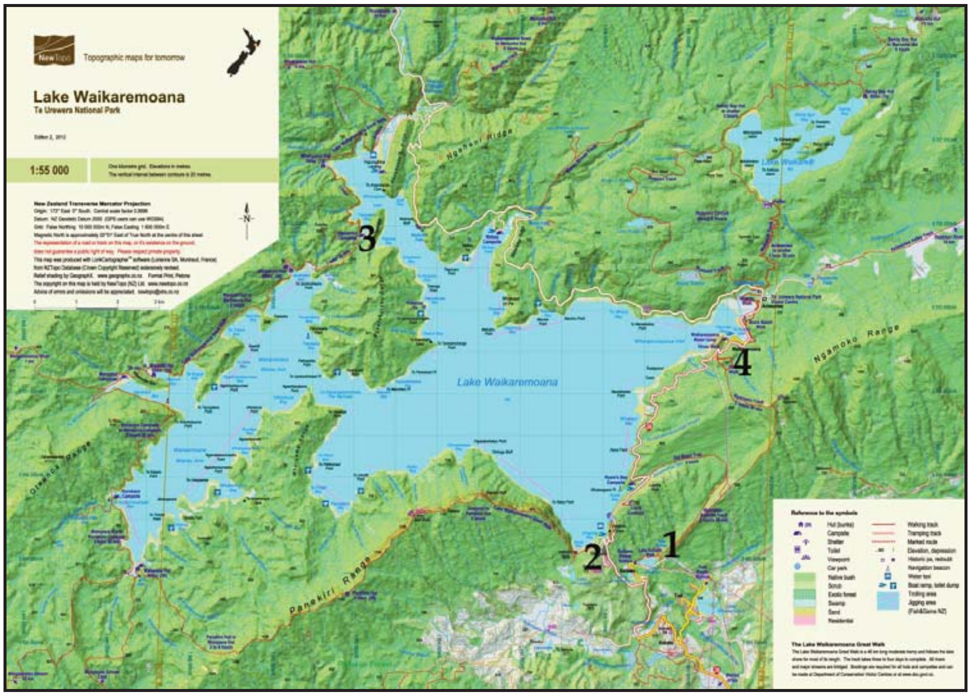


Figure 2.3 Lake Waikaremoana captive rat source and field experiment locations: 1=Kaitawa Lakes (PuM= possum-unmanaged / NC = near cat), 2= Onepoto (PM = possum-managed / NC = near cat), 3= Tapuaenui (SM = stoat-managed / FC = far cat), 4= Tawa Walk (SuM = stoat-unmanaged / FC= far cat). Map copyright of NewTopo (NZ). Each grid square = 1km distance.

Trapping of ship rats: For Phase 1 (Chapters 3 & 4) live capture trapping was undertaken at paired locations at each trapping session using "Rat Cage Trap 19RT10"(Pest Management Services, Paraparaumu, Kapiti, NZ). Individual trap positions within each study location remained consistent to within five to ten metres at each trapping session. Traps were placed at approximately 50m intervals following existing tracks or stoat trap lines and were pre-fed using chopped carrot and peanut butter for three to seven nights. Rat trapping occurred on a single night at each separate location. Traps were checked from first light the following day. Rats trapped had their sex and condition recorded; those animals that appeared particularly distressed, lethargic or otherwise unwell were released without further delay.

Eight animals (four per location) were selected per trapping session, to be taken into captivity; all surplus animals were released at point of capture. Captive research was undertaken in two phases: phase 1 between September and December 2009 and phase 2 between September and December 2010. Captive rats were selected to meet a number of criteria:

1. Adult: to increase the chance that all captive rats had encountered stoat predation, sub-adults and juveniles were excluded from captive selection.
2. Sex: I aimed to retain equal numbers of males and females from each trapping session wherever possible.
3. Healthy appearance: fit healthy animals should present normal behaviour and cope with the stress of capture. Also because of the close living captive conditions I wanted to avoid the risk of introducing any illness / contagion into the captive environment.
4. Calm demeanor within the trap: although this potentially biased the selection of captive rats by 'personality', rats that were highly distressed in the capture trap were considered more likely to succumb to capture stresses and die.

Transportation & preparation for captivity: Rats were transported in their capture traps back to a single holding location. After a minimum 24 hour capture recovery period phase 1 rats were anaesthetised, weighed, measured and PIT tagged (micro-chipped) and had their sex and sexual maturity confirmed. PIT tagging was done as I had hoped to measure arboreal versus ground activity within the enclosure however this was not achieved due to apparatus limitations and thus is not reported as part of this thesis. While anaesthetised, the rats were transferred into their nest boxes for the experimental phase of their captivity. Phase 2 rats were not PIT tagged as this was considered unnecessary for the planned experiments. The rats were instead restrained in a cloth bag for weighing and transfer into nest boxes. Additional morphological data for these individuals such as body length was obtained at the completion of the experiments when all rats were euthanised as release of an invasive pest species back into the wild was not an acceptable course of action in New Zealand. Rats in individual cages were transported back to Massey University by road in a darkened but well ventilated and secure traveling box, a three to six hour journey. Trapping, micro-chipping and transportation of rats was undertaken under MUAEC 07/135, 09/08 and 10/83.

2.2.2 *CAPTIVE ENCLOSURE*

The captive enclosure was a caged area (5.6m L x 2.1m W x 2.4m H) located at the Small Animal Production Unit at Massey University, Palmerston North (40° 21' 0" S, 175° 37' 0" E) (Figure 2.1). It had a sloping corrugated iron roof already in place and was positioned such that nearby buildings and a ca. 1.5m high wood panel fence provided some although incomplete, protection from weather. The internal area of the enclosure was divided into a housing area and four experimental compartments (Figure 2.4).

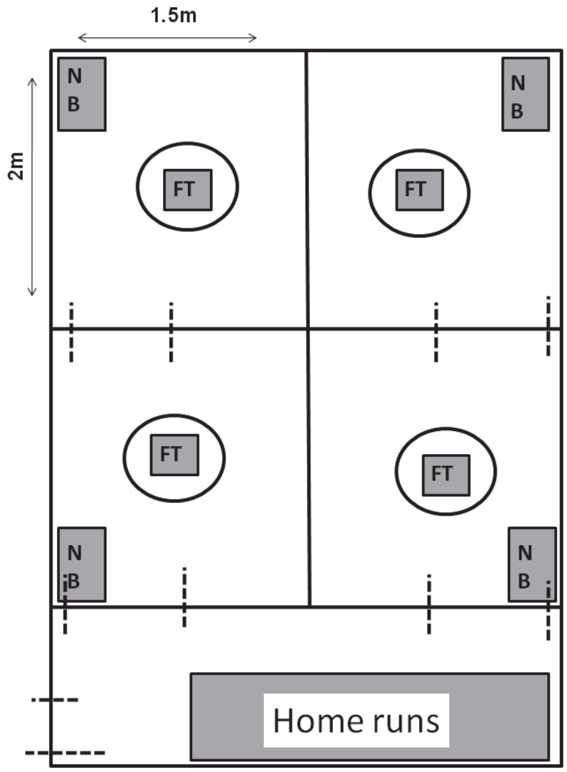


Figure 2.4 Plan view of experimental enclosures and rat housing area. FT= foraging tray; NB = nestbox location.

The housing comprised of eight individual runs, set out in a four up : four down arrangement. Each run was of wood and wire mesh construction and measured ca. 50cm deep x 45 cm wide x 90 cm tall. Each run was supplied with bamboo canes, rope and lengths of branch to allow three dimensional use of the space (Figure 2.5). Rats had access , *ad libitum* daily to carrot, apple, rodent nuts, grain and peanut butter and fresh water.

Figure 2.5 Housing runs for captive ship rats developed for the enclosure experiments.

Due to the sloping roof I standardised the compartment heights by fitting a wire mesh 'false ceiling'. Each compartment was fitted with branches to provide arboreal access across the area. The floor was lined with commercial garden bark. Centrally within each compartment a car tyre was positioned and filled with garden potting mix. During Phase 1 (Chapters 3 and 4) a number of rats (17/ 31) tunneled into the potting mix, of which ten did so routinely, often building nests within the tyre rims (pers obs) thus in phase 2 the apparatus was modified. Stones were used to pack the tyre rim and discourage this tunnelling and nesting behaviour. During phase 1 an inverted bucket feeding station as used in preliminary field trials (Carter, Barnes and Castro 2009) was positioned within the tyre, the bamboo canes used to hold the cameras could then be inserted into the potting mix substrate. Foraging trays (pumice filled ice-cream containers) were positioned within the feeding stations (Figures 2.6 and 2.7).

In phase 2, two bamboo canes were inserted into the potting mix substrate in a 'crossover' formation, from which the sample stimuli were suspended using tie-wire (Figure 2.8). Foraging trays (pumice filled 2 litre ice-cream containers) were again located centrally within the tyres with the stimuli suspended overhead at a height of no more than 10cm from tray surface (Figure 2.7 & 2.8). During both phases surveillance cameras (with integral infrared LEDs') were positioned within the enclosure to provide a clear view of the tyre, foraging tray and stimuli; and the DVR unit (Signet, JayCar Ltd, Palmerston North, New Zealand) was located in the housing area, (see Appendix 1 for full details of enclosure development and design).

The compartments were 'rat-proofed' with a layer of 13mm chicken mesh overlying the 'Hurricane' netting and steel frame main structure. Additional rat-proofing in the form of strips of aluminium sheeting was applied to any areas of ply-board where rats attempted to chew out (Figure 2.9). Additional weatherproofing was supplied on the three most exposed faces of the main enclosure using sheets of core-flute fixed to the outer walls from the top of the main enclosure to approx 70cm above ground level. Surveillance cameras were positioned to provide a clear view of the foraging trays and odour treatments (Figure 2.7 & 2.8) and the DVR unit (Swann DVR4 1000, Dick Smith Electronics, New Zealand) was located in the housing area (see Appendix 1 for full details of enclosure development and design).

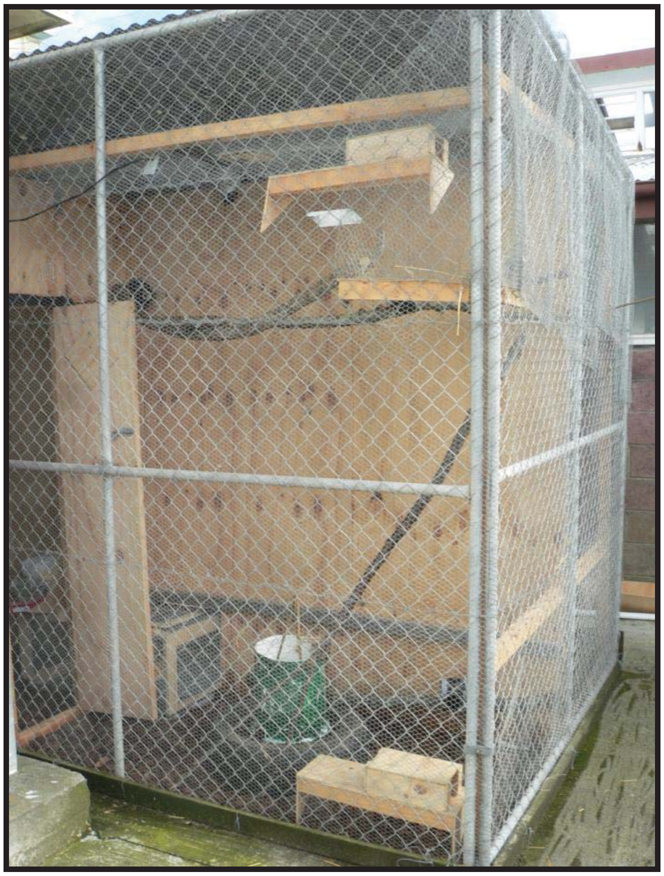
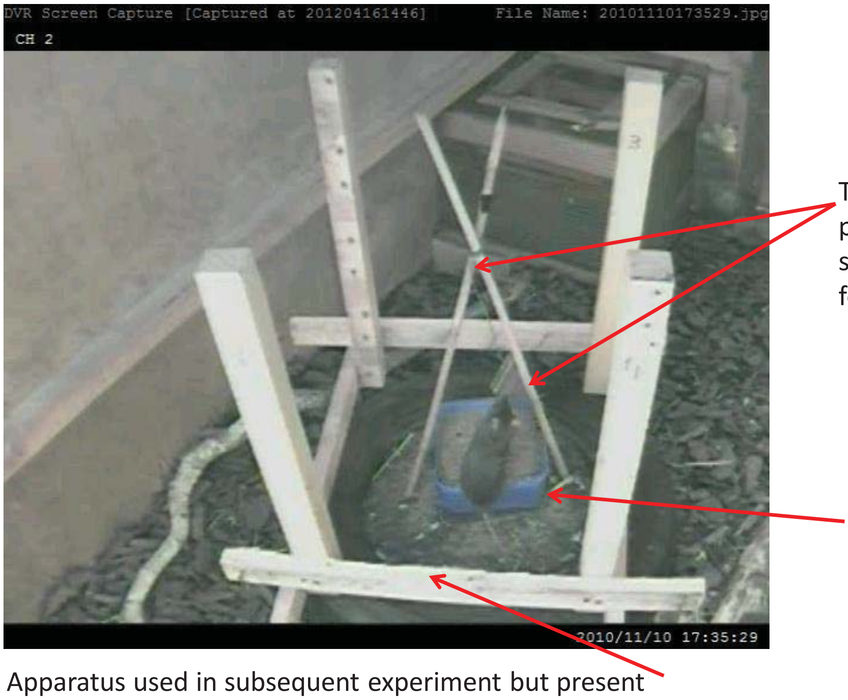


Figure 2.6 One of four outdoor experimental enclosures: featuring hide-boxes and branches to create a more naturalistic environment for captive rats during the odour experiments. Core-flute panels were attached to the exterior walls for additional weather-proofing.

Figure 2.7 Phase 1 enclosure foraging station set-up illustrating foraging tray within the inverted bucket, the overhead camera and the suspended zip-lock bag in which the odour samples were presented. In this example the blanket has been removed by the rat over the preceding night.



Treatment presentation suspended above foraging tray

Foraging tray supplied with sunflower seeds (20)

Apparatus used in subsequent experiment but present throughout this phase of captive research for continuity of environment for rat subjects.

Figure 2.8 Experimental apparatus used in phase 2 of the captive enclosure experiment, modified from the phase 1 apparatus.

Figure 2.9 Example of the extent of rat chewing through the wooden components of the enclosure construction. Aluminium sheeting can be seen where it had been fixed on the opposing side of the wall.

Acclimation phase: For phase 1 the rats were introduced individually to all of the four experimental compartments overnight to allow them to become familiar with the layout, the location of the food trays and the presence of sunflower seeds in the foraging trays. During the acclimation phase peanut butter was used to encourage investigation of the foraging station in preparation for the experiment phase. Ten sunflower seeds were sprinkled onto the surface of the foraging tray each night. The overhead cameras were activated during the acclimation sessions to familiarise the rats with the infra-red LEDs' in case they were able to see them. For phase 2 this procedure was ammended such that each rat was allocated to a single experimental compartment and introduced individually to the compartment prior to commencing the experiment. Twenty sunflower seeds were lightly mixed into the substrate of the foraging tray to a maximum depth of five centimetres (limited by 'false' bottoms) each night. Each rat was allowed three (alternate) nights in its assigned enclosure prior to experimentation commencing, and experiments commenced within six nights of the rats' arrival at Massey University.

Experimental predator cues: For phase 1 the test odours were obtained by incorporating strips of fleece blanket (approx. 5cm x 30cm) into the bedding of three treatment animals: stoat (familiar predator), chinchilla (novel herbivore) and possum (familiar competitor) for a minimum of one week and then stored frozen (stoat and possum samples were supplied by Landcare Research, Lincoln; chinchilla samples by Tracy Harris, Massey University Ecology technician). The number of strips per bed / donor animal varied according to animal and bed size ranging from 5 to 10 i.e. no bed / animal was so oversupplied that the blankets were in danger of not acquiring donor odour. As the behaviour of the donor animals could not be controlled it was assumed that over the course of 7 – 14 days all the strips present were odorous, unless the individual donor repeatedly rejected the blankets in which case those strips were not returned to me. Each of the three treatment samples had a corresponding control sample which was maintained under the same freezer conditions but was odour-free. All odour treated samples were kept boxed or bagged separately to avoid cross-contamination between treatments. This method of obtaining body odour samples had been trialled on laboratory rats using cat tainted blanket and found to be highly effective (see Appendix 2 for details of this experiment). For phase 2 the test odours were obtained by incorporating fleece blankets into the bedding of three treatment animals: individually housed captive stoats (familiar predator), colony housed domestic cats (alternative predator) and 'foreign' male ship rat (con-specific competitor and /or threat) for a minimum of one week and then stored frozen (stoat samples were supplied by Landcare Research, Lincoln; cat samples were supplied by the Feline Unit, Massey University, rat samples were sourced from bedding obtained from earlier subject animals kept in captivity during 2009 / 2010). All odour treated samples were kept boxed or bagged separately to avoid cross-contamination between treatments.

In preparation for the experiment small pieces (approximately 3 x 5 cm) were cut from a blanket strip derived from individual donor animals and control samples. For phase 1 the stoat and possum samples comprised one piece each from a male and female donor; the chinchilla samples comprise one piece each from two separate mixed sex donor colonies. For phase 2 the stoat and rat samples comprised one piece from each donor animal, the cat samples comprise one piece from mixed sex donor colonies. The same donor individuals

/colony were used for all the samples presented to rats in any given group. Each sample was bagged in an unused 7 cm x 5 cm zip-lock bag and labelled (Figure 2.10).

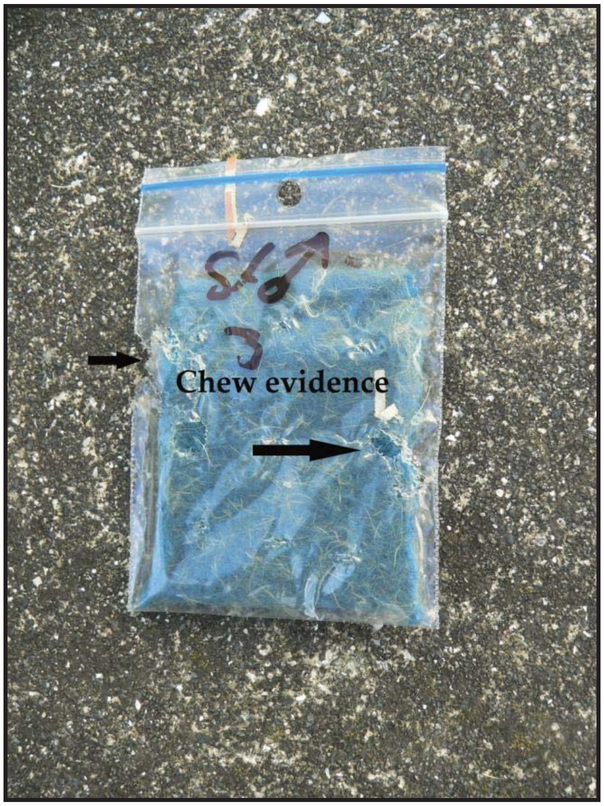


Figure 2.10 Odour cue presentation as used in both captive and field experimental protocols. Note the evidence of chewing (chew or remove behaviour) on this example taken from the 2011 Tawa Walk (SuM) experimental field trials.

Subject order: Each rat was randomly assigned an order of presentation for each of the treatment cues: control, chinchilla, stoat and possum. The first two groups (LW1 and ENP) were exposed to stoat, chinchilla and odour-free (control) cues; the second two groups (LW2 and LW3) were exposed to possum cues in addition to stoat, chinchilla and odour-free cues. Each rat was randomly assigned its experiment nights and compartment. Because of the number of variables associated with each treatment group, after seeking statistical advice (A. Noble, personal communication, March 6, 2009) I determined that random allocations were a better approach than a latin-square design.

Experiment protocol: Each experimental day the treatments assigned were placed in the appropriate enclosures. The bags holding the odour samples were perforated nine times on each side and suspended by tie wire above the feeding stations (Figure 2.6). While the experimental compartments were adjacent to each other, they were separated by solid ply walls (see Appendix 1). Thus while there no doubt was some potential for wafting of odour, the greatest concentration would be confined to the allocated enclosure. Un-husked sunflower seeds were used as a measure of foraging behaviour as these introduced a food handling component to the foraging and consumption behaviour. Rats might elect to remain at the source of the food supply and take the time to husk and consume the seeds in the location of my choice i.e. the foraging tray; or they may elect to individually remove the seeds to husk and consume in a location of their choice e.g. their nest-box.

Ten sunflower seeds were added to each foraging tray. Rats for that night were then relocated from their runs (in their nest boxes) to the start platform of their assigned compartment, along with their food and water dishes. They received the same food, in their own dishes as usual, with the exception that peanut butter was not provided in the foraging trays during the experimentation phase. This was in order to avoid the confounding effect of a particularly odorous and palatable food over-riding the treatment odours or affecting the sunflower seed consumption (Bramley, Waas & Henderson 2000). All rats were in position at least an hour before dark, and motion activated video recording was scheduled to commence at 4 – 5pm (depending on daylight savings) until 7 – 8am. The enclosure housing, husbandry and experimental protocol were covered under MUAEC 09/08, 10/83.

Data collection is described in the following experimental chapters.

2.2.3 Y-MAZE

The housing and experimental protocol described below were approved under MUAEC 09 /08 & amendments.

Subject animals & housing: At the completion of the phase 1 enclosure experiments (circa 21 days) each group of captive rats were transferred into standard plastic based, wire upper, laboratory cages (ca. 23cm wide x 45cm long x 22cm deep) and moved to an indoor location within the Ecology Department (Massey University). A total of 31 rats completed the phase 1 enclosure experiment, comprising three groups of eight and one group of seven. They remained on a natural light regime, with food consisting of *ad lib* rodent nuts; *ad lib* water via 'sippa' bottles (Critter Canteen 8oz; Premier 500ml; Living World 225ml, 450ml; Ferplast 150ml, 600ml, 1200ml) and supplementary carrot, apple and grain, with regular peanut butter. Bedding consisted of shavings, straw and remnants from their nest box bedding, plus additional blanket strips. Rats were checked and fed daily but remained largely undisturbed. Cages were cleaned every seven to ten days, or sooner if required. Experimentation did not commence for at least three weeks after introduction to this new environment.

Treatment samples: Odour samples were prepared and presented as per the preceding enclosure experiment. One sample from each treatment and its corresponding control (odour-free) sample were prepared for each night. Between rats the zip-lock bags were lightly wiped over with 70% ethanol and allowed to air-dry until required for another rat on that night. This ensured that any difference in response within nights was not due to variation in sample quality.

The samples presented to each group of rats were taken from a single donor animal (or male : female pair as discussed above). Where individual donors were identifiable (stoats), the same donor that had been used in the preceding enclosure experiment for any given group was also presented to those animals in the Y-maze experiment. This ensured that any difference in response between the two scenarios could not be ascribed to differences in donor quality.

Apparatus and protocol: The Y-maze was constructed from cut away 10cm diameter PVC piping, with removable Perspex lids for each arm. Each arm was approximately 45 cm long and removable Core-flute™ barriers allowed control of movement when necessary. The trials were undertaken in the same room as the animals were housed thus in terms of smells and general surroundings it was familiar. During the experimental period all the rats were moved to the semi-darkened holding room (an annex to the neighbouring laboratory) at approximately 4pm daily and allowed to settle. The experiment room was then dimmed by shuttering the windows, replicating a light level approximating that of dusk – early evening, a time when they had been observed in the enclosure and in the field to begin to become active. The adjacent laboratory served as a third room in which rats were held at the completion of their Y-maze run for each night to avoid repeated disturbance of those rats still awaiting their turn in the annexe room.

Rat groups 1 (LW1) and 2 (ENP) were exposed to chinchilla and stoat treatments, rat groups 3 (LW2) and 4 (LW3) were also exposed to possum treatments.

Rats were individually transported in their cages from the annexe holding room to the experimental apparatus, using a blanket to reduce exposure to light during transfer (<1.5m from door – door). Rats were captured in a cloth bag and introduced to the maze. I remained in the room and observed from behind the start position, endeavouring to disturb the rats as little as possible. At the cessation of the session the rat was moved back to the start arm and returned to its cage. After each rat the maze was wiped down with 70% ethanol and prepared for the next rat. The following protocol was used:

Night 1: Familiarisation: 5 minutes of exploration of the start arm + right or left arm, then start + alternate arm, then a final 5 minutes of free exploration.

Night 2: Lateralisation 1: 5 minute free exploration of the Y-maze. At the end of 5 minutes the rat was brought back to the start arm and allowed to make 5 free choices of left or right arm. Once 5 side choices were made the rat was returned to its cage.

Night 3: Lateralisation 2: As per night 2 to assess the likelihood that any side bias exhibited on night 1 is a genuine lateral bias rather than persistence behaviour for that night.

Night 4: Rest night

Night 5: Presentation 1: Each rat was presented with one randomly assigned treatment (stoat, possum or chinchilla) odour plus a control (odour-free) on each of the presentation nights. Likewise the treatment odour for each rat on each night was randomly assigned to the left or right arm of the maze. The samples were positioned at the far end of their assigned arm. Group 1 were allowed a five minute acclimation period in the start arm, however the rats tended to settle at the release point and were very reluctant to explore the maze once the arms were opened. Therefore the acclimation was reduced to two minutes for groups 2-4, a period of time which was observed to allow the rats to settle at the release point but still be willing to investigate the maze once access was granted. Each rat was allowed fifteen minutes of free maze exploration. On trial completion rats were temporarily held in the neighbouring laboratory to avoid excessive disturbance to those rats still awaiting their turn in the annexe. This third location, the far end of the neighbouring laboratory, was unlit and the rats were held there until all animals had completed the experiment for the night at which point they were all returned to the 'home room'.

Night 6: Presentation 2 (as per presentation 1)

Night 7: Presentation 3 (group 3 and 4 rats only; as per presentation 1)

Experimentation commenced between 5.00pm and 5.45pm nightly. All activity was recorded on video (Sony Handycam DCR-SR4) under infrared lighting (IRLamp6, Bat Conservation and Management Inc, Pensylvania) for later analysis and to reduce disturbance to the animals by recorder activity.

2.3 FIELD EXPERIMENT

Subject animals and locations: Ship rats were live captured, micro-chipped (PIT tagged) (see Carter et al. 2009 for full details of tagging procedure and use of PIT tags) and released, at four locations within the Lake Waikaremoana catchment, Te Urewera National Park, New Zealand (Latitude: 38° 46' 0 S, Longitude: 177° 4' 60 E) (Figure 2.3). PIT tags were used in favour of ear-tags or other external identification methods because they would 1) last the lifetime of the rat and 2) could be read electronically where ear-tag numbers would not be visible using the cameras I had. The Lake Waikaremoana location was selected because firstly ENP had recently undergone intensive, park-wide 1080 poisoning which would have removed a significant proportion of both rats and possums with stoats probably being reduced via secondary poisoning[1]; and secondly the LW stoat management program had been in place since 1995 (J. McLennan, personal communication, June 2010), and thus represented a rat population with a longstanding 'reduced' stoat exposure for the phase 1 experiments.

The four locations represent different background conditions in terms of pest management (SM, SuM; PM, PuM). These same four locations were used to source the rats taken into captivity for the preceding research phases, thus representing the same wider populations of animals that were used in captivity. The locations themselves are geographically separate enough that the populations, whilst not isolated, can be considered independent of each other for the duration of the research as any movement between locations would take a number of generations based on what is currently known of rat territory sizes (Innes, 2005).

Within each of the four locations (Kaitawa Lakes (PuM & NC), Onepoto (PM & NC), Tawa Walk (SuM & FC) and Tapuaenui (SM & FC)), two specific sites were selected, based upon trap capture success, for the positioning of the experimental apparatus. These experimental sites were selected as the best available to combine both high capture rate, ease of access (the experiment relied upon daily attention), and sufficient distance to minimise individual rat overlap in visitation between sites. Three of the four locations achieved all three criteria, the fourth, Tapuaenui (SM), returned only one area with acceptable accessibility and high capture rates thus the two experimental sites here were closer than ideal.

Trapping was undertaken during December 2009 and January 2010 (phase 1) over two nights at each location. Phase 2 trapping occured during Dec 2010 and January 2011 over a four to seven night period (depending on capture rates) at each location. There was a minimum 1 week pre-feed period prior to trapping activity. A total of 23 live capture ("Rat Cage Trap 19RT10"; Pest Management Services, Paraparaumu, Kapiti, NZ) traps were deployed at each location. Phase 1 traps were deployed at approximately 50 pace spacing linearly, whilst in phase 2 the traps were deployed in a concentration within an approximately 25m radius of the experimental location. This change in trapping approach was in hope of capturing more of the rats that were frequenting the immediate vicinity of the

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[1] this assumption was subsequently verified with pre and post 1080 monitoring data (DoC unpublished data)

apparatus as in phase 1 it had become apparent that only rats captured within the immediate vicinity of the apparatus were subsequently recorded via their PIT tags. The total number of rats captured varied between locations and is reported in subsequent chapters. All trapping was completed at least two weeks in advance of experimentation commencing in order to allow the rats time to recover from the capture and tagging experience.

Experimental set-up: At each of the selected experimental sites an array of four standard NZ tanalized pine fencing battens were positioned vertically in a square formation at two to three metre spacing depending upon vegetation and terrain (Figure 2.11). These battens were left in-situ throughout the research period (from January 2010 until April 2011).

At commencement of experimentation an infra-red LED surveillance camera was fixed to the top of each batten at the 'active site', the four cameras all linked back to a single DVR unit (Swann DVR 1000, Dick Smith Electronics, NZ), and a single 12 volt 40aH deep cycle battery powered the DVR / camera set up overnight. All four cameras were programmed to motion activate from dusk (8pm) to dawn (6am) each night, and camera position and functioning were checked daily using a small portable television (TEAC Axia 9", Norman Ross, NZ).

At the base of each batten a hoop antenna was positioned, behind which (between batten and antenna) the odour samples were secured with twist tie. The hoop antenna were connected to data-loggers (Department of Conservation, NZ), powered by individual 12 volt 7aH batteries which recorded the PIT tag number of any tagged animal interacting with the sample (see Carter et al. 2009 for a full description of the development of these techniques)(Figure 2.11). The apparatus described here was a refined version of that which we had previously used in apparatus trials and in our captive research. Because the antennae interfere with each other within a few metres proximity, only 1 antenna was able to be reliably activated on any one night, while the other three were positioned but not activated. At the first location, Tawa Walk (SuM), this was by chance the possum sample logger, thereafter the stoat sample logger was the only one activated as this was the sample of primary interest for non-avoidance / investigatory behaviours.

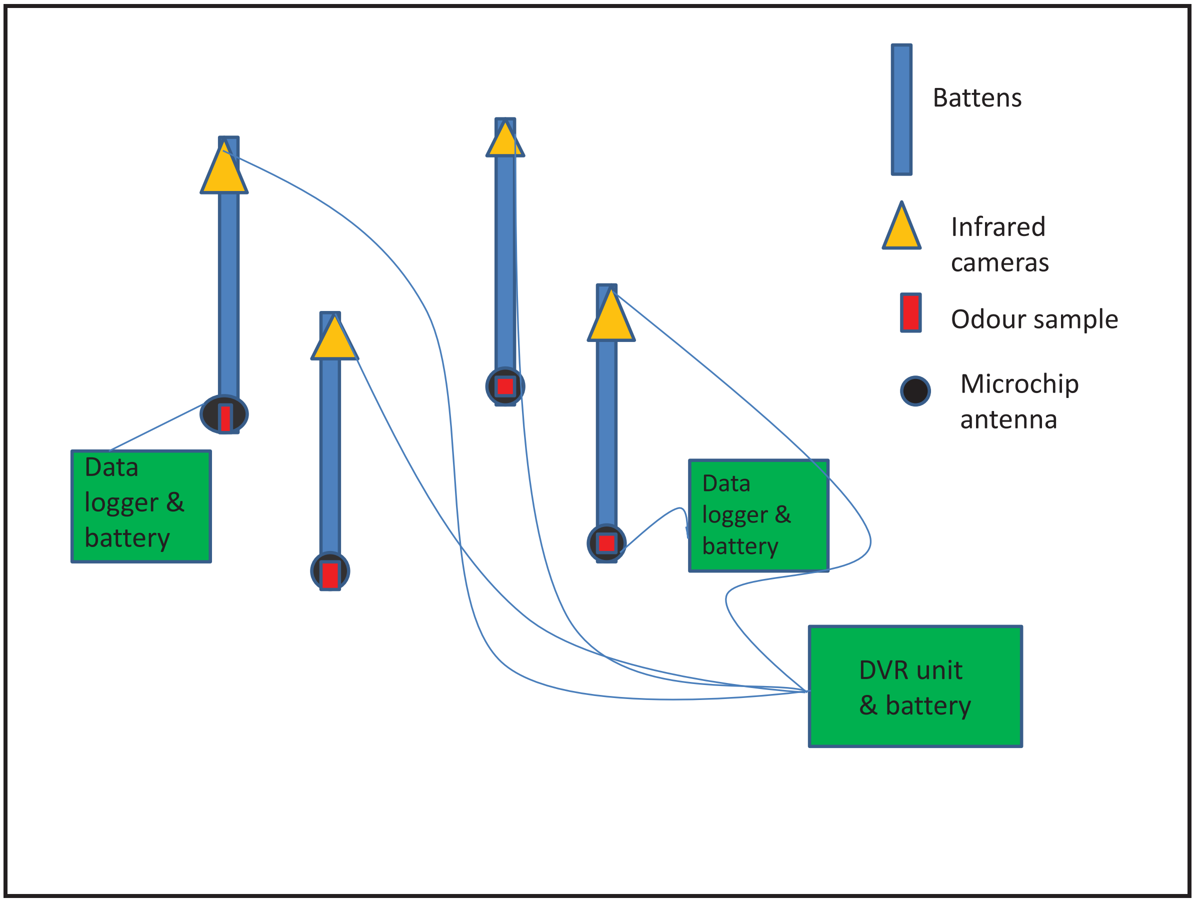

Figure 2.11 Layout of the field experiment batten array (not to scale). Battens when deployed were 2-3 m spaced dependent upon terrain and vegetation.

Experimental procedure: During phase 1 logistics and equipment availability dictated that the experiment was run sequentially between locations and sites within locations. During phase 2 the experiment ran concurrently at sites (A and B) within locations, but sequentially at each of the four locations (Figure 2.3). Weather conditions also affected when equipment could be deployed, thus the experiments were conducted between 18th January and 19 Feb 2010 (phase 1) and 1st February and 3rd March 2011 (phase 2).

1. On the night immediately preceding experiment night one peanut butter was applied to the top of each of the four battens to encourage rat visitation to the area.
2. Sample odours were permanently allocated to a batten / camera number. Each ca. 5 x 3 cm blanket sample was presented in a 5 x 7 cm zip-lock bag perforated with a small nail on the day of use (three x three times each side). 'Twist tie' was used to secure the zip-lock bags holding the sample to the base of the allocated batten as described above.
3. Camera positions and function were checked and the site left.

4. The following morning the samples were replaced and note made of any evidence of interaction such as chew marks on the bags. DVR / camera batteries were replaced and position and function rechecked.
5. During phase 1 after two nights the experiment was moved to the second site within the given location, during phase 2 the experiment was concluded after three consecutive nights at each location .
6. At the conclusion of the experiment at each location the DVR and data-logger were downloaded and backed up for analysis.

Samples: Odour samples for both phase 1 and 2 were taken from the same sources as in the preceding enclosure experiments. Commercially available fleece blankets were cut into strips and incorporated into the nest boxes / bedding of donor animals (stoats and possums, Landcare Research Lincoln, NZ; chinchilla, Tracy Harris, Massey University Ecology Department research technician) for a minimum of one week. The blankets were then held in zip-lock bags under freezer conditions until required. For this experiment the odour samples were, by necessity, held at ambient temperature, albeit in an 'out-room' until use which in some cases exceeded two weeks. I had previously experimentally tested the longer term efficacy of odour tainted blankets using Sprague Dawley strain laboratory (*Rattus norvegicus*) rats and found that it did not seem to diminish with transfer into ambient temperature (Appendix 2). Stoat samples comprised portions of both male and female blankets, as did possum, whilst chinchilla comprised blankets from two mixed sex colonies, control samples comprised a portion of non-tainted blanket controls associated with each of the three odour sources, as per the preceding enclosure experiment.

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Chapter 3 DOES REDUCING PREDATOR EXPOSURE AFFECT THE AVOIDANCE RESPONSE OF PREDATOR-EXPERIENCED PREY?

3.1 ABSTRACT

Prey response to predator cues may be driven by genetics, experience or a combination of both. This experiment tests whether the avoidance behaviour of a prey species might be affected by reduced learning opportunities i.e. obtaining experience. I used a New Zealand forest ecosystem in which the predator and prey species had not co-evolved, thus reducing the influence of a predator specific innate response; and whereby a single species is the primary predator thus reducing the influence of generalised learning. In New Zealand, with the exception of two bat species, all terrestrial mammals have been introduced. In the forest two of the most widespread and important exotic mammalian pest species are ship rats and stoats. Both species are the target of control operations reducing their numbers to allow the survival of native fauna and to improve seed viability in native flora. I used wild ship rats from both stoat-unmanaged and reduced predator exposure (stoat-managed) locations and tested their avoidance responses under two captive protocols.

Under Y-maze conditions the rats exhibited no avoidance response to stoat odour. Under enclosure conditions the rats exhibited no avoidance response to stoat odour but the rats from stoat-managed areas exhibited a modified foraging behaviour in the presence of stoat odour. The changes in behaviour are a greater frequency of entries to the foraging tray, and a greater seed consumption rate. I suggest that the enclosure results provide evidence of two different risk-assessment responses to predator odour in terms of foraging behaviour and that the Y-maze results are largely artefacts of the experimental procedure. I conclude that under reduced predation conditions opportunities to learn from experience are limited and this is reflected in behavioural changes such as more cautious foraging strategies when predator odour is present, but not a difference in overall food consumption.

3.2 INTRODUCTION

Predator-prey interactions, particularly the response of prey species to the cues of predators has been a popular subject of research for many years within both the ecological and psychological or neuroscience literature (see Apfelbach et al. 2005; Kats & Dill 1998 for comprehensive reviews). Ecological research has tended to focus on behavioural effects e.g. defensive behaviour, avoidance behaviour (including habitat use and foraging); physiological effects such as changes in size and weight in the presence of predators and the implications for populations such as breeding suppression and population stability (see Apfelbach et al 2005; Kats & Dill 1998 for comprehensive reviews). The psychological and neuroscience researchers have largely focused on understanding what they call the "fear response" via behavioural, hormonal or neural changes (Adamec, Blundell & Burton 2006; Adamec, Blundell & Collins 2001; Dielenberg & McGregor 1999; Guimaraes-Costa, et al. 2007; Holmes & Galea 2002; Mitra, Adamec & Sapolsky 2009).

The main mammalian subject species has been the laboratory rat, (Adamec & Shallow 1993; Blanchard, et al. 2001; Dielenberg & McGregor 1999; Endres, Apfelbach & Fendt 2005; Hubbard et al. 2004; McGregor et al. 2002; Naidenko et al. 2003; Voznessenskaya et al. 2003); however a range of other small mammals including ship (black, roof) rats (Bramley & Waas 2001; Burwash et al. 1998a, 1998b; Leung & Clarke 2005), kiore (Pacific rat; *R. exulans*) (Bramley & Waas 2001), wild Norway rat (*R. norvegicus*) (Bramley, Waas & Henderson 2000; Klemann & Pelz 2006), marmots (e.g. *Marmota flaviventris*) (Blumstein et al. 2008), European rabbits (*Oryctolagus cuniculus*) (Monclus, Rode, & von Holst 2006; Monclus et al. 2005), hedgehogs (*Erinaceus europaeus*) (Ward, MacDonald & Doncaster, 1997), bats (Chiroptera) (Baxter et al. 2006), and voles and lemmings (Cricetidae) (Borowski 1998; Fletcher & Boonstra 2006; Fuelling & Halle 2004; Norrdahl et al. 2004; Sullivan et al. 2004; Sundell, 2006) have also been studied.

Much of the behavioural research to date has concentrated on addressing two main questions: whether the responses of the prey to the predator cue were learned or innate, and whether the fear or avoidance responses of the prey could be utilised for the protection of valuable economic or ecological resources. Overall these studies have tended to show that a fear or avoidance response towards predators is innate in many species. However the utilisation of predator cues in deterrents or repellents for the prey has had very mixed success particularly under field conditions (as reviewed by Apfelbach et al. 2005; Kats & Dill 1998).

Within most natural predator – prey systems, each prey species has multiple potential (co-evolved) predators, this being particularly true for smaller prey species including the many rodent species upon which most laboratory behavioural research is based (Sinclair 2003). This situation could complicate any research that seeks to understand the predator-prey response mechanisms of free-living animals because co-evolution is a possible pre-requisite to innate avoidance responses to specific predators. For example Orkney voles (Orcadian *Microtus arvalis*), whose ancestral population was introduced to the stoat and weasel free Orkney Islands from mainland United Kingdom an estimated 5000 years ago, continue to exhibit an avoidance response to stoat odour under both captive and field experimental conditions (Gorman 1984).

While much research, particularly that involving domesticated laboratory rats, mice and guinea pigs, indicates that the avoidance response to predators is innate, there is also a considerable body of evidence, primarily non-mammalian, indicating that experience affects the response of individuals. Some predator-prey interactions appear to be the result of learned rather than innate behaviour resulting in the demonstration of 'risk assessment' behaviour. In some species this learning may extend to recognition of hetero-specific alarm cues and in-utero learning (Blumstein et al. 2008; Brown & Chivers 2005; Brown 2003; Darwish et al. 2005; Ferrari & Chivers 2010; Gonzalo, Lopez & Martin 2007; Griffin, Evans & Blumstein 2001; Holmes & McCormick 2010; Kelley & Magurran 2003; McLean, Lundie-Jenkins & Jarman 1996; McLean et al. 2000; Muller & Manser 2008; Nocera & Taylor 2008; Turner, Turner & Lappi 2006; Vieth, Curio & Ernst 1980; Wisenden, Chivers & Smith 1997; Woody & Mathis 1998).

New Zealand, where all terrestrial mammals except two bat species are introduced, offers a rare opportunity to address the question of the effect of experience upon prey animals in a relatively simple predator-prey system involving two non-co-evolved species: the ship rat (Indian sub-continental evolutionary origins (Innes 2005)) and stoats (continental European evolutionary origins (King & Powell 2007)). These two species have co-existed in the mainland New Zealand forest for approximately 130 years, stoats being the later arrival, introduced to NZ in 1884 (King & Murphy 2005). Since stoats and ship rats have not co-evolved, the potential for an innate stoat specific avoidance response is limited. However other mustelids are endemic to the Indian subcontinent so an innate mustelid-generalised response cannot be totally excluded without specific investigation.

Stoats, cats and ship rats are a recognised threat to the endemic flora and fauna of NZ, but stoats in particular are intensively managed by kill trapping in a number of locations for the protection of highly threatened endemic avifauna such as kiwi (*Apteryx* spp.) and whio (*Hymenolaimus malocorhynchos*) (Anon, n.d.; Heather & Robertson 1996). This situation allows large populations of ship rats to live under conditions of significantly reduced stoat predation threat and therefore presumably reduced opportunities to gain experience of surviving / avoiding stoat predation. Thus populations of both stoat-managed and non-stoat-managed ship rats are accessible to investigate the effects of predator experience upon their prey. Similarly, whilst feral cats (*Felis catus*) are also present in New Zealand mainland forest, based upon trapping data their numbers in deep forest and away from farmland or urban / forest transition zones are often low (C. Gillies, personal communication, May 18 2012), thus stoats are the primary predator of ship rats in much deep forest habitat and the predator most likely to elicit a learned response.

In addition to the predator-prey interaction, the Australian brush-tail possum (*Trichosurus vulpecula*, Australian evolutionary origin) co-exists alongside stoats, cats and rats. Although not a predator of ship rats, the possum has both habitat and dietary overlap with rats (Ruscoe et al. 2011). Possums are also considered a major ecological and economic threat and are also routinely controlled via lethal trapping and poisoning creating both possum-managed and unmanaged zones for rat populations. Possums were included in this research to ensure that any response seen in the rats to the treatments was not due to (a) shifts in competition pressure since there is also some dietary overlap between ship rats and stoats, for example invertebrates; or (b) 'management' activity per se.

The aim of this research was to determine whether animals from stoat-managed areas exhibited a behaviourally different response to a familiar predator (stoat) cue than those from stoat-unmanaged areas. I predicted that rats sourced from stoat-managed backgrounds would be less responsive to the stoat predator cue presented because they were less experienced and thus less likely to be able to recognise the threat represented by stoat odour. To distinguish a generalised response to 'management' or reduced competition rather than reduced predation, I predicted that animals from a reduced possum (competitor) zone would not exhibit any behavioural difference to the possum odours presented.

3.3 METHODS

Captive experimentation was undertaken using wild-caught ship rats under two protocols: an enclosure approach aimed at allowing freedom of expression of behaviour (c.f. Blanchard and Blanchard 2003) that I expected would elicit responses reflective of a free-living population; and a Y-maze experiment representative of much captive predator response research to date (see Appendix 1 for discussion of this).

A total of 31 mixed sex rats rats from six sites and two management backgrounds were analysed in the Phase 1 captive experiments (Table 3.1).

Table 3.1 Composition of the Phase 1 captive rat sample set analysed. Source codes relate to Figures 2.2 and 2.3.

Source	LW1	LW2	LW3	LW4	ENP1	ENP2	**Totals**
Management	PuM	PM	SM	SuM	SM	SuM	
Males	1	1	6	5	1	2	**16**
Females	3	2	2	3	3	2	**15**
Totals	**4**	**3**	**8**	**8**	**4**	**4**	**31**

3.3.1 EXPERIMENT 1: AVOIDANCE RESPONSES BY RATS IN CAPTIVE ENCLOSURES

The methods used are those outlined in Chapter 2 General Methods, Captive Experiments (2.2).

Data analysis: Avoidance behaviour was assessed using two independent measures: frequency of entry into the foraging station which I interpret as representing the rats willingness to approach an area tainted by the treatment odour; and seed consumption which I considered represented the rats willingness to engage in a behaviour that was in conflict with predator avoidance. Entry frequency was assessed from the video recording while seed consumption was assessed by direct count of those remaining in the foraging tray each morning. Only once all the assessments were completed were the camera and foraging station identifications reconciled with rat identifications and the treatment that was presented, thus all rat behaviour was effectively scored blind. Scored data were collated (Excel 2007 Student Version) for statistical analysis.

Statistical analyses: General exploration of the foraging station entry frequency and seed consumption data (dependent variables) against treatment (stoat, chinchilla, possum and control) and management background (managed / unmanaged) (factors) were explored. From this an assessment was made as to what test approaches would be most suitable. Exploration confirmed that in most cases the data were not normally distributed and could not be transformed.

In addition, the rat sample was statistically unbalanced in terms of rat sex, source location and management background (Table 3.1), and there was high individual variability in responses. For these reasons a non-parametric statistical approach was undertaken. Kruskal-Wallis analyses were used to determine whether either treatment or management background affected the frequency of entry into the foraging station and consumption of sunflower seeds. Mann-Whitney U-test analyses were used to determine if management background affected the frequency of entry into the foraging station or consumption of sunflower seeds in the presence of any treatment specifically i.e. did stoat-managed / unmanaged rats differ in their response to stoat odour and did possum managed/ unmanaged rats differ in their response to possum odour. The average number of entries per sunflower seed consumed was also calculated from these rat sample groups. Mean, median, range, standard deviation and interquartile ranges are all presented where appropriate. Statistical analyses were carried out using SPSS 16.0 (Student version) and Bonferroni corrections were then calculated and presented where necessary.

3.3.2 EXPERIMENT 2: Y-MAZE AVOIDANCE RESPONSES

The experimental procedure was that described in Chapter 2 General Methods, Y-maze (2.2.3).

Data gathering: Lateral bias was assumed at an 80% threshold based on statistical advice (A. Noble, personal communication, March 6, 2009) i.e eight or more selections of the same arm indicated a lateral bias in favour of that side. In order to analyse the responses of the rats under the Y-maze conditions the following data were collated:

- First response (enter or avoid treatment arm)
- Entry treatment arm (frequency)
- Entry control arm (frequency)

Statistical analyses: Eight rats exhibited lateral bias and were excluded from subsequent analyses. Expected and observed values, to determine whether the first response of the rat (enter or avoid treatment arm) followed a random distribution or not, were calculated. Because 'avoid' comprised two possible responses: remain in the start arm or move into the non-treatment arm; whereas 'enter' comprised only one possible response: to enter the treatment arm; the expected values were adjusted to meet a 2 (avoid):1 (enter) ratio. To determine whether first response to treatment might be affected by the source management background of the rats this test was repeated on each management pairing i.e: stoat treatment for the stoat-managed and unmanaged populations only; possum treatment for possum-managed and unmanaged populations only.

Non-parametric tests were used because of the high variability and extreme poisson distribution of much of the data. Transformation options were explored but found to be inadequate. Kruskal-Wallis analyses were used to determine whether either treatment or management background (grouping variable) significantly affected the frequency of entry into the treatment and non-treatment Y-maze arms (test variables). Mann-Whitney U-tests determined if management background (stoat-managed versus unmanaged; possum-managed versus unmanaged) affected the frequency of entry into the treatment and non-treatment Y-maze arms in the presence of stoat and possum odour specifically.

Statistical analyses were carried out using SPSS 16.0 (Student version) and Bonferroni corrections were then calculated and presented where necessary.

3.4 RESULTS

3.4.1 EXPERIMENT 1: CAPTIVE ENCLOSURE AVOIDANCE RESPONSES

Entry to foraging station: The frequency of entry into the foraging station was highly variable both in terms of odour treatment and management background. The treatment entries ranged from a minimum of 3 (control) to a maximum of 130 (control); background management entries ranged from a minimum of 3 (stoat-managed and unmanaged areas) to a maximum of 130 (stoat-managed area) (see Table 3.2 for full details).

There were no significant differences in the number of entries to the foraging stations between the different treatments (Kruskal-Wallis test; all comparisons $P \geq 0.05$; Table 3.3). Likewise there were no significant differences in the number of entries to the foraging stations between the different management backgrounds (Kruskal-Wallis test: all comparisons $P \geq 0.05$; Table 3.3). Nor were there significant differences in the number of entries to the foraging station of rats from either possum or stoat-managed versus unmanaged areas in the presence of any treatment odour (Mann-Whitney U-test; all comparisons $P \geq 0.05$ (Table 3.4).

Table 3.2 Descriptive statistics for entry frequency and seed consumption according to management background and treatments (n= 31 rats).

	Entry Frequency				
Factor	**Mean**	**Median**	**Range**	**Standard deviation**	**Interquartile range**
Stoat-managed	39.1	28.0	3 – 130	28.4	40
Stoat-unmanaged	27.0	23.5	3 – 114	19.6	16
Possum-managed	25.4	15.5	10 - 52	17.1	34
Possum-unmanaged	21.4	20.0	8 – 43	9.1	10
Control treatment	36.0	26.5	3 – 130	30.2	34
Chinchilla treatment	30.1	26.0	8 – 85	18.1	20
Stoat treatment	28.2	24	7 - 90	20.2	15
Possum treatment	21.9	19	3 - 54	15.5	21
	Seed Consumption*				
Factor	**Mean**	**Median**	**Range**	**Standard deviation**	**Interquartile range**
Stoat-managed	9.3	10	0 - 10	2.2	0
Stoat-unmanaged	9.8	10	2 – 10	1.3	0
Possum-managed	9.4	10	3 – 10	2.0	0
Possum-unmanaged	9.9	10	8 – 10	0.5	0
Control treatment	9.4	10	0 – 10	2.0	0
Chinchilla treatment	9.7	10	2 – 10	1.5	0
Stoat treatment	9.5	10	2 – 10	1.9	0
Possum treatment	9.9	10	8 – 10	0.5	0

*Maximum of ten seeds

Table 3.3 Results of Kruskal-Wallis testing for entry frequency and seed consumption by the rats in response to treatment cues and management background (n=31 rats).

Factor tested	**Entry to foraging station**	**Sunflower seed consumption**
Treatment (all)	H(3) = 3.473; P = 0.324	H(3) = 0.355; P = 0.949
Background management (all)	H(3) = 6.773; P = 0.079	H(3) = 1.057; P = 0.787

Table 3.4 Results of Mann-Whitney U-tests for entry frequency and seed consumption for rats from managed and unmanaged areas in the presence of stoat and possum cues (n=31 rats).

Factor tested	**Treatment**	**Entry to foraging station**	**Seed consumption**
Stoat-managed / unmanaged	Control	U= 48.5; P=0.288	U = 48.0; P= 0.288
	Chinchilla	U = 47.0; P= 0.260	U = 65.0; P= 0.976
	Stoat	U = 60.0; P= 0.740	U = 60.5; P = 0.740
	Possum	U = 7.0; P= 0.886	U = 8.0; P = 1.000
Possum-managed / unmanaged	Control	U = 4.0; P= 0.629	U = 6.0; P= 1.000
	Chinchilla	U = 6.0; P= 1.00	U = 6.0; P= 1.000
	Possum	U = 2.0; P = 0.229	U = 4.5; P = 0.629
	Stoat	U = 4.0; P= 0.629	U = 4.0; P= 0.629

Seed consumption: The consumption of sunflower seeds was variable both in terms of treatments and management background. Treatment consumption ranged from a minimum of 0 (control) to a maximum of 10 (all four treatments); rat background seed consumption ranged from a minimum of 0 (stoat-managed) to a maximum of 10 (all management backgrounds) see Table 3.2 for full details. Neither treatment odours nor management background generated significant differences in the number of sunflower seeds consumed by the overall rat dataset (Kruskal-Wallis test; all comparisons P≥ 0.05; Table 3.3). There were no significant differences in the number of sunflower seeds consumed by either stoat or possum-managed versus unmanaged rats in the presence of any odour (Mann-Whitney U-test; all comparisons P≥0.0125 (Bonferonni corrected) (Table 3.4)).

3.4.2 EXPERIMENT 2: Y-MAZE AVOIDANCE RESPONSES

Lateral bias: 24% of rats (8) exhibited lateral bias, of which 9% (3) were right biased and 15% (5) were left biased. These rats were excluded from the following analyses.

First response: The majority of rats elected to avoid the treatment arm as their first response (Table 3.5). Chi square testing could not be undertaken on this data due to the occurrence of categories with < 5 observations. A Fishers exact test was considered but because the expected values were not 1 : 1 this test was also deemed inappropriate. Instead I present the observed and expected values for comparison (Table 3.5). From this it is clear that no combination of rat background and treatment produced entry or avoidance results that were potentially significantly different from the calculated expected values.

Table 3.5 Rats choosing to enter or avoid the treatment arm of the Y-maze with observed and expected values adjusted for 1 (enter): 2 (avoid) ratio) (n=23).

Population	**Treatment**	**Enter treatment arm**		**Avoid treatment arm**		**Number of events**
		Observed	**Expected**	**Observed**	**Expected**	
All rats	All treatments	19	19	38	38	57
Stoat-managed / stoat-unmanaged	Stoat treatment	4	5.3	12	10.7	16
Stoat-managed / stoat-unmanaged	Possum treatment	5	2.3	2	4.7	7
Possum-managed / possum-unmanaged	Possum treatment	2	2	4	4	6
Possum-managed / possum-unmanaged	Stoat treatment	2	2	4	4	6

Effect of treatment upon entry frequency: There was considerable variation in the frequency with which rats entered either the treatment or non-treatment arms, and this was evident across the dataset with a minimum of 0 entries recorded for all treatments in both arms regardless of treatment and a maximum of 17 entries recorded for the stoat treatment arm (Table 3.6). Similarly, rats from both the stoat and possum-managed and unmanaged areas exhibited great variation in their entry frequency in response to stoat and possum odour respectively (Tables 3.7 & 3.8).

Table 3.6 Descriptive statistics for frequency of entry into the treatment and non-treatment arms of the Y-maze for the overall rat dataset (n= 23 rats).

Factor	Mean	Median	Range	Standard deviation	Interquartile range
			Enter treatment Arm		
Stoat-managed	3.6	2.0	0 - 17	4.3	7
Stoat-unmanaged	2.9	1.0	0 -7	2.2	4
Possum-managed	4.3	6.0	0 -11	4.4	.
Possum-unmanaged	2.3	0	0 - 15	4.5	11
			Enter non-treatment arm		
Stoat-managed	3.5	2.0	0 – 14	4.2	4
Stoat-unmanaged	2.9	1.0	0 – 7	2.7	4
Possum-managed	3.5	4.5	0 – 8	3.5	.
Possum-unmanaged	2.3	1.0	0 - 11	3.5	8

Table 3.7 Descriptive statistics for the entry frequency into the treatment and non-treatment Y-maze arms by the stoat and possum-managed and unmanaged populations when exposed to stoat odour (n=23 rats).

Factor	Mean	Median	Range	Standard deviation	Interquartile range
			Enter treatment Arm		
Stoat-managed	3.8	2	0 – 17	5.1	7
Stoat-unmanaged	2.2	1	0 – 7	2.8	4
Possum-managed	6.0	6	4 – 8	2.8	.
Possum-unmanaged	3.8	0	0 – 15	7.5	4
			Enter non-treatment arm		
Stoat-managed	3.4	2	0 – 13	4.5	4
Stoat-unmanaged	1.8	1	0 – 6	2.5	4
Possum-managed	4.5	4.5	2 – 7	3.5	.
Possum-unmanaged	2.6	1.0	0 - 11	5.2	8

Table 3.8 Descriptive statistics for the entry frequency into the treatment and non-treatment Y-maze arms by the possum and stoat-managed and unmanaged populations when exposed to possum odour (n= 15 rats).

Factor	Mean	Median	Range	Standard deviation	Interquartile range
			Enter treatment Arm		
Possum-managed	6.5	5.5	0 – 11	7.8	.
Possum-unmanaged	2.0	0.5	0 - 7	3.4	6.0
Stoat-managed	3.3	3.0	0 - 7	3.8	7.0
Stoat-unmanaged	3.7	4.0	1 - 6	2.5	.
			Enter non-treatment arm		
Possum-managed	4.0	4.0	0 - 8	5.7	.
Possum-unmanaged	2.5	1.0	0 - 8	3.8	6.0
Stoat-managed	3.0	3.0	0 - 6	3.5	6.0
Stoat-unmanaged	3.7	4.0	0 - 7	3.5	.

Table 3.9 Results of Kruskal-Wallis testing for the frequency of entry to the treatment or non-treatment Y-maze arm by the rats in response to treatment cues and management background (n=23 rats).

Factor tested	Entry to treatment arm	Entry to non-treatment arm
Treatment (all)	H (2) = 0.018; P = 0.991	H (2) = 0.07; P = 0.967
Background management (all)	H (3) = 3.790; P= 0.285	H (3) = 0.89; P= 0.828

Table 3.10 Results of the Mann-Whitney U-testing for the frequency of entry to the treatment or non-treatment Y-maze arm by the rats from stoat and possum-managed and unmanaged populations in response to stoat and possum odours (n=23 rats).

Factor tested	Treatment	Entry to treatment arm	Entry to non-treatment arm
Stoat-managed / unmanaged	Stoat	U = 23.5; P= 0.661	U = 22.0; P = 0.583
	Possum	U = 5.5; P= 0.858	U = 5.0; P = 0.857
Possum-managed / unmanaged	Possum	U = 3.0; P = 0.8	U = 3.5; P = 0.800
	Stoat	U = 2.0; P= 0.533	U = 2.0; P= 0.533

There were no significant differences in the entry frequency to the treatment or non-treatment arm evident for any of the four backgrounds or three presented treatments (Kruskal-Wallis test; all comparisons $P \geq 0.05$) (Table 3.9). Nor was there any significant difference in the entry frequency to either treatment or non-treatment arm in the presence of stoat or possum odour exhibited by the rats from either stoat or possum-managed and unmanaged areas (Mann-Whitney U-test; all comparisons $P \geq 0.025$ Bonferonni corrected) (Table 3.10).

There was a considerably greater mean entry frequency into the treatment arm by the rats from the possum-managed area than from the possum-unmanaged area when the rats were exposed to possum odour (Table 3.8).

3.5 DISCUSSION

Contrary to my prediction there was no difference in avoidance responses between rats from the stoat-managed and the stoat-unmanaged areas. As predicted there was no difference in avoidance response between the possum-managed or unmanaged rat sample populations. However there were some results of note in terms of foraging patterns. It was evident, albeit not significant statistically, that rats from stoat-managed backgrounds exhibited a greater number of entries into the foraging trays and that their entry rate per seed consumed was also higher than other groups. This I interpret as evidence of a carry and consume foraging strategy which is discussed in more detail below.

The results of neither the enclosure nor the Y-maze offer any evidence of the expected avoidance response towards predator odour. In the enclosure experiment avoidance responses were assessed using two measures, entry into a foraging station or consumption of sunflower seeds in the presence of stoat, chinchilla and possum odour cues. Since the rats had been introduced to the presence of the seeds in the foraging stations during the acclimation phase, and had willingly consumed the seeds during this phase, I presumed that any change in behaviour or consumption would be attributable to the presence of the treatment odour cue. A reduction in entry events in response to a treatment odour would indicate that the rats were exhibiting an avoidance behaviour that prevented them from accessing the known food resource.

Based upon the majority of current predator response literature (see Apfelbach, et al., 2005; Kats & Dill, 1998 for reviews) I expected that the rats would avoid areas tainted with a predator cue, and predicted a lower entry rate in the presence of stoat odour. This prediction was not supported by the data with no apparent avoidance behaviour in the form of entry frequency being found in response to any treatment odour. I also predicted that since predator –prey interactions have both learned and innate components that the rats that were most familiar with stoat predators i.e. those from areas which were stoat-unmanaged , would exhibit greater avoidance behaviour than those from areas with reduced stoat (managed) densities. This prediction was not supported by the results; rather the greatest mean entry frequency was exhibited by rats from the areas with reduced predator and competitor exposure.

Foraging is a behaviour that is often cited as being affected by predator cues (see Apfelbach et al. 2005; Kats & Dill 1998 for reviewed examples). I predicted that the rats would reduce their consumption of sunflower seeds in the presence of stoat odour but not in the presence of any of the other three treatments. The results did not support this prediction with no difference in the number of seeds consumed according to treatment. I further predicted that seed consumption in the presence of stoat odour would be most reduced in the rats most familiar with stoat predators (the stoat-unmanaged groups); the results did not support this prediction. Overall, seed consumption did not diminish under any treatment or with any management background in response to stoat or possum odour indicating that the presence of a predator or competitor odour cue in the form presented here was not sufficient to affect this aspect of ship rat behaviour.

In the Y-maze apparatus first response and number of entries into the treatment and non-treatment arm were used to assess rat response to the treatment cues. Both these measures are potentially susceptible to individual lateral biases therefore individuals exhibiting this behaviour were identified and excluded from the final analyses.

I expected that the arm entered first would be significantly affected by the treatment presented, with rats actively avoiding the treatment arm in the presence of the predator (stoat) odour, and exhibiting no such differentiation when presented with the possum or chinchilla odour treatments. The absence of such a choice could still be deemed a response in terms of predator odour as 'freezing' is a valid response to predation threat (Blanchard & Blanchard 2003; Blanchard et al. 2001; Endres et al. 2005; Hubbard et al. 2004; Ribeiro-Barbosa et al. 2005; Sousa, Almeida & Wotjak 2006), especially where no escape is possible.

Bramley & Waas (2001) did similar experiments and based on those results I predicted that under the Y-maze experimental conditions ship rats would actively choose to avoid the predator odour either by moving into the non-threatening control arm, or by simply freezing in the start arm. Therefore a 'first response': enter or avoid; is the most appropriate response parameter to report. Neither the overall dataset result nor the individual treatment or background analyses of the data indicate that the first response differed from random. It is of note here that when individuals froze in the start arm, they tended to do so at the release point where the cloth bag in which they had been transferred to the maze remained in-situ offering a limited amount of cover. Furthermore they tended to face towards the maze i.e. the direction from which the threat would come. This is consistent with observations reported by Blanchard and Blanchard (2003) where they report a similar behaviour in colony living laboratory rats housed in a visible burrow system when exposed to cat threat.

However, 7/23 (30%) rats elected to enter the treatment arm in the stoat presentation, a response contrary to initial expectations. It may be that under the experimental conditions the rats were unable to clearly differentiate which arm was the source of the odour, and were simply 'panicked' into escaping the start arm (and the observer) making no conscious odour related choice. Alternatively they may have genuinely elected to investigate the odour source, deliberately moving in an 'up-wind' fashion towards the threat (Bramley et al. 2000). Examining the remaining treatments it is apparent that in all cases the rats are most likely to demonstrate an avoidance response as their initial reaction regardless of the treatment cue presented. As with stoat treatment a minority of rats did enter the treatment arm as a first response. This might also simply be consistent with a random distribution of responses.

Activity as a response parameter was measured in this experiment via frequency of entries into each arm I decided *a priori* that the frequency of visits was a better measure of activity than time spent in each arm because in many instances the rat rapidly moved into the first choice arm and simply stayed there, thus these rats would record a high duration of time in a single arm which is not a true reflection of their activity or necessarily an accurate reflection on their ease in that arm (Bramley et al. 2000). For example rats may move rapidly from the start arm to 'escape' the experimenters' presence, stop in the first arm selected, and then simply 'freeze' in response to the predator or other odours presented. That there was no significant difference reported in entry frequency between the treatment and non-treatment arms under any of the odours tested may indicate that the active rats are randomly exploring the maze and were neither deterred nor attracted by any one treatment.

There was no significant difference in overall seed consumption or entry frequency by the rats, however while not statistically significant, the entry frequency of the rats from stoat-managed areas in the presence of stoat odour was considerably higher than that of rats from the non stoat-managed areas. The rats did appear to exhibit high individual variability, which is likely to affect the ability of statistical tests to report a significant result without considerably larger sample sizes. This was also apparent in previous research but I had, based upon Bramleys' sample sizes of 15 mixed sex rats per species used (Bramley & Waas 2001 p. 1032), considered my sample size to be appropriate within the scope of the resources available.

The raw data suggests a greater entry frequency per unit consumed in the stoat-managed rats (3.6 entries per seed consumed) than the stoat-unmanaged rats (2.6 entries per seed consumed). Similarly possum-managed rats exhibit a greater entry frequency per unit consumed (3.2 entries per seed consumed) than do the possum-unmanaged rats (1.8 entries per seed consumed). Thus the enclosure experiment data suggested that rats with reduced exposure to both stoats and possums were entering the foraging stations more frequently than their counterparts from unmanaged areas. Although unsubstantiated it is possible that this is evidence of different foraging strategies as a result of the management conditions. There are sound ecological reasons why such differences in foraging behaviour might develop, and such adaptations have been previously reported in Norway rats (*Rattus norvegicus*) (Klemann & Pelz 2006; Fenn & MacDonald 1995). While not statistically verified at this point, largely due to sample size and behavioural variability in the rats, I consider the 'mean entry frequency per seed consumed' data warrants further discussion here.

As the sunflower seeds used were un-husked the rats had to take the time to access and consume the seed in-situ, or remove the seeds individually then husk and consume them elsewhere. Although carrying behaviour was difficult to identify reliably on video, rats removing seeds individually might be expected to generate a greater entry frequency than those that husked and consumed multiple seeds at any one entry. The greater entry frequency among rats from stoat-managed areas in response to stoat odour may result from their lack of familiarity with the stoat predator leading to a more cautious foraging behaviour in the form of seed removal for consumption elsewhere, reflected in the higher mean entry per seed consumed rate. Such behaviour has been observed in laboratory studies utilising ‘risk-approaching’ signals (Onuki & Makino 2005). Wild Norway rats living in high and low disturbance habitats on a pig farm were also seen to adjust both temporal activity (daytime foraging) and visitation pattern with the high disturbance rats exhibiting frequent short visits compared to the low disturbance habitat where rats spent longer foraging at each visit (Klemann & Pelz 2006). Wild Norway rats have also been reported to temporally shift foraging to daylight hours in response to increased nocturnal predation threat (Fenn & MacDonald 1995).

Prey animals that are regularly exposed to predator cues, but without a reinforcer: e.g. an actual encounter, may become habituated to the cue and thus demonstrate reduced responses. Parsons and Blumstein (2010) discuss this phenomenon in some depth and concluded that for a number of species, including several macropodids, such habituation does not occur (Parsons & Blumstein, 2010). The ship rats from the stoat-managed areas are assumed to have a lower exposure to stoats due to the lower stoat populations, thus there could be less opportunity for habituation to develop, which would explain their apparently greater response in the form of entry frequency rates. I suggest that under reduced stoat densities an encounter with a stoat cue, particularly a body odour cue as I have used here, is both rare, and likely to be followed by a reinforcer such as an actual encounter, further reducing any opportunity for habituation.

The response of the rats from the possum-managed area to possum odour might similarly be viewed as a response to the threat of a larger competitor, a similar behaviour has been seen in response to con-specific competitors in wild Norway rats (Whishaw & Whishaw 1996). Those rats that do not routinely encounter possum cues in their habitat may elect to carry the food resource away to a ‘safe’ place to process and consume, whilst those that are more accustomed to possum presence, the rats from the possum-unmanaged area, might be more inclined to consume food rewards in situ, having assessed the risk of interference as low.

An alternative, but complementary explanation, may be that the rats from the managed backgrounds were more inclined to return to a known food resource location than were those from unmanaged backgrounds. For the rats from the stoat-unmanaged areas, regularly returning to the same location might be a high risk strategy because stoats could ‘learn’ the location of likely rat prey (pers obs.; stoat predation on live-trapped rats and as ‘by-catch’ in rat cage traps) and include these locations in their own hunting strategy. Thus these rats might best minimise the number of visits to the foraging stations, whilst maximising their intake of available food: i.e. the 'sit and eat' strategy.

Possums are not known to prey upon adult ship rats and the two species do forage in close proximity (pers obs.), but if mutually accessible resources are scarce or particularly desirable a possum would likely out-compete a ship rat. As with stoats, possums were observed (both on video and as trapping 'by-catch') over the course of this study to 'learn' the location of food resources (intended for the ship rats) and make repeat visits to these locations (pers obs.). Thus the rats from the possum-unmanaged area might also be best to engage in a 'sit and eat' strategy to maximise consumption of the food resource.

For the rats from the stoat-managed areas in particular, where predation rather than foraging interference is the threat, the rarity with which these encounters occur would make cautionary behaviour an adaptive response. However, for those rats living under more frequent exposure conditions a cautionary response at every encounter would soon prove maladaptive, costing too much time in terms of foraging, reproduction and territory defence. These animals, to be successful, need to develop rapid and accurate risk assessment skills enabling them to avoid any real predation risk but rapidly continue with everyday life when the risk is low.

I suggest that the rats from the stoat-unmanaged areas, under enclosure conditions, where there was neither spatial nor temporal restraint imposed, assessed the risk posed by the odour cue presented, determined it to be low and thus did not exhibit any response measureable by this experiment. The rats from the stoat-managed areas, being less experienced with stoat predation, may have responded with a quantifiable degree of caution in engaging in their foraging behaviour evidenced by the number of entries per seed consumed.

In the Y-maze experiment the rats again did not exhibit significant avoidance of the predator cues. Y-maze, and other similar experiments often generate avoidance type responses, or results which are largely inconclusive and which are not supported by field results (Bramley & Waas 2001; Bramley et al. 2000; Burwash et al. 1998a, 1998b). Blanchard and Blanchard (2003) provide a thought provoking discussion of the shortcomings of laboratory based predator-prey research.

The choice of subject animal, wild caught ship rats, may have resulted in the lack of evidence for predator avoidance behaviour exhibited because none of these individuals could be considered predator naive, unlike a significant proportion of the study animals in other published literature. Comparative studies using captive ship rats are rare as this is not a species that has been widely domesticated for either laboratory or pet populations. However, Norway rats (*R. norvegicus*), are found wild in New Zealand and one study which examined the response of wild-caught Norway rats to predator cues found that those captured from a predator-free background exhibited avoidance to five of six predator odours (natural and synthetic), whilst those captured from multi-predator backgrounds exhibited limited avoidance to carnivore odours (Bramley et al. 2000). These authors also suggest that predator experience may be a major contributory factor in behavioural modification from the innate and generalised fear exhibited by the predator naive individual to a learned and more situation appropriate response of more experienced individuals.

3.6 CONCLUSION

The aim of this research was to determine whether animals with presumably limited exposure to predators exhibited a behaviourally different response to a familiar predator cue to those with presumably greater predator exposure (thesis objectives A and C). I predicted that rats sourced from backgrounds thought to have reduced predation would be less responsive to the stoat odour presented because they were less experienced and thus less able to recognise the threat represented by stoat odour. This prediction was not supported by the evidence of either experiment.

There was no significant avoidance response shown by the rats I tested overall, but there was some evidence of a shift in foraging strategy by the rats from stoat and possum-managed areas in the form of increased feeding station entry frequency per seed consumed which may have been indicative of a ‘carry and consume’ strategy for the sunflower seeds rather than a ‘sit and eat’ strategy.

All the rats in this research had potentially been exposed to some level of stoat threat because the stoat management strategies reduce rather than eliminate the resident stoat population. Therefore they were potentially all predator-experienced to some extent, unlike domestic laboratory rodents that are totally predator naive. These two experiments show that an avoidance response may be more typical of totally predator naive domestic laboratory subjects than it is of wild captured prey animals and that experimental protocols may greatly influence the behaviours exhibited by predator-experienced animals. I conclude that experience, even at what might be perceived as a reduced level does affect a prey individuals’ behaviour, via risk assessment skill, when faced with a predator cue.

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Chapter 4 DO SHIP RATS (*RATTUS RATTUS*) EXHIBIT NON-AVOIDANCE RESPONSES TO A FAMILIAR PREDATOR?

4.1 ABSTRACT

The response of prey species to indirect predator cues such as odour is a well established field of research with a large focus on laboratory or other small captive rodents exhibiting avoidance behaviour, while piscean, avian and larger mammalian research often reports non-avoidance responses including mobbing, individual attack and inspection behaviour. Much of the non-avoidance, and predator inspection behavioural research in particular, has focussed on group living species, but despite the colonial living tendencies of many rodents e.g. Norway rats (*R. norvegicus*) this form of predator response is rarely reported. One possible explanation for this is that if predator inspection is a learnt behaviour, predator-naive laboratory rodents are incapable of exhibiting the behaviour, however predator-experienced wild animals should.

I undertook three experiments, two in captivity and one in the field, to investigate whether non-avoidance behaviour in wild ship rats in response to a familiar predator occurred. For these experiments I used ship rats (*R. rattus*) that could be expected to be familiar with predators. Based on my observations I predicted that these predator-experienced prey animals would exhibit non-avoidance responses akin to predator inspection and any predator inspection behaviours would be less apparent in a population of wild rats that had lived under reduced predator exposure conditions because they had less opportunity to learn from experience. Two of my experiments, field and captive enclosure, supported my first prediction with significantly more sniff approach and mouthing behaviours directed towards stoat (*Mustela erminea*) odour. My second prediction was not supported because there was no discernible difference in the frequency of the mouthing behaviour between unmanaged or reduced predator (stoat-managed) populations.

I conclude that there is evidence in wild ship rats for a risk assessment motivated predator inspection behaviour, and that even reduced predator exposure is sufficient to develop this response.

4.2 INTRODUCTION

The response of prey animals to their predators has been well studied and a wide range of responses reported including changes in foraging and reproductive behaviour, both avoidance and aggressive e.g. mobbing behaviour, and paradoxically investigative or inspection behaviour (as reviewed by Apfelbach et al. 2005; Kats & Dill, 1998). Predator inspection behaviour has been reported in species including: Thomson's gazelle (FitzGibbon 1994), black-tailed prairie dogs (Owings & Owings 1979) and a wide range of fish (Brosnan, Earley & Dugatkin 2003; Brown & Dreier 2002; Dugatkin et al. 2005; Haberli, Aeschlimann & Milinski 2005; Thomas et al. 2008; Walling et al. 2004). Mobbing in birds, and more rarely in fish has also been reported, but this is usually interpreted as aggressive defensive (anti-predator) behaviour rather than an investigatory behaviour (Hein 1996; McLean, Smith & Stewart 1986; Smith, Arcese & McLean 1984). Typically these are behaviours that are engaged in by multiple individuals in an 'organised' manner which culminates in the attack of the potential predator; whereas 'inspection' behaviour, when dealing with live predators, involves approach and retreat but not attack per se (Hein 1996). Predator inspection differs from predator directed aggression in that it is generally exhibited by a small number of individuals within a larger group, and is very much an individual activity when compared to the more organised mobbing behaviour. Unlike aggressive interactions, predator inspection does not culminate in an attack on the predator.

Generally the role of predator inspection behaviour is thought to be related to increasing the preys' ability to detect a predator, improving recognition of predators, assessing the risk posed, and improving attack avoidance (Kelley & Magurran 2003), i.e. it is a largely experience driven learned behaviour. In some cases the inspection behaviour has been related to mate selection and social status within the group dynamic (Gomez-Laplaza 2002). Whilst predator inspection behaviour has primarily been investigated under laboratory conditions, it has often involved wild captured subjects rather than domesticated species (Brown & Cowan 2000; Malavasi et al. 2004; Thomas et al. 2008; Walling et al. 2004). Predator inspection behaviour has also been reported in free-living populations (FitzGibbon 1994; Pelligrini, Wisenden & Sorensen 2010), thus making it a compelling example of a predator instigated response that can be seen to occur under both captive and wild conditions and in both wild captured and domesticated subjects.

Predator inspection behaviour by rodents has not been reported in mainstream behavioural literature, although aggressive confrontations between predators and rodent prey have been reported (Guimaraes-Costa et al. 2007; Owings et al. 2001; Owings & Owings 1979;

Swaisgood, Owings & Rowe 1999); and risk assessment as a defensive behaviour in the presence of a threat has been described (Blanchard & Blanchard 2005). Given the extensive literature investigating predator response with rodents as a prey species, and the colony–living nature of the wild pro-genitor of the domestic laboratory rat (*R. norvegicus*); it seems unusual that examples of an investigatory behaviour in response to predators have not been reported. One possible explanation is that in the predator-naive individual the avoidance response is effectively the 'default setting' when encountering a predator cue, thus in predator-naive laboratory rodents there has been no opportunity for a learned behaviour, such as inspection, to develop.

New Zealand, where all obligate terrestrial mammals except two bat species are introduced, offers an opportunity to investigate whether a rodent species, represented by the ship rat (*Rattus rattus*), exhibits a non-avoidance or inspection type behaviour towards a primary forest predator, the stoat (*Mustela erminea*) both in captivity and in the wild. New Zealand also allows investigation of whether any such behaviour might be modified by prey experience via predator exposure levels. These two species have co-existed in the mainland New Zealand forest for approximately 130 years, stoats being the later arrival, introduced to NZ in 1884 (King & Murphy 2005). Ship rats are usually solitary as adults, although mothers and juvenile / sub adult offspring are often found together (Innes 2005: Carter pers. obs.) in the NZ forest. Ship rats are occasionally found nesting together and can co-exist at very high densities on islands and within human habitation (Innes 2005). Thus as a species, they may have evolved some of the same group living attributes that contribute to the exhibition of predator inspection behaviour in other species.

Both stoats and ship rats are a recognised threat to the endemic flora and fauna of NZ (Innes 2005; King & Murphy 2005). Stoats in particular are intensively managed by kill trapping at a number of locations for the protection of highly threatened endemic avifauna and restoration of ecosystem function. Although managed throughout parts of the country, rat control is seldom so intensive. This management situation allows large populations of ship rats to live under conditions of significantly reduced predation threat and therefore reduced opportunities to gain experience of surviving or avoiding stoat predation. Thus ship rat populations in both normal predator density areas and reduced predator density areas, are accessible to investigate the effects of predator experience upon prey animals.

In addition to the predator-prey interaction, the Australian brush tail possum (*Trichosurus vulpecula*) co-exists alongside stoats and rats and has both habitat and dietary overlap with rats (Ruscoe et al. 2011). The possum is physically much larger than either stoats or rats but does not pose a known predatory threat to the adult of either species, although it is likely to be a major resource competitor for rats. Possums are also considered a major ecological and economic threat and are routinely controlled via lethal trapping and poisoning creating both unmanaged and reduced-competitor density zones for rat populations.

In light of these conditions the aims of this research were to: (1) investigate whether non-avoidance responses to predators occurred in wild caught or free-living ship rats; and (2) determine whether any such responses differed in intensity or expression between rats sourced from stoat-unmanaged areas and those from reduced (managed) stoat density areas. I predicted that a non-avoidance response would be apparent in these wild, and to some (unknown) extent predator-experienced animals. However, since these behaviours are thought to be learned, I predicted that responses would be less apparent in rats sourced from the stoat-managed areas because these animals would have had less predator exposure and less opportunity to learn investigatory behaviour. I expected no difference in behaviour between rats from possum-managed and possum-unmanaged areas and those from stoat-unmanaged areas because possum management should not affect exposure to stoats. Based upon the limited captive research using ship rats, and the literature about wild-captured subjects more generally, I predicted that the enclosure and field experiments would generate comparable results, whilst the Y-maze would generate more mixed results.

4.3 METHODS

A mixture of wild-caught and free-living ship rats were used in three experiments: a captive enclosure experiment, using wild captured ship rats, aimed at allowing freedom of expression of behaviour (c.f. Blanchard and Blanchard 2003). I expected that this approach would elicit responses that were reflective of a free-living population; a Y-maze laboratory experiment representative of past predator response research with ship rats specifically; and a field experiment undertaken at the source locations of the captive rats, aimed at representing the responses of the captive rats under free-living conditions. The Y-maze experiments were conducted on the same captive rats approximately three weeks after the conclusion of the enclosure experiment. These two experiments, using four groups of rats, took place between July 2009 and January 2010. The field experiments took place over January and February 2010. To date, and to the best of my knowledge, neither 'mouthing' or 'chew or remove' behaviours that I report here have previously been reported in either predator inspection or predator – prey interaction literature. I believe that these behaviours are an important and currently underestimated component of the ship rat predator response repertoire that warrants closer investigation. That these behaviours have not been reported in the vast laboratory rat literature is discussed in depth in the discussion section.

4.3.1 EXPERIMENT 1: INVESTIGATIVE RESPONSES OF SHIP RATS: CAPTIVE ENCLOSURE

The methods outlined here are those outlined in Chapter 2 General Methods: section for captive experiments (2.2.2).

Data analysis: Approach and investigation type behaviour was assessed by scoring the frequency of behaviours directed at the treatment odour cue.

Only once all the assessments were completed were the camera and foraging station identifications reconciled with the rat's identification and the treatment that was presented, thus all rat behaviour was effectively scored blind. Scored data were entered into an Excel 2007 Student Version spreadsheet..

Statistical analyses: General exploration of the investigatorybehaviours (see 'Results') against treatment and management background were undertaken using SPSS (Version 16.0 Student). From this an assessment was made as to what test approaches, parametric or non-parametric, would be most suitable. Exploration confirmed that in most cases the data were not amenable to distribution correcting transformation, were highly variable, had unequal dataset sizes or small dataset sizes thus non-parametric tests have primarily been utilized. Mean, median, range, standard deviation and interquartile ranges for each treatment and background are presented in the results.

Kruskal-Wallis analyses were used to determine whether behaviours were affected by the treatment odour presented or management background. Where the Kruskal –Wallis test returned a significant result, Mann-Whitney U-tests were used to determine which background or treatment pairings were significantly different. Stoat odour was the primary treatment of interest thus the remaining three treatments were tested against this.

Mann-Whitney U-tests were used to determine if management background (managed or unmanaged), affected investigative behaviour in the presence of any of the treatment odours. These statistical analyses were carried out using SPSS 16.0 (Student version) and Bonferroni corrections were then calculated and presented where necessary.

For the most extreme of the frequent resultant investigative behaviours (mouthing) I converted the observed frequencies to a binary value: 0 = 0 or 1 event or 1 = 2+ events per rat per night. This is presented as a bar chart and was examined using a G-test (Excel 2007 Student Version). This was done as I considered that the distinction between a cursory investigation and a repeated investigation was important, this is discussed in more detail below. The demographics and response frequencies of rats exhibiting this behaviour are also reported on. Additional generalised mixed linear modelling using the 'R' statistical package was undertaken by Tian Mao and Leiyan Wang under the supervision of Professor Steve Haslett (Massey University Statistics Department). Factors tested include treatment, order of presentation, source location of rats and the effect of a stoat presentation on later treatment presentations. The results are reported in brief in the results section and the full reports are presented as Appendix 3.

4.3.2 EXPERIMENT 2: INVESTIGATIVE RESPONSES OF SHIP RATS: CAPTIVE Y-MAZE

The preparatory and procedural methods used are those outlined in Chapter 2 General Methods: Y-maze (2.2.3).

Data collection: The most clearly definable non-avoidance response previously found (this chapter, Experiment 1) was the mouthing behaviour. Because arm entry and first response had been previously analysed in terms of avoidance behaviours (Chapter 3, Experiment 2: Y-maze avoidance responses), only the investigatory mouthing behaviour is considered here for comparison with the results of the alternative captive procedure, the enclosure (this chapter, Experiment 1).

Statistical analyses: As in the analyses of mouthing in Experiment 1, the behaviour was converted to a binary value: 0 = 0 or 1 event or 1 = ≥2 events; in order to distinguish a perfunctory investigation from sustained interest, and the data was tested by a G-test (Excel 2007 Student Version) with the null hypothesis that there would be no difference in repeated mouthing (≥2 events) frequency between treatment and control odour presentations. The number of times ≥2 mouthing events occurred for both treatment and control at the same presentation is also calculated.

The demographics and management background of rats exhibiting the mouthing behaviour are also reported on.

4.3.3 EXPERIMENT 3: INVESTIGATIVE RESPONSES OF SHIP RATS: FREE-LIVING

The methods outlined here are those outlined in the General Methods section for field experiments (2.3).

Data collection: Investigatory behaviour was assessed by scoring the frequency of approach and investigation type behaviours directed at the odour cue. The frequency of three specific behaviours and one form of physical evidence was recorded. Scored data were entered into an Excel 2007 Student Version spreadsheet for statistical analysis.

Analyses: The number of individual ship rats interacting with the experimental apparatus was estimated by comparing video and data logger evidence of rat presence. The prevalence of the investigatory behaviours across the sites and recording nights is also reported.

General exploration of the resultant non-avoidance behaviours (see 'Results') against treatment and management background were undertaken using the SPSS statistical package "Explore" function. Mean, median, range, interquartile range and standard deviation for each treatment and background are presented in the results.

To minimize the number of statistical tests carried out on an already small dataset, the most extreme of the frequently occurring investigatory behaviours (mouthing) was analysed via the Wilcoxon signed-rank test only between stoat and the treatment(s) with the closest reported mean frequency value, chinchilla and possum.

Within each management pair: stoat-managed and stoat-unmanaged; possum-managed and possum-unmanaged , three dependant variables (approach, sniff, mouth) were tested using the Mann-Whitney U-test for differences in response to the odours presented. This was to specifically determine whether there were any differences in these behaviours attributable to the exposure levels of the resident populations to predators (stoat) or competitors (possum), and whether a novel odour (chinchilla) would elicit an investigatory response. Bonferroni corrections were then calculated and presented where necessary.

Mouthing data were transformed into a binary frequency table: 0 = 1 or 0 events per location per night and 1 = ≥2 events per location per night, this data along with frequency of physical evidence, damage to the zip-lock presentation bags, are presented graphically due to the small sample sizes.

4.4 RESULTS

1. Rearing approach (RA): the rat approaches the treatment odour presentation (to within approximately 20cm) and rears onto its hindquarters with its attention apparently focused on the presentation .
2. Approach (A): the rat enters the foraging tray directly below the test treatment odour; or the rat approaches the batten to which the treatment odour is attached to within approximately 20cm.
3. Sniff approach (SA): the rat approaches the treatment odour presentation and reaches towards it, either on all fours or in a rearing position to investigate closely (within 5cm) but without physical contact with the treatment presentation.
4. Mouthing (M): the rat approaches the treatment odour presentation and reaches towards it, either on all fours or in the rearing position, and physically takes the odour presentation into its mouth. This sometimes escalates from a 'mouthing' to a vigorous tugging of the treatment presentation (See Figure 4.1 and CD-R Video 1 & 2).

5. Chew or remove (CR): the zip-lock bag containing the sample blanket exhibited evidence of a physical interaction in the form of tooth marks or tears, was physically removed from the batten, or the sample blanket was removed from the zip-lock bag.

In total there were five behaviours recorded, four on video and one in the form of physical evidence, damage to the zip-lock presentation bag. Because of the differing experimental set-up between captive and field experimentation there was slight variation in the variables recorded: captive enclosure 1, 2, 3 & 4; y-maze 4; field 2, 3, 4 & 5.

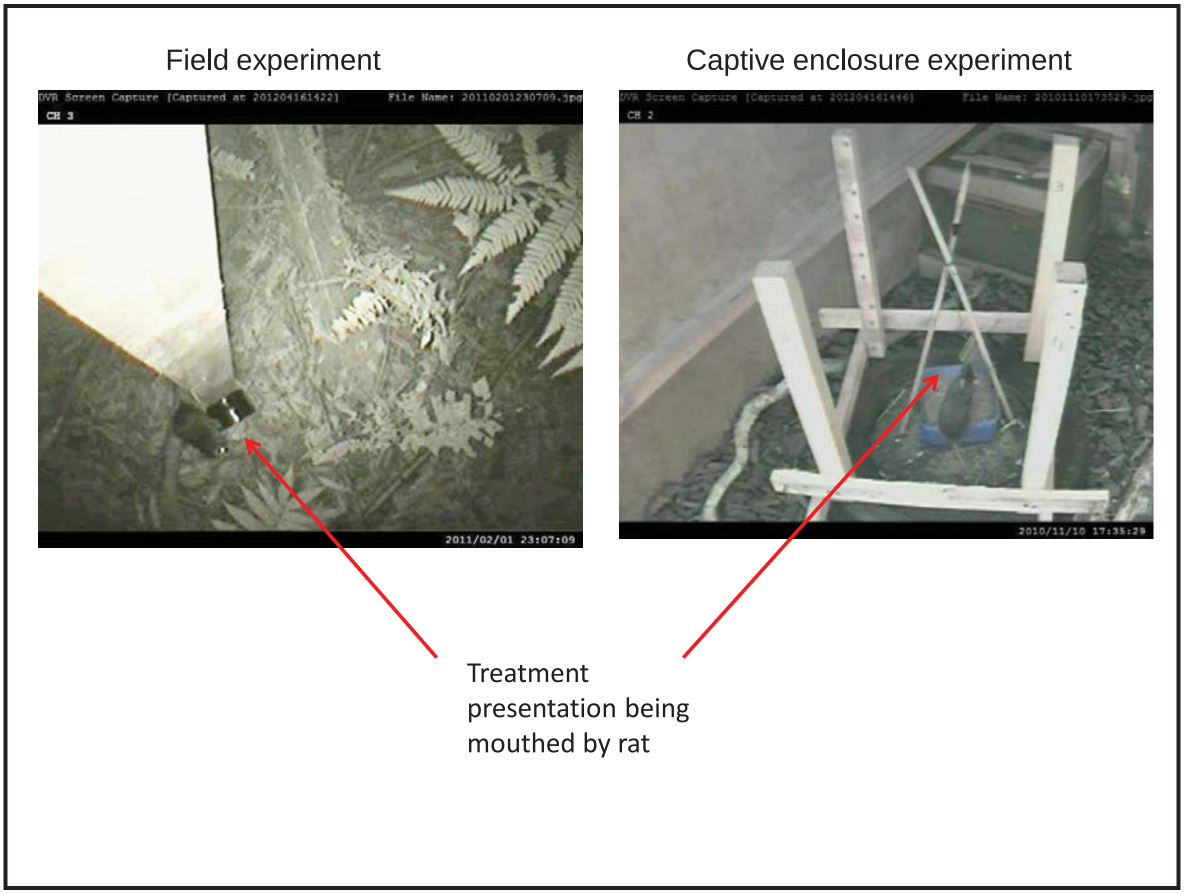

Figure 4.1 Mouthing behaviour as exhibited by wild captured rats in the captive enclosure and free living rats in the field experiments.

4.4.1 Experiment 1: : Investigative responses of ship rats: Captive enclosure

Responder analyses: A total of 21 / 31 rats exhibited the mouthing response to one or more treatment cues. Nine rats responded to control odour of which two exhibited > 1 mouthing event and two responded only to control and not to any other odour. Six rats responded to chinchilla odour including one rat that exhibited > 1 mouthing event and one rat that responded only to chinchilla odour and not any other. Two (/15) rats responded to possum odour, but neither exhibited >1 mouthing event and one responded only to possum odour.

Sixteen rats (eleven male and five female) responded to stoat odour, of which 13 exhibited >1 mouthing event and eight responded only to stoat odour (Table 4.1).

Table 4.1 Individual mouthing responses by male and female rats towards novel and familiar odours.

Responder Rats	Sex	Control	Chinchilla	Stoat	Possum
		Number of individually scored mouthing events			
1	Female	1			NA
2	Male	1	1		NA
3	Male			7	NA
4	Male			1	NA
5	Male			1	NA
6	Male		1	6	NA
7	Female	1			NA
8	Female	5	10	4	NA
9	Male	1		2	NA
10	Female	4	1	7	
11	Male	1		4	
12	Male			7	
13	Male			2	
14	Male			1	
15	Male		1	2	
16	Female			2	
17	Female		1		
18	Male		1		
19	Female	1		4	
20	Female				1
21	Female	1		2	1

<u>Overall responses:</u> There was a significant difference in the event frequency for sniff approach (p= 0.001) and mouthing (p= 0.001) (Kruskal-Wallis test, P≤0.05) against odour presented. Rearing approach was also significantly different (p=0.001) according to the background management of the rats. However Mann-Whitney U testing to establish which pairings differed significantly in rearing returned no significant results after Bonferonni correction (p≤ 0.0083; 6 tests; Tables 4.2 – 4.5). There was also significantly more frequent sniff approaches and mouthing in response to stoat odour than any either possum or chinchilla odour, but not the control after Bonferoni correction (p≤ 0.016; 3 tests; Table 4.5)

There was no significant difference in any of the three response variables (rearing approach, sniff approach or mouthing) towards the different treatments within management background pairs: stoat-managed / stoat-unmanaged or possum-managed / possum-unmanaged (Mann-Whitney U-test; all comparisons P≥0.025 Bonferroni corrected; 2 tests) (Table 4.6).

Table 4.2 The event frequency of three behavioural responses (rearing approach, sniff approach, mouthing) against odour and background management (n= 31 rats).

Factor	Mean	Median	Range	Standard Deviation	Interquartile range
			Rearing approach		
Control odour	2.3	1.0	0 - 7	2.507	5.0
Chinchilla odour	2.5	2.0	0 - 15	3.020	2.0
Stoat odour	4.7	3.0	0 - 26	5.658	7.0
Possum odour	1.2	1.0	0 - 3	1.014	2.0
			Sniff approach		
Control odour	2.8	1.0	0 - 15	3.737	5.0
Chinchilla odour	2.5	1.0	0 - 15	3.254	3.0
Stoat odour	5.3	4.0	0 - 17	5.068	7.0
Possum odour	.9	1.0	0 - 3	0.915	1.0
			Mouthing		
Control odour	.50	0	0 - 5	1.136	1.0
Chinchilla odour	.48	0	0 - 10	1.805	0
Stoat odour	1.7	1.0	0 - 7	2.338	2.0
Possum odour	.13	0	0 - 1	0.352	0
			Rearing approach		
Stoat-managed	3.7	2.0	0 - 26	4.878	4.0
Stoat-unmanaged	3.5	2.5	0 - 14	3.501	5.0
Possum-managed	1.2	1.0	0 - 4	1.405	2.0
Possum-unmanaged	0.7	0	0 - 3	1.078	1.0
			Sniff approach		
Stoat-managed	3.7	2.0	0 - 17	4.468	5.0
Stoat-unmanaged	3.8	2.0	0 - 15	4.506	5.0
Possum-managed	1.4	1.0	0 - 5	1.557	2.0
Possum-unmanaged	1.5	1.0	0 - 8	1.966	2.0
			Mouthing		
Stoat-managed	1.0	0	0 - 10	2.236	1.0
Stoat-unmanaged	0.8	0	0 - 7	1.747	1.0
Possum-managed	0.2	0	0 - 2	0.599	0
Possum-unmanaged	0.8	0	0 - 4	1.125	1.0

Table 4.3 Results of Kruskal - Wallis tests for behavioural responses (rearing approach, sniff approach, mouthing) to odour and background management (n=31 rats).

Response variable	H(3)	P
	Treatment	
Rearing approach	5.523	0.137
Sniff approach	14.581	**0.002**
Mouthing	14.058	**0.003**
	Management Background	
Rearing approach	16.89	**0.001**
Sniff approach	4.976	0.174
Mouthing	2.277	0.517

Table 4.4 Results of the Mann-Whitney U - testing of those management background pairs most likely to have caused the significant results reported in the Kruskal Wallis testing (n=31 rats; Bonferroni corrected p= 0.0083).

Test Variable	Grouping variable	U	P
Rearing approach	Possum-managed : possum-unmanaged	69.0	0.174
Rearing approach	Stoat-unmanaged : possum-unmanaged	132.5	0.001
Rearing approach	Possum-managed : stoat-unmanaged	142.5	0.031
Rearing approach	Stoat-managed : stoat - unmanaged	781.5	0.857
Rearing approach	Stoat –managed : possum-unmanaged	142.0	0.030
Rearing approach	Stoat-managed : possum-managed	141.0	**0.001**

Table 4.5 Results of the Mann-Whitney U - testing of those treatment pairs most likely to have caused the significant results reported in the Kruskal Wallis testing, focusing upon stoat odour as the treatment of primary interest (n=31 rats; Bonferroni corrected p= 0.016). Significant results are in bold.

Test Variable	Grouping variable	U	P
Sniff	Possum : Stoat	84.0	**<0.001**
Sniff	Chinchilla : Stoat	303.0	**0.012**
Sniff	Control : Stoat	324.0	0.026
Mouthing	Possum : Stoat	130.5	**0.006**
Mouthing	Chinchilla : Stoat	301.0	**0.003**
Mouthing	Control : Stoat	333.0	0.019

Table 4.6 Mann-Whitney U-tests to determine whether odour affects the behaviour (rearing approach, sniff approach, mouthing) of rats from different management background pairs (n=31 rats; Bonferonni corrected p=0.025).

Response Variable	Treatment	Management pair	U	P
Rearing approach	Control	Stoat-managed : Stoat-unmanaged	69.0	0.860
	Chinchilla		69.5	0.883
	Stoat		63.0	0.601
	Possum		6.5	0.617
Rearing approach	Control	Possum-managed : Possum-unmanaged	6.0	1.000
	Chinchilla		1.0	0.056
	Stoat		4.5	0.578
	Possum		5.5	0.853
Sniff approach	Control	Stoat-managed : Stoat-unmanaged	71.0	0.953
	Chinchilla		60.0	0.481
	Stoat		67.5	0.794
	Possum		5.0	0.343
Sniff approach	Control	Possum-managed : Possum-unmanaged	3.5	0.307
	Chinchilla		4.0	0.467
	Stoat		5.5	0.853
	Possum		5.5	0.853
Mouthing	Control	Stoat-managed : Stoat-unmanaged	67.0	0.497
	Chinchilla		65.0	0.852
	Stoat		67.0	0.624
	Possum		8.0	1.000
Mouthing	Control	Possum-managed : Possum-unmanaged	3.0	0.180
	Chinchilla		4.0	0.248
	Stoat		3.0	0.252
	Possum		3.0	0.180

<u>Mouthing response:</u> The frequency of repeated mouthing (≥2 events) clearly shows a significant predominance of this behaviour in response to stoat odour (Figure 4.2) (G=20.09, d.f.=3, P< 0.0001).

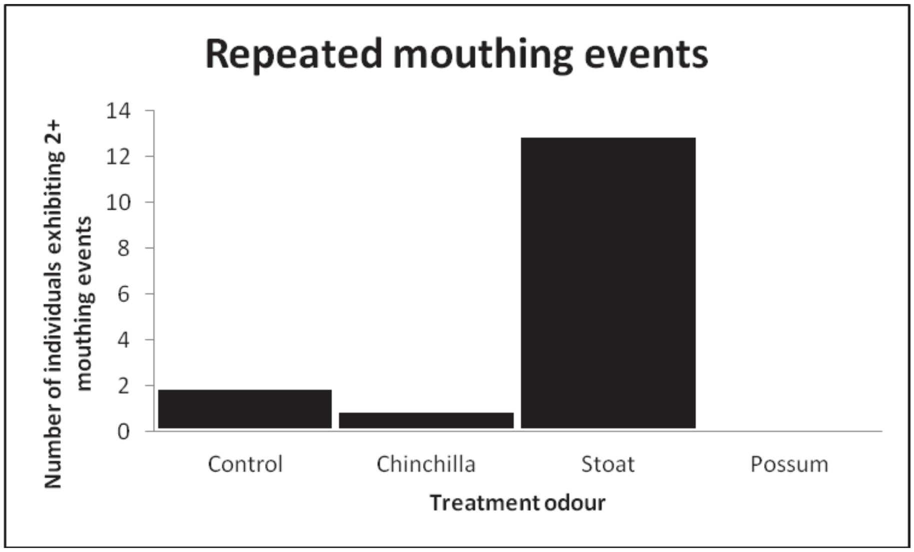

Figure 4.2 Number of rats exhibiting a repeated (≥2 event) mouthing response towards the odours presented.

Such a marked investigative response towards a predator odour is unprecedented in ship rat research and therefore in order to confirm that the response seen was due to the stoat odour and not any other confounding factor I enlisted assistance (Professor Steve Haslett, Massey University Department of Mathematics and Statistics) to undertake more comprehensive statistical analyses on the data. Using conservative quassipoisson without random effects modelling (S. Haslett and L. Wang personal communication, January 14, 2011), incorporating all four rat groups (LW 1- 3, ENP) for stoat, chinchilla and control treatments only (rats n=31), the mouthing behaviour was found to be significantly greater with stoat treatment (GLM z= 2.617; p=0.0104). Regardless of source location the ship rats exhibited significantly greater inclination to engage in the mouthing behaviour in response to stoat than any of the other treatments presented (GLM z= 3.196; p=0.00139).

They also investigated the possibility that the randomly assigned order in which the odours were presented may have affected the mouthing (tug/taste as it is termed in the reports) response. Inclusion of all 4 locations and orders of odour presentation (1st, 2nd and 3rd) shows that the ENP rats exhibited significantly more mouthing behaviour than the three groups of LW rats (GLM Laplace approximation: z=1.974, p=0.0484). The order of presentation did not significantly affect the model, indicating that the mouthing response was not directly affected by the order in which the control, chinchilla or stoat treatments were presented. Considering only LW 2 and 3, and orders of presentation (1st – 4th) indicates that source location is not a significant factor either, however in this model an order effect for 2nd and 4th is significant. There is no ready explanation for this but it was suggested (L. Wang, personal communication, January 14, 2011) that it may simply be an artefact of the random allocation of treatment order.

This result with respect to the order of presentation did raise some concerns and further models were developed to specifically test whether exposure to the stoat treatment influenced the response of rats to later treatments. All four additional models indicated no significant effect of source location or prior exposure to the stoat treatment. Thus whilst I still cannot rule out that these significant results were generated in response to the order of presentation positions, I can be sure that prior exposure to the stoat treatment was not the causal factor.

The full reports by Wang et al are presented in Appendix 3.

4.4.2 EXPERIMENT 2: INVESTIGATIVE RESPONSES OF SHIP RATS: CAPTIVE Y-MAZE

Mouthing response: The frequency of repeated mouthing (≥2 events) was widespread across all treatments including the control (control = 14/61; chinchilla = 8/ 24; stoat = 7/24; possum = 5/13). There was no significant difference in the repeated mouthing events between any of the treatments (G-test, G=3.44, d.f.=3, $P > 0.05$). Ten of the rats responded to predator (stoat) odour including six males and four females. Of the 10 that responded, four were from each of the stoat-managed / unmanaged backgrounds. One rat that responded to stoat odour was from a possum-managed background, and one was from a possum-unmanaged background.

4.4.3 EXPERIMENT 3: INVESTIGATIVE RESPONSES OF SHIP RATS: FREE-LIVING

Sample size: Only three individually tagged rats were recorded interacting with the apparatus (one per location) and all three were males. There was no video evidence to confirm the presence of more than one rat at any of these locations, however except for in the stoat-managed sites, video evidence and the distance between sites suggested that at least four other rats interacted with the apparatus. They may not however have approached close enough for the tag to be read i.e. they had not placed their heads through the hoop antenna to closely investigate the sample. The trap capture location for the three tagged rats which were positively identified and the location of the experimental sites where they were recorded indicated that all three rats were captured in the immediate vicinity (within ca. 50m) of the apparatus. Thus I conservatively estimate an interacting sample population of at least seven individual rats (three tagged, four untagged or unread).

Prevalence of the mouthing behaviour across sites: There were a total of 64 camera nights available for data analysis (16 camera nights per treatment). However of these two camera nights were lost due to recording failures, one due to the camera being flipped over, and two nights when no rats visited. Therefore a total of 59 camera nights, with a rat presence were available for analysis. The mouthing behaviour was the least displayed of the three behaviours recorded on video, and was only witnessed on 10 of the 59 possible camera nights. Mouthing was witnessed at one site each within the possum-unmanaged and stoat-unmanaged locations, and both sites at the stoat-managed location, but was not seen at the possum-managed location (Figure 4.3).

Exploratory data analysis of the event frequency data suggested a response to the stoat treatment odour in the three behaviours assessed (approach, sniffing, mouthing). However, no trend was visible due to the background management for the three behaviours (approach, sniff, mouth) (Table 4.7).

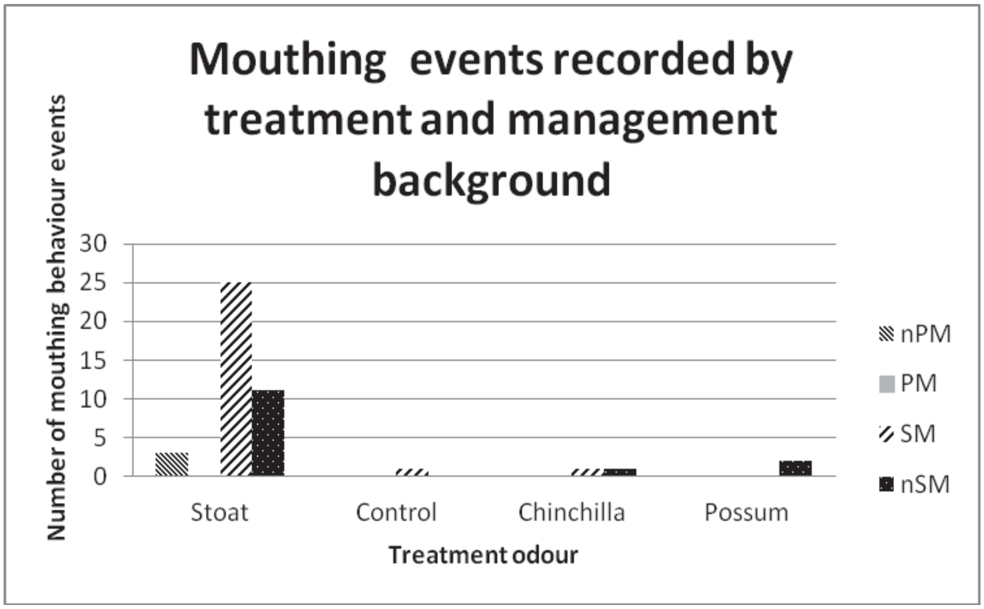

Figure 4.3 Frequency of mouthing events in response to simultaneously presented odours at the four experimental locations: possum-unmanaged (PuM), possum-managed (PM), stoat-unmanaged (SuM), stoat-managed (SM).

Table 4.7 Descriptive data for the approaching, sniffing and mouthing behaviours in response to odour and background management factors under field conditions.

Factor	Mean	Median	Range	Standard deviation	Interquartile Range
			Approach		
Stoat odour	5.2	1.5	0 – 22	7.859	10.0
Control odour	2.8	2.0	0 – 9	2.808	5.0
Chinchilla odour	2.1	1.0	0 – 9	2.475	4.0
Possum odour	3.1	3.0	0 – 10	2.863	5.0
			Sniff		
Stoat odour	3.0	0	0 - 15	4.926	7.0
Control odour	0.2	0	0 - 1	0.414	0
Chinchilla odour	0.6	0	0 - 2	0.828	1.0
Possum odour	0.8	0	0 - 4	1.328	1.0
			Mouthing		
Stoat odour	2.4	0	0 - 14	4.442	4.0
Control odour	0.07	0	0 - 1	0.258	0
Chinchilla odour	0.07	0	0 - 1	0.258	0
Possum odour	0.2	0	0 - 1	0.403	0
			Approach		
Stoat-managed	4.9	4.0	0 - 14	4.827	9.0
Stoat-unmanaged	5.6	3.5	0 - 22	6.830	4.0
Possum-managed	2.3	2.0	0 - 6	2.082	4.0
Possum-unmanaged	0.7	0.5	0 - 2	0.793	1.0
			Sniff		
Stoat-managed	1.8	0.5	0 - 12	3.599	1.0
Stoat-unmanaged	2.44	1.0	0 - 15	3.983	3.0
Possum-managed	0.19	0	0 - 2	0.544	0
Possum-unmanaged	0.38	0	0 - 3	0.806	1.0
			Mouthing		
Stoat-managed	2.00	0	0 - 14	4.506	1.0
Stoat-unmanaged	0.81	0	0 - 7	1.940	1.0
Possum-managed	0	0	0	NA	NA
Possum-unmanaged	0.19	0	0 -3	0.750	0

<u>Approaching, sniffing and mouthing behaviour:</u> The results of the Wilcoxon-signed ranks tests showed significant differences in both sniffing and mouthing but not approaching in response to odour. More mouthing was directed at stoat odour than possum and chinchilla odours which had the closest mean event frequency (Z= -1.992, n = 16, P= 0.046). I found no significant differences (Mann –Whitney U tests p≤ 0.025 after Bonferroni correction) in behaviour (approaching, sniffing, mouthing) within management background pairs (stoat-managed : stoat-unmanaged; possum-managed : possum-unmanaged) (Table 4.7).

The repeated mouthing response data shows that this behaviour is exclusively exhibited towards stoat odour, occurring on five video nights (Figure 4.4).

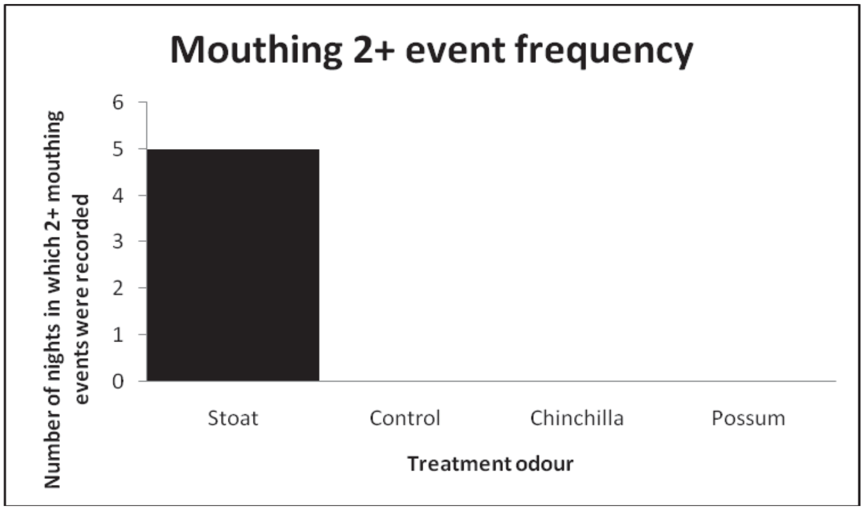

Figure 4.4 Number of nights on which repeated (2+ events) mouthing behaviour was recorded in response to simultaneously presented treatment odours in field trials.

Table 4.8 Mann-Whitney U-test to investigate whether exposure to odours affects the behaviours (approaching, sniffing, mouthing) between the different management pairs (n= 24 exposure nights).

Response Variable	Treatment	Management pair	U	P
Approach	Stoat	Stoat-managed : Stoat-unmanaged	4.0	0.243
	Control		7.5	0.884
	Chinchilla		7.5	0.884
	Possum		2.5	0.108
Approach	Stoat	Possum-managed : Possum-unmanaged	5.0	0.369
	Control		5.0	0.369
	Chinchilla		5.0	0.369
	Possum		2.5	0.091
Sniff approach	Stoat	Stoat-managed : Stoat-unmanaged	6.5	0.655
	Control		2.5	0.190
	Chinchilla		2.5	0.190
	Possum		3.0	0.134
Sniff approach	Stoat	Possum-managed : Possum-unmanaged	7.5	0.850
	Control		7.5	0.850
	Chinchilla		7.5	0.850
	Possum		6.0	0.317
Mouthing	Stoat	Stoat-managed : Stoat-unmanaged	6.0	0.538
	Control		4.0	0.248
	Chinchilla		4.0	0.248
	Possum		6.0	0.495
Mouthing	Stoat	Possum-managed : Possum-unmanaged	6.0	0.317
	Control		8.0	1.000
	Chinchilla		8.0	1.000
	Possum		2.5	0.091

Physical evidence: Chewing evidence on the sample bags was recorded on four nights, two each for stoat-unmanaged (both site A) and stoat-managed (both site B). In all cases the chewing occurred on the stoat odour sample bag and no other bags were affected (Figure 4.5). This observation concurs with video analyses which showed frequent and intensive mouthing behaviour at those locations. It is likely that it was the same individual responsible for both events at each location: i.e. two individuals exhibiting the behaviour.

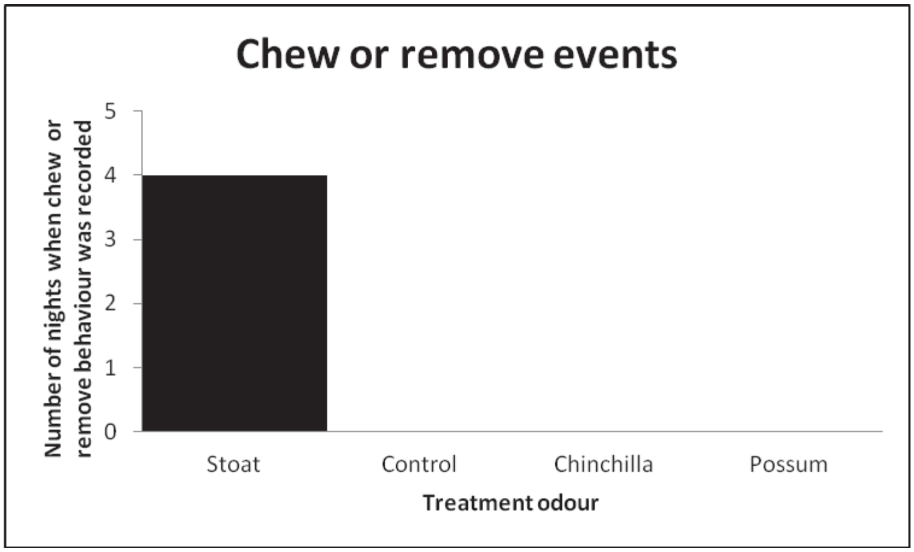

Figure 4.5 Number of nights when chew or remove behaviour was recorded in response to the simultaneously presented treatment odours in field trials.

4.5 DISCUSSION

As predicted both the enclosure and field experiments produced evidence of investigative responses significantly more often directed at stoat odours than at the chinchilla or possum odours. After the Bonferoni correction was applied there was no significant difference in mouthing or sniffing behaviour between stoat and control treatments, although in both cases the values fell close to reaching the significance levels required. I suggest that this result is in part down to the small sample sizes in conjunction with the conservative nature of the Bonferoni testing. In part the lack of any odour associated with the control treatment may also have driven the rats to investigate it more closely, but not repeatedly, as was borne out by the repeated mouthing analyses. The Y-maze experiment produced no evidence of investigation via the mouthing behaviour. Contrary to expectation there was no difference detected in the frequency of the investigative behaviours, specifically mouthing in any of the experiments according to predator exposure background. As predicted the captive enclosure and field experiments generated similar results whilst the captive Y-maze experiment generated inconsistent results.

Of the three behaviours analysed (rear approach, sniff approach and mouthing), mouthing is the most investigative and least ambiguous in terms of video scoring and analyses. Therefore I have foccussed the discussion on mouthing, with approaching, rearing and sniffing being considered as prerequisite behaviours in the build-up to the expression of mouthing. In other words my observations lead me to hypothesize that: a rat must first make some kind of investigatory approach (which may or may not be related to the treatment odour), it may or may not investigate more closely by sniffing, and finally it may or may not escalate to the mouthing behaviour. More rarely a rat may approach and engage in mouthing without a sniffing behaviour, but it is possible that in these cases the rat had engaged in the sniff behaviour but it had not been detected by the observer during the analyses of the video. The ship rats in this study exhibited an investigatory behaviour, mouthing directed towards predator (stoat) odour under captive and field conditions regardless of geographic or management background.

Non-avoidance behaviours generally take two forms, that of aggressive interactions or investigatory / inspection type interactions. Aggressive interactions are typified by mobbing behaviours, in which multiple individuals act together to drive off a potential predator. Mobbing is often reported in avian species (e.g. Bērziņša et al. 2010), and has also been observed in ungulate species (e.g. Caro et al. 2004). These behaviours have also been reported in group living rodents towards snake predators, but in these cases the prey had evolved some immunity to the predatory snake venom thus physiologically reducing the risk of fatal interactions (Owings, et al. 2001; Owings & Owings 1979; Swaisgood et al. 1999). A second form of aggressive behaviour, and one more commonly demonstrated by prey animals acting alone is that of maternal aggression whereby a mother will act to protect her offspring (Caro et al. 2004; Swaisgood et al. 1999).

The behaviours I observed were witnessed in both sexes, and no nursing females were used in the captive experiment, thus it cannot be ascribed to maternal aggression. Even under field conditions the behaviours only ever appeared to involve one individual and thus cannot be described as mobbing. Whilst the mouthing behaviour could be described as aggressive, it is directed at an odour source not at a real predator, thus such behaviour is unlikely to result in an aggressive attack on the predator itself. To the contrary, under field conditions a rat has been witnessed fleeing from a stoat (pers obs.) and whilst larger prey such as rabbits may, as adult individuals, be capable of successfully fending off a stoat attack by an aggressive counter attack (King & Powell 2007), the relatively small ship rat would be highly unlikely to do so. The observed mouthing behaviour seems more likely to represent a form of predator inspection behaviour directed towards the odour source. In this case the response was greatest towards an assumed familiar predator.

Although predator inspection is primarily reported in group living species, it is only ever undertaken by some members of the group. In my experiments the prey species, ship rats, although largely solitary in the forest habitat, is commonly found living at extremely high densities in island situations or in human habitation (Innes 2005). Ship rats are known to be present at high densities in houses within one kilometre of the possum-unmanaged location (pers obs.), thus it may be that the wild rats used in the field study were predisposed to expressing group living behaviours if circumstances dictate or it is advantageous to the rats to do so. Not all the rats that were captured on video expressed this behaviour, consistent with the group living scenario where not all individuals express predator inspection behaviour.

Predator inspection has been considered to be a largely learnt behaviour (see Brown and Chivers 2005 for discussion) and has been shown to be modified by predation experience. For example a number of studies, primarily piscean, have demonstrated that predator-sympatric populations exhibit significantly greater predator inspection behaviour than predator-naive populations do (see Walling et al. 2004 for a review). Walling et al. (2004) also suggest that inappropriate inspection behaviour may play a role in the development of inspection behaviour within and between populations i.e. experience and selective mortality play a part in developing appropriate inspection behaviour. They found that there was little difference in inspection behaviour between small and large sticklebacks (*Gasterosteus aculeatus*) from predator-sympatric populations and suggest that whilst it is possible that experience was not a factor in stickleback predator inspection, it was more likely that these small fish had gained sufficient predator experience prior to capture to develop the inspection behaviour (Walling et al 2004). Magurran (1990) found that in laboratory reared European minnows (*Phoxinus phoxinus*) antipredator behaviour is inherited, but is adjusted with early experiences, increasing the inspection rate. Similarly Csânyi, Csizmadia and Mikloski (1989) found that even limited (60 second) exposures to a non-predatory species, common goldfish (*Carassius auratus*) could result in habituation in the paradise fish (*Macropodus opercularis*), while a five minute exposure led to a long-term (three months) memory formation.

The role of genetics versus experience in predator inspection behaviour remains ambiguous . For example, juvenile hatchery reared sea bass (*Dicentrarchus labrax*) of wild parentage exhibit predator inspection behaviour, as do wild captured individuals (Malavasi et al. 2004).

Studies with other species however indicate that experience significantly affects the expression of predator inspection behaviour (e.g. Magurran 1986; Magurran & Seghers 1990; Magurran 1990; McCartt, Lynch & Johnson 1997). My results indicate that the mouthing behaviour is expressed in captive populations that were sourced from geographically disparate locations (Egmont National Park / Lake Waikaremoana) and thus if the behaviour is genetically based in the ship rat, it is now widespread within the North Island population.

If predator inspection is occurring in the ship rat there must be a sound ecological driving mechanism. Understanding the motivation for predator inspection behaviours has been a recurring theme in the literature to date with suggestions including: reducing the predation risk to the inspecting individual, acquiring information and informing others (Brown, Paige & Godin 2000; FitzGibbon 1994; Haberli, Aeschlimann & Milinski 2005); risk assessment (Fishman 1999); mate selection (Godin and Dugatkin, 1996); reciprocal altruism or cooperative behaviour (Bleakley & Brodie 2009; Thomas et al. 2008); and foraging trade-offs (Brown & Cowan 2000). Others have suggested that predator inspection is a behaviour that is indicative of personality traits which themselves have survival value, for example boldness, learning ability, adaptability or fighting prowess (Brick & Jakobsson 2002; Dugatkin & Alfieri 2003; Pelligrini et al. 2010; Quinn & Cresswell 2005; Webster, Ward & Hart 2009). Predator inspection behaviour and how it is expressed by an individual has also been suggested to be indicative of an individuals' social status within a group (Godin & Davis 1995; Gomez-Laplaza 2002). In some fish species, sex and size have been found to be important factors in predator inspection, the larger sex – tending to be the more profitable prey- also exhibit enhanced anti-predator behaviour including predator inspection (Kelley & Magurran 2003).

As the ship rats in my research were not group or colony living, motivations that involve an audience effect such as warning others, reciprocal or co-operative behaviour or gaining social status within a group are unlikely to apply. Benefits occurring to an individual are more likely to explain the persistence of predator inspection. Mate selection cannot be discounted because although the rats were tested in isolation both in captivity and in the wild, it is possible that the captive rats were aware of rats of the opposite sex in neighbouring experimental enclosures or in the housing runs. Similarly it is possible that rats of the opposing sex were in the vicinity of the field apparatus but not within camera view. If the indication that this is a primarily male behaviour is correct; then mate selection might be a strong, but not exclusive, motivator.

A more generally applicable mechanism might be that of risk assessment, incorporating foraging trade-off. Ship rats in much New Zealand mainland forest probably encounter predator cues on a routine basis as stoats are almost as ubiquitous a presence as the rats. If the prey species were to take avoidance action at every cue encounter, the individuals' ability to engage in essential activities such as territory defence, foraging and mate searching might be seriously compromised reducing not only their own personal fitness, but also their lifetime reproductive fitness (Brown & Chivers 2005). Those individuals that are able to undertake a risk assessment that enables them to rapidly resume their normal behaviour would be at an ecological advantage. The propensity to display the mouthing behaviour might be linked to a personality trait such as boldness since in experiment one (captive enclosure) over half the individuals that exhibited mouthing towards a non-stoat treatment odour also exhibited this response towards stoat odour; while half the stoat odour responders responded only to stoat; i.e. the most threatening cue.

The investigation of non-stoat odours was markedly less apparent in free-living animals in experiment three (field) where there was virtually no mouthing interaction seen towards any odour except stoat, despite all four cues being available simultaneously for inspection by any interacting rat. I suggest that the increased interest exhibited by the captive enclosure rats towards the non-stoat odour cues is an artefact of the captive environment and the experimental design as the rats were induced to approach the odour cue by the knowledge of a food resource being present in the form of sunflower seeds. Thus the rats cursorily investigated the non-stoat treatment odour presented, usually with no more than one mouthing interaction, before continuing with other activities such as foraging.

In contrast, when presented with the stoat odour half the individuals engaged in a mouthing behaviour, and a clear majority of those engaged in more than one mouthing interaction. I interpret this as evidence that whilst the rats may investigate any odour or object that is close to a foraging source for example, they only exhibit repeated or sustained interest in the predator odour. Comparing the responses of rats in the captive enclosures to the free-living rat responses, free-living rats showed virtually no mouthing interaction with non-stoat odour in experiment three, nor was there any food resource present, thus the rats that interacted with the treatment odours were not influenced by any other factor such as a foraging opportunity.

In experiment two (captive Y-maze), there was no significant difference in the frequency of repeated mouthing between treatment odours, and in most cases where repeated mouthing was observed for the treatment sample, there was also repeated mouthing directed at the control sample (14/20 cases). I suggest this reflects the contrived and confined nature of the Y-maze experiment wherein the rats are both temporally and spatially restricted and may be responding more to the experimental environment than the cues presented. I suggest that the results of this experiment, when compared to those of experiments one and three, provides evidence of the unsuitability of this type of experimental approach when investigating the predator response behaviours of wild captured subjects.

A fourth behaviour was witnessed in all three experiments, but only recorded and analysed in experiment three (free-living rats), that of chew or remove. This was an infrequent but notable behaviour because it was only recorded in response to the stoat odour treatment. This chew or remove is an escalation of the mouthing behaviour such that tooth marks and / or tears are clearly evident on the sample presentation zip-lock bag. In some instances the sample blanket was partially or completely removed.

The drive to access the source of the sample odour might be related to the role of the vomeronasal organ whereby further information can be gained by 'taste' in addition to olfaction (Wyatt 2003). Alternatively a phenomenon known as 'defensive burying' is widely reported in laboratory rodents whereby the individuals attempt to remove by burying objects that they perceive as threatening in their environment (De Boer & Koolhaas 2003; Pinel & Treit 1983). This chew or remove behaviour was recorded more frequently in subsequent experiments and is discussed in more depth in Chapter 5. Although it was exhibited by rats in the Y-maze I elected not to analyse it because it was not a behaviour that I had thought to record in experiment one and thus had no comparative data across the three experimental protocols. Observationally the chew or remove behaviour in the Y-maze was expressed as randomly as the mouthing behaviour, which of itself is a pre-requisite to chew or remove.

My prediction that a non-avoidance or investigatory behaviour could be seen in rodent prey species in response to predators has been supported by experiments one and three. The second of my predictions, that as a learned behaviour such responses would be less apparent in populations of reduced predator exposure has not been supported.

Overall the possum-managed and possum-unmanaged populations seem to be less responsive to any presented treatment than the stoat-managed and stoat-unmanaged populations, although why this should be currently remains unclear. Both these possum sites are located closer to human settlement than are the stoat-managed / stoat-unmanaged sites thus it is possible that these rats are exposed to a predator suite where feral and house cats are a more prominent feature and that this affects their responses as cats and stoats may utilise quite different hunting strategies. The small sample size for these two management strategies in captivity (four : three), is problematic, but that the same pattern is seen in the free-living animals suggests that there is a genuine 'other' factor at play.

Despite this apparent anomaly, these populations still exhibit the same mouthing response towards stoat odour in captivity, although the free-living rats from the possum-managed site did not exhibit the mouthing behaviour. The interactions between possums and rats in the New Zealand forest have only recently begun to be investigated, and already there is compelling evidence that there are significant population dynamic interactions (Ruscoe et al. 2011; Sweetapple & Nugent, 2007). I predict that further research will demonstrate that there are significant behavioural interactions between these two species that have not yet been reported

The lack of support for my hypothesis that the more stoat experienced rats (stoat-unmanaged; possum-managed; possum-unmanaged) would exhibit significantly more non-avoidance behaviour than the less predator-experienced (stoat-managed) population may be explained by two alternative hypotheses. Firstly, that the behaviour is a genetically heritable trait as would seem to be the case in for example juvenile sea bass (Malavasi et al. 2004); or that only a very small number of exposures is sufficient to develop the behaviour (Walling et al. 2004). I favour the latter as rats in the stoat-managed population are not predator-naive; rather they live under conditions of reduced predation threat and thus remain predator-experienced.

It may be that the reason predator inspection behaviour has not been reported in the plethora of predator odour response research utilising laboratory rodents is that these animals are genuinely predator-naive. Without any access to predator experiences such individuals might exhibit a default response which is that of predator avoidance. Since laboratory rodents have been domesticated for hundreds of generations, they may have lost the propensity to inspect, this behaviour may be considered 'abnormal' in captive populations and deliberately bred out during the domestication process. However my own observations, particularly in the field experiments, lead me to believe that experience is important, especially in juveniles and young sub-adults which were conspicuously absent from the odour experiments and yet were subsequently seen interacting with foraging apparatus at the same locations (Carter, unpublished data; and Chapter 6, Figure 4.5).

4.6 CONCLUSION

The results of these experiments have largely supported my hypothesis that ship rats exhibit investigatory responses to predator odours under both captive and wild conditions (thesis objectives A and B). I have interpreted mouthing as a form of predator inspection, which is driven by individual, rather than group related, benefits. I consider that the most likely is risk assessment.

My second hypothesis, that learned predator inspection would be less evident in the population living under reduced stoat exposure conditions, was not supported (thesis objective C). This might be explained by predator inspection being a genetically heritable characteristic rather than a learned behaviour; or that predator inspection behaviour can be developed in response to very few experiences, and hence even reduced exposure populations will have developed the behaviour. I favour the latter suggestion and posit that only truly predator-naive populations will fail to exhibit predator inspection.

If risk assessment is the primary driving mechanism, then predator inspection behaviour should be observed in response to other predators and not stoats alone. This is investigated in Chapter 5. There are other possible causal mechanisms for the investigatory or non-avoidance behaviour such as chemical cue overlaps, neophillic behaviour or parasite infection and these are addressed in Chapters 6 and 7.

Finally I have compared the results of three experimental protocols: captive enclosure, captive Y-maze and field trials and found that whilst the captive enclosure and field trials generated comparable results, the captive Y-maze does not. I suggest that: (1) the experimental environment and protocol might have a profound impact upon the expression of behaviours, particularly in wild caught subjects, potentially generating misleading results; and (2) my captive enclosure results can be viewed as a reliable proxy for the behaviours that will be observed in free-living ship rat populations.

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Chapter 5 DO INVESTIGATORY RESPONSES TOWARDS STOAT (*MUSTELA ERMINEA*) ODOURS IN SHIP RATS (*RATTUS RATTUS*) REPRESENT PREDATOR INSPECTION BEHAVIOUR?

5.1 ABSTRACT

Previous research (described in Chapters 3 & 4) revealed several investigatory behaviours exhibited by free-living and captive ship rats (*Rattus rattus*) in response to stoat (*Mustela erminea*) odour. I interpreted these behaviours as a form of predator inspection, a phenomenon which has been widely reported in piscean species, but more rarely in mammals including rodents. If my interpretation was correct then the investigatory behaviours, exemplified by mouthing and chewing or removing odour cues should be a generalised response to other familiar predators.

Using captive and free-living rats I investigated whether these two responses were also elicited towards cat (*Felis catus*) odour. In addition to the presentation of male stoat, female stoat and mixed sex cat odour I also present the odour of an unfamiliar male con-specific to determine whether the behaviours are specifically directed towards predators or are generalised across other forms of threat. I found that in captivity mouthing behaviour was more frequently directed at male stoat and cat odours than towards female stoat and male con-specific odours. The chew or remove behaviour was more frequently observed in response to the three predator odours than the con-specific odour. Under field conditions both behaviours were more commonly; and almost exclusively directed towards the male stoat odour. I found little evidence that the management background of the rat source populations (possum, *Trichosurus vulpecul*a or stoat control); or the proximity to known house and feral cat populations affected the exhibition of either behaviour.

The results of these two experiments continue to support my interpretation that a form of predator inspection explains the investigatory responses of ship rats to predator odours, although further work is required to further substantiate this or rule out alternative hypotheses, primarily those of a chemical cue overlap or parasite infection.

5.2 INTRODUCTION

Predator inspection is an investigatory behaviour reported in a number of prey species in response to the presence of a predator. This behaviour, although primarily reported in the piscean literature (see Brown & Chivers 2005; Malavasi et al. 2004; Walling et al. 2004 for comprehensive discussions), has also been reported in some mammalian species including artiodactyls (Caro et al. 2004) and gazelles (FitzGibbon 1994). Although the majority of the piscean research has been conducted under laboratory conditions, the subject populations have often been sourced from wild stock (Brown & Cowan 2000; Malavasi et al. 2004; Thomas et al. 2008; Walling et al. 2004); and predator inspection has also been reported amongst free-living species (Caro et al. 2004; FitzGibbon 1994; Pelligrini, Wisenden & Sorensen 2010). Whilst predator inspection is widely considered to be a learned response (see Brown & Chivers 2005 for a detailed discussion), there is some evidence that there is also a genetic component to its development (e.g. Malavasi, et al. 2004).

Any behaviour that involves a prey animal deliberately approaching a predator would seem to carry a significant degree of risk and thus there must be an appropriately significant fitness benefit from doing so. In the case of aggressive responses such as mobbing and maternal aggression, there might be safety in numbers, or elements of defence of resources and protection of offspring which might outweigh the risk to the approaching prey animal. Predator inspection however is characterised by individuals approaching the potential predator in a non-aggressive manner. The benefits of predator inspection to the inspecting individual might include: reducing the predation risk, acquiring information, informing others (Brown, Paige & Godin 2000; FitzGibbon 1994; Haberli, Aeschlimann & Milinski 2005); risk assessment (Fishman 1999); or improved mate selection (Godin and Dugatkin 1996), reciprocal altruism or cooperative behaviour (Bleakley & Brodie 2009; Thomas et al. 2008); and foraging trade-offs (Brown & Cowan 2000). Many of these potential benefits are more relevant to group living prey, where there is an audience or a degree of relatedness between individuals but whether this accurately reflects the motivation behind the behaviour or the species chosen for study is unknown.

One group of prey species that is poorly represented in the predator inspection literature is rodents. This seems unusual because there are many examples of both colony living and co-operative rodents, and rodent species are routinely utilised in predator – prey research. The non-avoidance behaviour that has been reported in rodent species has more often been interpreted as aggressive interactions than as investigative behaviour, although investigation may form a component of these responses (Guimaraes-Costa et al. 2007; Owings et al. 2001;

Owings & Owings 1979; Swaisgood, Owings & Rowe 1999). I suggest that there are three possible explanations for this lack of reporting of investigatory behaviour in rodent research to date: (1) that the propensity for predator inspection is a genetic trait and has effectively been bred out of domestic laboratory populations; (2) that predator inspection is learned and thus predator-naive laboratory or captive bred populations have not had the opportunity to learn the behaviour; (3) that investigatory behaviours have been observed but not interpreted or reported as such.

The ship rat (*Rattus rattus*) is a common rodent species in New Zealand and provides a model species for investigating predator inspection behaviour in predator-experienced rodents. Although primarily solitary in forest habitat, ship rats can and do live at high density on islands and in human habitations (Innes 2005), thus do have colony or group living tendencies. In New Zealand all obligate terrestrial mammals, except two bat species, are introduced. In mainland forest habitats there now exists a suite of mammalian pests and predators that are variously the target of lethal management programs for the protection of the endemic flora and fauna.

Four of the main forest predator and pest species in NZ are the ship rat, stoat (*Mustela erminea*), feral cat (*Felis catus*) and the brush-tail possum (*Trichosurus vulpecula*). In most mainland forests stoats and rats represent predator and prey, with possums existing as competitors of rats. Cats in deep forest, away from farmland and human habitation are present only in low numbers and thus are a less important rat predator (C. Gillies, personal communication, May 18, 2012). In a number of locations in New Zealand populations of ship rats live under conditions of reduced predation exposure due to the intensive and targeted lethal management of stoat and cat populations for the protection of endemic species. This situation allows the opportunity to investigate predator inspection in a rodent prey species (ship rat) at a species level, and specifically in populations sourced from both unmanaged and ‘reduced’ (managed) predator exposure backgrounds.

My previous research (Chapter 4) revealed a behavioural response to stoat odour which I interpret as predator inspection. If this interpretation is correct then I expect that the behaviour would be generalised to the odour of other familiar predators. Because the mammalian predator-prey suite in New Zealand mainland forest is comprised of introduced species that have not strictly co-evolved, any genetic component to the behaviour would have had to develop over an evolutionarily short timescale. Cats are believed to have arrived in NZ from 1769 (Gillies & Fitzgerald 2005), ship rats were reported as present from the mid 1800’s (Innes 2005) and stoats were deliberately introduced and released in 1885 (King &

Murphy 2005). Since cats were present on board ships for the purpose of rat control (Gillies & Fitzgerald 2005), rats surviving early journeys could be considered genetically or behaviourally predisposed to avoid cat predation. If cat recognition is innate, as suggested by laboratory rat research e.g. (Adamec & Shallow 1993; Blanchard et al. 2001; Hubbard, et al. 2004; McGregor et al. 2002) an avoidance behaviour towards cats should be expressed by all rats regardless of the source populations' proximity to known cat populations. If there is a learned modification to an innate predator avoidance response in ship rats it might be expected that any investigatory responses would be most evident towards the predator species that the rats have the most contact with, stoats or cat. However it might still be exhibited towards both predators if the rats had experienced even a limited exposure to the secondary predator.

Predator inspection is not the only possible explanation for the response described in Chapter 4. At least two significant alternative mechanisms could be operating including a chemical cue overlap (such as sex pheromones) between the prey and the predator species (e.g. Zhang, Sun & Novotny 2007) or a behaviour modifying parasite infection such as toxoplasmosis (Webster 2001).

The aims of this research were to: (1) ascertain whether the mouthing response previously reported was a generalised response to familiar predator odour cues (cat and stoat), and therefore consistent with being a predator inspection behaviour; and (2) ascertain whether the source location (management background and inferred cat proximity) of the rats affected their responses. I hypothesized that ship rats would exhibit the mouthing response towards both stoat and cat odour. I also predicted that source location in terms of stoat or possum management, or proximity to a 'known cat population' would not affect mouthing behaviour. Based on my previous experiments I also predicted that my captive enclosure and field trials would generate results which were comparable with each other.

5.3 METHODS

I used two experimental approaches: a captive enclosure experiment, using wild-captured ship rats, which allowed freedom of expression of behaviour (c.f. Blanchard & Blanchard 2003) that I expected would elicit responses that were more reflective of a free-living population than a captive one; and a field experiment undertaken at the source locations of the captive rats, which aimed to validate the responses of the captive rats under free-living conditions.

5.3.1 EXPERIMENT 1: MULTI-PREDATOR INVESTIGATORY RESPONSES – CAPTIVE ENCLOSURE

The methods used here were those outlined in Chapter 2.2.2 (General Methods for captive experiments) with the following exceptions:

Subject animals: Ship rats were captured at each of four separate locations within the Lake Waikaremoana (LW) area of Te Urewera National Park, New Zealand (Latitude: 38° 46' 0 S, Longitude: 177° 4' 60 E) between September and December 2010. The four capture sites represented reduced predator exposure (stoat-managed; Tapuaenui) and unmanaged predator (stoat; Tawa Walk) exposure sites; reduced competitor exposure (possum-managed; Onepoto) and unmanaged possum (Kaitawa Lakes) exposure. The possum-managed and unmanaged sites were also close to human habitation and farmland where populations of both tame and feral cats were present, thus representing 'near cat habitat' for this experiment; whilst the stoat-managed and stoat-unmanaged sites were deep within the national park and thus 'far cat habitat' (Chapter 2: Figure 2.3).

Prior predator exposure was expected at all four locations because predator management did not achieve elimination, however the stoat-managed rats were expected to have only limited experience of stoats and cats; stoat-unmanaged rats were expected to have 'normal' experience of stoats and limited exposure to cats; both possum-managed and possum-unmanaged rats were expected to have 'normal' (unmanaged) experience of stoat exposure and more than 'normal'cat exposure. The possum-managed rats were also expected to have been exposed to less interference competition from possums than any of the other three populations.

Three groups of eight animals were captured and retained over the course of the experiment, two stoat-managed / stoat-unmanaged (far-cat) pairings and one possum-managed /possum-unmanaged (near-cat) pairing.

Because the preceding experiments had indicated that males were the most likely to exhibit the mouthing behaviour, I aimed to use only male rats in this experiment to increase the responsiveness to the stimuli and thus the statistical robustness. A total of 20 adult males from the four source populations completed the experiments.

Subject order: Each group of eight rats was allocated to two experimental blocks according to home run block, upper (assigned as rat cage 1, 3, 5, 7) or lower (assigned as rat cage 2, 4, 6, 8) . A latin–square design was then used to allocate treatment order.

Experimental protocol: All rats were placed in the enclosures at least an hour before dark, and motion activated video recording occurred between 2.00 pm and 10.00 am. These hours were extended from the preceding experiment as I had witnessed occasional rats active much earlier in the day than I originally anticipated and wanted to be sure that I was not missing any important interactions.

Data analysis: Both avoidance and investigatory behaviour was assessed by scoring the event frequency of the following behaviours:

1/ Approach frequency (A): the rat enters the foraging tray directly below the test treatment odour.

2/ Seed consumption (SC): the number of sunflower seeds consumed in the presence of each treatment odour.

3/ Mouthing frequency (M): the rat approaches the odour presentation and reaches towards it (either on all fours or in a rearing position) and physically takes the odour presentation into its mouth, this sometimes escalated from a 'mouthing' to a vigorous tugging of the treatment presentation (Figure 4.1 & CD-R videos 1 & 2).

4/ Chew or remove (CR): the zip-lock bag containing the sample blanket exhibits evidence of a physical interaction in the form of tooth marks or tears; had been physically removed from the bamboo canes; or the sample blanket had been removed from the zip-lock bag (Figure 2.10).

Statistical analyses: Preliminary analyses and exploration of the behaviours (Approach, Mouthing, Seed Consumption) were undertaken using the SPSS statistical package. From this an assessment was made as to what test approaches, parametric or non-parametric, would be most suitable. Exploration confirmed that in most cases the data were not amenable to distribution correcting transformation, were highly variable, had unequal dataset sizes or small dataset sizes thus non-parametric tests have primarily been utilized. Mean, range and standard deviations for each treatment and background are presented in the results.

The non-parametric Kruskal-Wallis and Mann-Whitney U-tests were used to identify significant differences in response variable due to treatment, management background and proximity to known cat populations.

Where Kruskal –Wallis identified a significant difference further pair-wise Mann-Whitney U-tests were performed on pairings which showed the greatest (mean and median) difference until a non-significant pairing was reached. Specific treatment-focussed testing of significant avoidance responses associated with potential predator exposure was also undertaken. The data was grouped by treatment and Mann-Whitney U-tests undertaken to determine whether the response of rats sourced from specific management background or cat proximity pairs differed in the presence of any individual treatment. Where necessary Bonferroni corrections have been applied.

The number of rats exhibiting the mouthing behaviour was counted and converted into a binary value representing <2 events or ≥2 (repeated) events per rat per treatment as a means of distinguishing a perfunctory investigation from a repeated interest in the odour. This data was subjected to a G-test (Excel 2007 Student Version) and is presented graphically.

Chew or remove returned a small number of events and is also presented graphically, the data was also subjected to a G-test (Excel 2007 Student Version) test for significant differences in occurrence between odours..

5.3.2 EXPERIMENT 2: MULTI-PREDATOR INVESTIGATORY RESPONSES: FIELD

The methods used here are those outlined in the General Methods section for field experiments except the changes noted below:

Data analysis: The number of interacting rats was estimated via video and data logger evidence, and species other than rats seen on the video were also are recorded.

Both avoidance and investigative behaviour was assessed by counting the event frequency of the following behaviours:

(1) Approach frequency (A): the rat approached to within ca. 20cm of the batten and odour presentation.

(2) Mouthing frequency (M): the rat approached the odour presentation and reached towards it, either on all fours or in a rearing position, and physically took the odour presentation into its mouth, this sometimes escalated from a 'mouthing' to a vigorous tugging of the zip-lock bag (Figure 4.1 & CD-R videos 1 & 2).

(3) Chew or remove (CR): the zip-lock bag containing the sample blanket exhibit evidence of a physical interaction in the form of tooth marks or tears; had been removed from the batten; or the sample blanket had been removed from the zip-lock bag (Figure 2.10).

Statistical analyses: Preliminary analyses of the approach and mouthing variables were undertaken using the SPSS (version 16 Student) statistical package. General exploration of approach and mouthing against odour, management background and proximity to known cat populations were undertaken using the SPSS 'Explore' function.

The non-parametric Friedman's signed rank test was used to determine if significant differences in either approach or mouthing behaviour occurred in response to the odours presented. Where this returned a significant result the Wilcoxon signed-ranks test was used to seperate significant differences in the response between treatment pairs. The data was then grouped according to treatment presented and the non-parametric Mann-Whitney U-test was used to test for significant differences in response variables (approach and mouthing) between stoat and possum-managed and unmanaged rats, and the near cat / far cat rats. Where necessary Bonferroni corrections have been applied. The distribution of mouthing events across odour, management background and inferred cat population proximity is presented graphically.

The mouthing behaviour was converted into a binary value representing <2 events or ≥2 (repeated) events per night per odour as a means of distinguishing a perfunctory investigation from a repeated interest in the odour. This data was subjected to a G-test to determine whether there was any significant difference in this behaviour between the treatment odours presented (Excel 2007 Student Version). This data is also presented graphically. Chew or remove behaviours only seldomly occurred so its occurrence according to the treatment to which it was directed is presented graphically. This data was also subjected to a G-test to test for any differences in this behaviour attributable to the treatment odours presented (Excel 2007 Student Version).

5.4 RESULTS

5.4.1 EXPERIMENT 1: MULTI-PREDATOR INVESTIGATORY BEHAVIOURS – CAPTIVE ENCLOSURE

<u>Overall responses:</u> The rats' approach and seed consumption varied in response to treatment and background (Table 5.1 & 5.2). Seed consumption was most variable in response to cat proximity, with both approach and mouthing less affected (Tables 5.2 & 5.3).

<u>Avoidance behaviours</u>: Seed consumption was significantly lower in the stoat-managed population than the possum-managed and possum-unmanaged populations (all comparisons $P \leq 0.025$ Bonferroni corrected; Tables 5.1 and 5.4). Rats sourced from populations with inferred higher cat populations made significantly more approaches and consumed significantly more sunflower seeds overall (Mann-Whitney U-test, Tables 5.1 and 5.5). However the presence of cat odour specifically did not affect seed consumption for any source population pairings (near / far cat populations: Mann-Whitney-U test: U=23.00,

P=0.065, N=20; stoat and possum management pairings:Kruskal-Wallis, H(3)= 4.431, P= 0.218).

Rats from the possum-unmanaged sample exhibited a significantly higher approach frequency to the odours in general than the possum-unmanaged and stoat-managed rat populations (all comparisons p≤ 0.025 Bonferroni corrected; Table 5.4). There was a significantly greater frequency of approach behaviour in the possum-unmanaged rat population than the possum-managed rat population in response to female stoat odour Table 5.6). There were no other significant differences reported in either seed consumption or approach response variables within management background pairs: stoat-managed and stoat-unmanaged or possum-managed and possum-unmanaged; or in response to cat proximity (Mann-Whitney U-test; all comparisons P ≥ 0.0125 Bonferroni corrected) (Tables 5.6 & 5.7).

Table 5.1 The frequency of approach, mouthing and seed consumption by ship rats in the enclosure in response to odour and background management (n= 20 rats).

Factor	Mean	Median	Range	Standard deviation	Interquartile range
			Approach		
Rat odour	22.25	14.0	0 - 82	24.144	22.0
Cat odour	25.75	17.5	0 -106	28.840	24.0
Stoat male odour	22.35	14.5	0 - 88	20.098	20.0
Stoat female odour	18.26	14.0	0 - 58	16.716	14.0
			Mouthing		
Rat odour	1.25	1.0	0 - 4	1.482	2.0
Cat odour	3.45	1.5	0 - 16	4.466	5.0
Stoat male odour	5.95	3.0	0 - 31	8.494	6.0
Stoat female odour	1.21	1.0	0 - 6	1.619	2.0
			Seed consumption		
Rat odour	13.8	16.5	0 - 20	7.252	13.0
Cat odour	13.0	19.0	0 - 20	8.645	18.0
Stoat male odour	13.5	18.0	0 - 20	7.709	13.0
Stoat female odour	14.95	19.0	0 - 20	7.420	11.0
			Approach		
Stoat-managed	17	11.0	0 - 88	21.801	15.0
Stoat-unmanaged	24.14	13.5	0 - 106	25.868	27.0
Possum-managed	17.88	15.0	8 – 49	10.295	9.0
Possum-unmanaged	33.42	24.5	12 – 73	22.084	35.0
			Mouthing		
Stoat-managed	1.43	0	0 - 6	1.903	3.0
Stoat-unmanaged	4.64	1.5	0 - 31	7.851	4.0
Possum-managed	2.12	1.5	0 - 6	1.996	2.0
Possum unmanaged	3.25	2.5	0 - 14	4.159	4.0
			Seed consumption		
Stoat-managed	9.91	12.0	0 - 20	9.288	19.0
Stoat-unmanaged	13.18	17.0	0 - 20	7.344	14.0
Possum-managed	17.5	19.5	7 – 20	4.336	2.0
Possum-unmanaged	17.75	20.0	7 - 20	4.025	4.0

Table 5.2 The frequency of approach, mouthing and seed consumption by ship rats in the enclosure in response to the source rat populations inferred proximity to known feral and house cat populations (n= 20 rats).

Factor	Mean	Median	Range	Standard deviation	Interquartile range
			Approach		
Near cat	24.54	17.5	8 - 73	17.858	17.0
Far cat	20.92	13.0	0 - 106	24.153	26.0
			Mouthing		
Near cat	2.61	2.0	0 - 14	3.095	3.0
Far cat	3.2	1.0	0 - 31	6.122	4.0
			Seed consumption		
Near cat	17.61	20.0	7 – 20	4.131	2.0
Far cat	11.71	15.0	0 - 20	8.353	19.0

Table 5.3 Kruskal-Wallis testing for evidence of significant differences in variables (approach, mouthing and seed consumption) in response to background management conditions of rat populations or treatment presented (n=20 rats).

Response variable	H	P
Background H(3)		
Approach	9.243	**0.026**
Mouthing	3.319	0.345
Seed consumption	11.41	**0.010**
Treatment H(3)		
Approach	0.987	0.804
Mouthing	9.955	**0.019**
Seed consumption	0.213	0.975

Table 5.4 Results of Mann-Whitney U-test analyses of factorial pairs most likely to be responsible for the significant response variable results generated under Kruskal- Wallis testing (Bonferoni correction applied at p= 0.025) (n=20 rats).

Response variable	Test variable pair	U	P
Approach	Possum-managed : Possum-unmanaged	44.5	**0.017**
	Stoat-managed : Possum-unmanaged	57.5	**0.005**
Seed Consumption	Stoat-managed : Possum-unmanaged	67.5	**0.012**
	Stoat-managed : Possum-managed	96.5	**0.010**
Mouthing	Male stoat odour : rat odour	111.0	**0.014**
	Male stoat odour : female stoat odour	102.5	**0.012**

5.5 Mann-Whitney U-tests for evidence of significant differences in variables (approach, mouthing and seed consumption) in response to the proximity of known feral and house cat populations to subject rat populations (n=20 rats).

Response variable	U	P
Approach	498.0	0.27
Mouthing	631.5	0.385
Seed consumption	418.0	**0.002**

Table 5.6 Results of Mann-Whitney U-tests to determine whether approach, mouthing and seed consumption differed between management pairs(SM: SuM; PM:PuM) in response to odour (n=20 rats).

Response variable	Treatment	Management pair	U	P
Approach	Rat	Stoat-managed : stoat-unmanaged	19.0	0.775
	Cat		19.5	0.830
	Male stoat		18.5	0.721
	Female stoat		7.5	0.104
	Rat	Possum-managed : Possum-unmanaged	3.0	0.289
	Cat		4.0	0.480
	Male stoat		4.0	0.480
	Female stoat		0.00	**0.032**
Mouthing	Rat	Stoat-managed : Stoat-unmanaged	11.0	0.117
	Cat		19.5	0.825
	Male stoat		10.5	0.131
	Female stoat		15.5	0.723
	Rat	Possum-managed : Possum-unmanaged	4.0	0.459
	Cat		4.0	0.463
	Male stoat		5.0	0.719
	Female stoat		5.5	0.853
Seed consumption	Rat	Stoat-managed : Stoat-unmanaged	18.0	0.663
	Cat		19.0	0.769
	Male stoat		16.5	0.517
	Female stoat		9.5	0.180
	Rat	Possum-managed : Possum-unmanaged	5.0	0.459
	Cat		3.0	0.237
	Male stoat		5.0	0.696
	Female stoat		4.0	0.459

Table 5.7 Results of Mann-Whitney U-tests to determine whether approach, mouthing and seed consumption by ship rats differ significantly according to inferred proximity to known feral and house cat populations in response to specific treatments (n=20 rats).

Response variable	Treatment	U	P
Approach	Rat	30.5	0.234
	Cat	28.5	0.178
	Male stoat	37.0	0.500
	Female stoat	27.7	0.220
Mouthing	Rat	42.5	0.802
	Cat	30.5	0.227
	Male stoat	37.0	0.496
	Female stoat	32.0	0.368
Seed consumption	Rat	28.0	0.152
	Cat	23.0	0.065
	Male stoat	26.0	0.114
	Female stoat	28.5	0.239

Investigatory behaviours: Odour affected the frequency of mouthing events with more mouthing observed in response to male stoat odour and cat odour regardless of rat experience with predators (Table 5.1). No such difference was observed for female stoat or male rat odour.

When the data was grouped by treatment there were no significant differences in mouthing according to management background pairs: stoat-managed and stoat-unmanaged, and possum-managed and possum-unmanaged responded to the odours in the same way. Inferred cat proximity did not affect mouthing behaviour either (Mann-Whitney U-test; all comparisons $P \geq 0.0125$ Bonferroni corrected) (Tables 5.6 & 5.7).

G-testing of the repeated mouthing data returned a non-significant result ($G=4.067$, d.f.=3, $P=0.254$) when all four treatments were included, however graphing of the data illustrated that both male stoat and cat odours generate the most mouthing, and that male stoat odour was more likely to generate repeated mouthing events than was either male rat or female stoat odour (Figure 5.1). The data was retested excluding cat odour to determine if male stoat odour generates significantly greater repeated mouthing than female stoat and male rat odours, the G-test result is significant ($G= 13.03$, d.f.=2, $P=0.0014$).

Chew or remove responses were analysed in a binary manner (present / absent). Graphing of this data shows that the response is directed towards all three predator odours (cat, male stoat, female stoat) more so than male rat odour (Figure 5.2). G –test of this data returned a non significant result ($G= 1.40$, df = 3, $p = 0.706$).

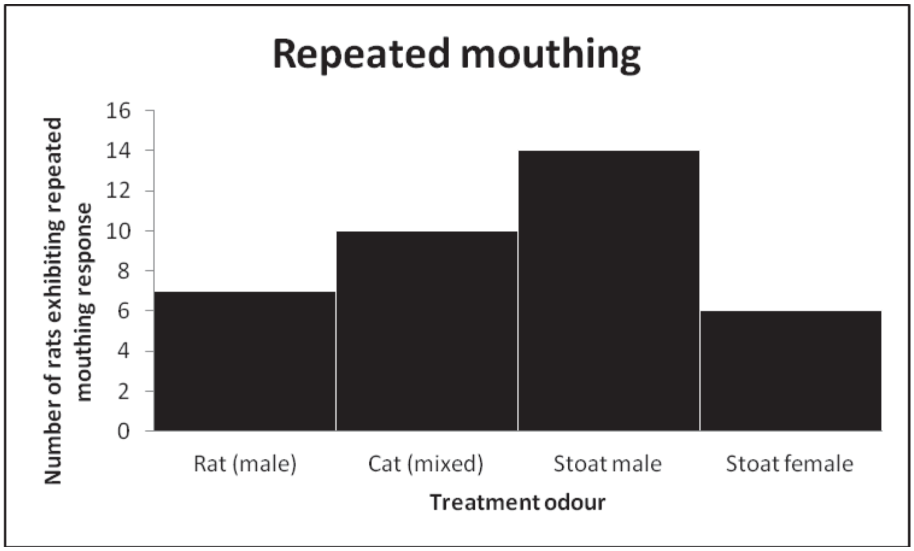

Figure 5.1 Number of rats exhibiting repeated mouthing response towards individual odours.

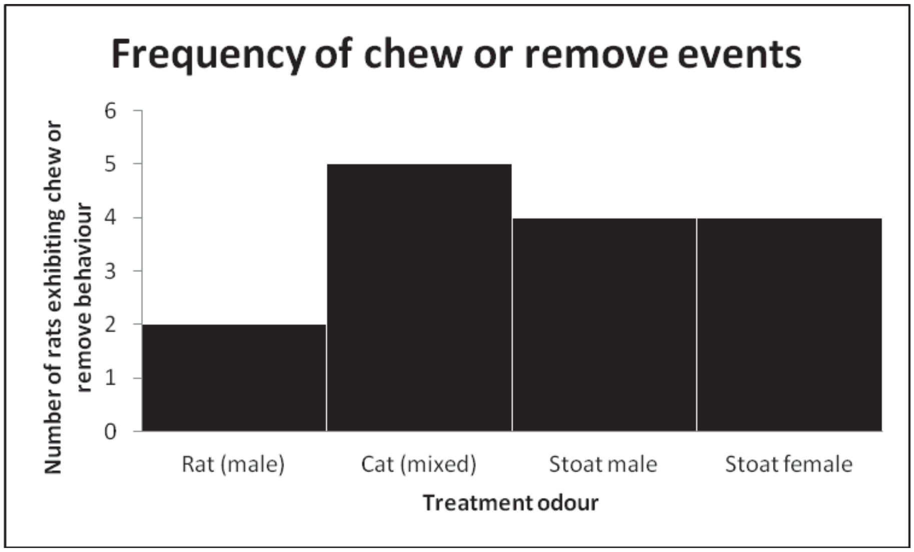

Figure 5.2 Number of rats exhibiting chew remove events towards individual odours.

5.4.2 *EXPERIMENT 2: MULTI-PREDATOR INVESTIGATORY RESPONSE - FIELD*

Trapping: A total of 37 rats were trapped and micro-chipped across the four experimental locations (Table 5.8).

Table 5.8 Total number and sex of rats captured and micro-chipped at each of the experimental sites preceding the 2011 odour trials.

Location	Site	Rats tagged	Male	Female
Kaitawa Lakes	A	7	5	2
	B	9	7	2
Onepoto	A	5	3	2
	B	6	3	3
Tawa Walk	A	5	3	2
	B	3	2	1
Tapuaenui*	A	0	0	0
	B	2	2	0
Totals		**37**	**25**	**12**

*Significant possum interference with traps causing most (90 – 100%) traps to be triggered with bait removed each night, possums captured in rat cages on 2 nights.

Data logger & Video evidence: Only three tagged rats were recorded interacting with the apparatus, two (one male, one female) on night two at the possum-unmanaged / near-cat site on the logger associated with male stoat odour (Kaitawa Lakes A); and one (male) on nights one and two at the stoat-unmanaged / far-cat site on the logger associated with cat odour (Tawa Walk B). While there was no video evidence to confirm the presence of more than one rat at any of these locations the data-logger evidence confirms the presence of two rats at Kaitawa Lakes A. There was video evidence of rat visitations to each of the experimental sites and so I estimate a minimum sample population of eight individuals based on two rats confirmed at Kaitawa Lakes A, plus one rat per site except Tapuaenui A which may have been visited by rats from Tapuaenui B.

<u>Other species:</u> In addition to the rats, several other species were captured on video in the vicinity of the experimental apparatus: possum (Kaitawa Lakes B, Onepoto A, Tawa Walk B and Tapuaenui A & B) and hedgehog (Onepoto B). Rats were observed on the video both before and after visits by these non-target species. Stoat presence was confirmed visually and from strong target indications by a trainee mustelid detection dog during the trapping phase at both Kaitawa Lakes A and Tawa Walk B (pers. obs.). Both trapping and experimentation at the stoat-managed sites were strongly affected by possum interference with up 90% of traps triggerred on some nights, accidental capture of several possums in the rat traps on different nights, and frequent observation of possums on the video. Possums visited both the traps and the experimental apparatus and there was considerably less rat activity recorded than at any of the other three sites.

<u>Overall responses:</u> Assessment of the mean, minimum, maximum frequency and standard errors for the data (SPSS 16 "Explore" function) showed considerable variability in the mean event frequency of both mouthing (M) and approach (A) in response to all three factors (treatment, background and cat proximity) (Table 5.9 & 5.10).

<u>Avoidance behaviours:</u> Exploration of the data showed that male stoat odour consistently elicited the greatest mean frequency of approaches (Table 5.10). Friedman's test indicated that there was a significant difference in the event frequency for approach ($x^2 = 9.037$, d.f.=3, n=24, P= 0.029) according to odour. Wilcoxon signed-rank tests showed that after Bonferroni correction male stoat odour was significantly ($p \leq 0.008$) more likely to elicit the approach response than female stoat but not cat or male rat odours (Table 5.11 & 5.12). There were no significant differences in approach frequency towards the odours within management background pairs: stoat-managed / stoat-unmanaged or possum-managed / possum-unmanaged; or inferred cat proximity (Mann-Whitney U-test; all comparisons $P \geq 0.025$) (Tables 5.13-5.15).

Table 5.9 Overall frequency of approach and mouthing in relation to management background and inferred cat proximity under field conditions (n= 24 treatment presentation nights).

Response variable	Management	Mean	Median	Range	Standard deviation	Interquartile range
Approach	Possum-unmanaged	2.75	0.5	0 - 19	4.767	4
	Possum-managed	1.48	0	0 - 9	2.447	2
	Stoat-managed	0.62	0	0 - 4	1.117	1
	Stoat-unmanaged	3.20	1.5	0 - 25	5.809	3
Mouthing	Possum-unmanaged	1.1	0	0 – 12	2.918	1
	Possum-managed	0	.	0	0	.
	Stoat-managed	0.24	0	0 – 2	0.625	0
	Stoat unmanaged	3.45	0	0 – 55	12.288	1
Approach	Near cat	2.07	0	0 - 19	3.719	3
	Far cat	1.88	0	0 - 25	4.285	2
Mouthing	Near cat	0.51	0	0 - 12	2.040	0
	Far cat	1.8	0	0 - 55	8.635	0

Table 5.10 Event frequency of approach and mouthing in response to odour presented under field conditions (n = 24 treatment presentation nights).

Response variable	Treatment	Mean	Median	Range	Standard deviation	Interquartile range
Approach	Rat	1.17	0	0 -7	1.855	2
	Cat	1.68	0	0 – 9	2.533	3
	Male stoat	4.23	1.5	0 – 25	6.633	6
	Female stoat	0.68	0	0 – 5	1.323	1
Mouthing	Rat	0.06	0	0 – 1	0.236	0
	Cat	0.27	0	0 – 4	0.883	0
	Male stoat	3.95	0	0 – 55	11.826	2
	Female stoat	0.09	0	0 – 1	0.294	0

Table 5.11 Results of Wilcoxon signed-rank tests comparing frequency of approaches in response to odours , Bonferonni correction applied at p=0.008 (n=24 treatment presentation nights).

Treatment comparison	Z	P
Cat : rat	-1.143	0.253
Male stoat : rat	-1.987	0.047
Female stoat : rat	-0.310	0.757
Male stoat : cat	-2.064	0.039
Female stoat : cat	-1.890	0.059
Male stoat : female stoat	-2.633	**0.008**

Table 5.12 Results of Wilcoxon signed rank tests comparing mouthing behaviour in response to odours, Bonferroni correction applied at p= 0.008 (n=24 treatment presentation nights).

Treatment pair	Z	P
Cat : rat	-1.000	0.317
Male stoat : rat	-2.226	0.026
Female stoat : rat	-1.414	0.157
Male stoat : cat	-2.213	0.027
Female stoat : cat	-0.577	0.564
Male stoat : female stoat	-2.113	0.035

Table 5.13 Results of Mann-Whitney U-tests carried out to detect differences in the response of stoat-managed and stoat-unmanaged rats to odours (n=24 treatment presentation nights).

Response variable	Treatment	U	P
Approach	Rat	7.0	0.877
	Cat	6.0	0.085
	Male stoat	13.5	0.765
	Female stoat	9.0	0.104
Mouthing	Rat	6.0	0.439
	Cat	11.0	0.351
	Male stoat	12.0	0.525
	Female stoat	15.0	1.000

Table 5.14 Results of Mann-Whitney U-tests carried out to detect differences in the response of possum-managed and possum-unmanaged rats to odours (n=24 nights).

Response variable	Treatment	U	P
Approach	Rat	12.0	0.526
	Cat	10.0	0.521
	Male stoat	12.5	0.647
	Female stoat	8.5	0.168
Mouthing	Rat	15.0	1.000
	Cat	12.5	1.000
	Male stoat	6.0	**0.037**
	Female stoat	9.0	0.102

Table 5.15 Results of Mann-Whitney U-tests carried out to detect differences in the response of inferred near and far cat population rats(n=24 nights).

Response variable	Treatment	U	P
Approach	Rat	29.5	0.303
	Cat	46.5	0.315
	Male stoat	34.0	0.074
	Female stoat	50.5	0.402
Mouthing	Rat	35.0	0.264
	Cat	44.0	0.069
	Male stoat	55.0	0.662
	Female stoat	49.5	0.147

Investigatory behaviour: Exploration of the data showed that male stoat odour elicited the greatest mean and maximum frequency of mouthing responses (Table 5.10). There was a significant difference in the frequency for mouthing (Friedman's test, x^2= 9.222, d.f.=3, n=24, P= 0.026) according to treatment. After Bonferroni corrections were applied to there were no significant differences between the treatment odours presented (Wilcoxon signed-rank pair tests; Table 5.12). There was no significant difference in either approach or mouthing between stoat-managed and stoat-unmanaged background rats (Mann-Whitney U tests; P ≤ 0.05; Table 5.13) for any treatment odour. There was a significant difference in mouthing frequency in response to male stoat odour between the possum-managed and possum-unmanaged background rat populations (Mann-Whitney U tests; P ≤ 0.05; Table 5.14). There was no significant difference in either approach or mouthing between near-cat and far-cat background rats (Mann-Whitney U tests; P ≤ 0.05; Table 5.15) for any treatment odour.

Graphing of the mouthing response data clearly demonstrates that male stoat odour is most likely to elicit a response with the non-stoat-managed and far-cat background population rats (Figures 5.3 & 5.4). Analysis of the repeated mouthing data indicates that male stoat odour was markedly more likely to generate repeated mouthing than any of the remaining odours (G-test, G= 12.64, d.f.=3, P=0.005; Figure 5.5).

Chew or remove was significantly more often directed towards male stoat odour, than any of the remaining three odours (G –test, G = 22.11, df = 3, P= 0.00006; Figure 5.6).

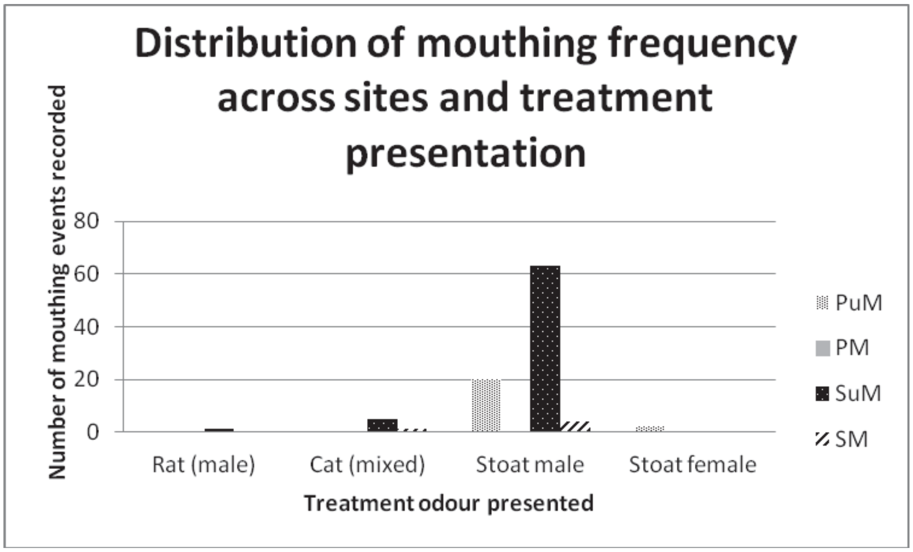

Figure 5.3 Mouthing behaviour across all management backgrounds and odours.

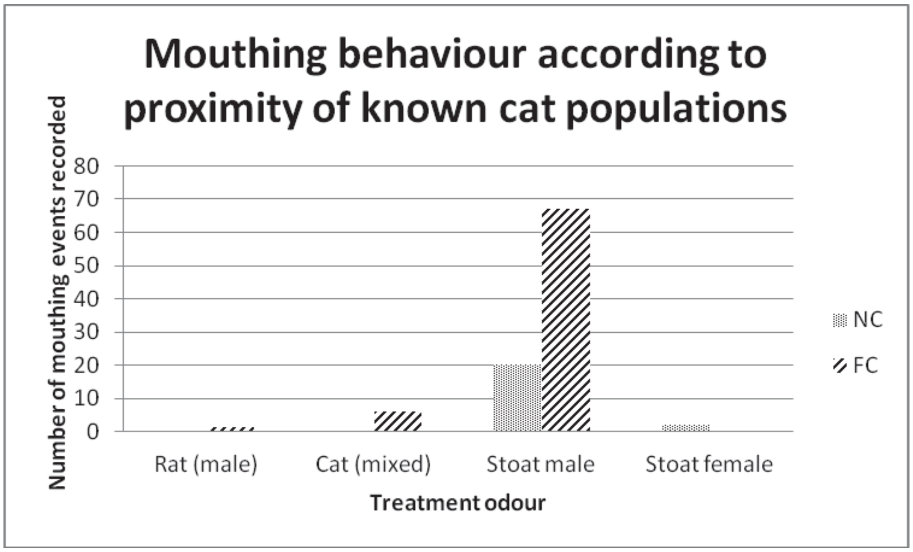

Figure 5.4 Mouthing behaviour across odour and inferred proximity to known cat populations.

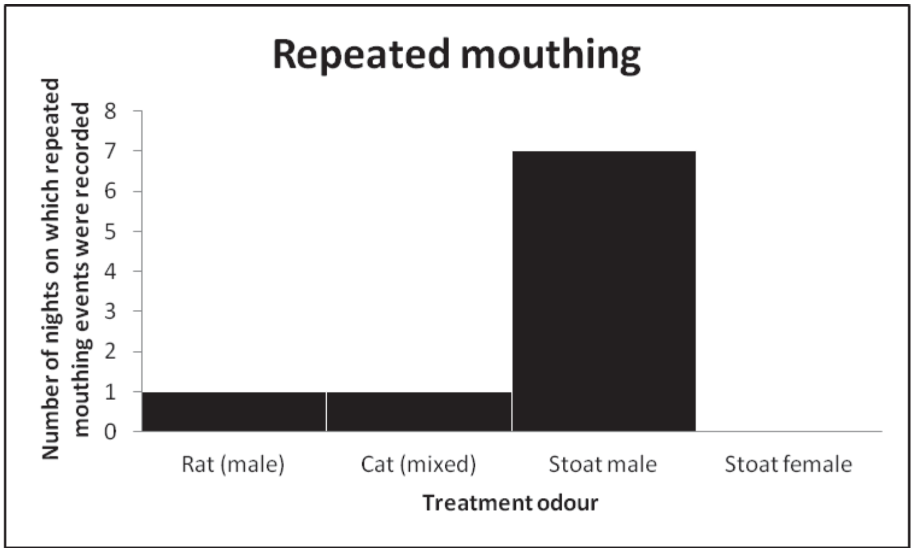

Figure 5.5 Number of nights when repeated (≥2) mouthing behaviour was recorded towards odours.

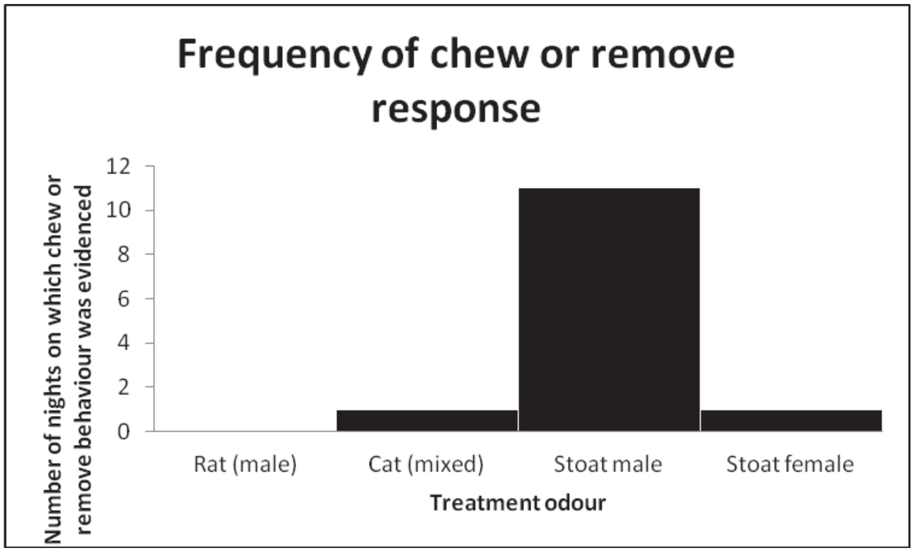

Figure 5.6 Number of nights on which chew / remove behaviour was observed. Note the the frequency includes nights for which there is no video evidence due to record failures.

5.5 DISCUSSION

The overall aim of this phase of research was to test the hypothesis that ship rats exhibited predator inspection in response to stoat odour. I suggested that if the mouthing behaviour could be shown to be generalised to include another predator (cat), then the behaviour would be consistent with a learned form of predator inspection behaviour, possibly motivated by risk assessment. If however the rats were found to exhibit the behaviour only towards stoat or more strongly / frequently towards a less familiar predator other explanations such as parasite infection or chemical cue overlaps would have to be more thoroughly investigated.

Overall my prediction that response would be generalized to include other predator odours was upheld with both cat and male stoat odour in particular eliciting strong responses. My prediction that the background management and hence experience with predators would affect investigatory responses was not supported. My third prediction, that the enclosure and field experiments would produce similar results was partially supported because some comparable results were obtained in both experiments between my experiments. My results are at odds with other rodent research such as reports of aggressive responses in rock squirrels towards snakes (Owings et al. 2001) and defensive responses in laboratory rats towards cat and a range of synthetic odours (MacGregor et al. 2002). Other researchers (e.g. Bramley & Waas 2001) report inconsistent responses between both rat individuals and results from two different experimental approaches to a range of natural and synthetic odours.

Multi-predator investigatory behaviour: Data from field studies suggests that predator inspection is not exhibited by all individuals within a given species or group within species, and there has been considerable discussion in the predator inspection literature as to the motivating factors for a behaviour that would seem to put those who exhibit it at significantly increased risk of predation (Dugatkin et al. 2005; Fishman 1999; FitzGibbon 1994; Godin & Davis 1995). My results suggest that predator inspection in ship rats is a learned behaviour, thus an individuals' propensity to exhibit it would vary according to that individuals' previous experiences. Others have suggested that predator inspection and its expression by any given individual is affected by "personality" (Dugatkin & Alfieri 2003; Dugatkin et al. 2005; Pelligrini et al. 2010; Quinn & Cresswell 2005), for example attributes such as 'boldness' may be intimately connected to an individuals' propensity to exhibit a behaviour. Boldness may be either a driver - 'bold individuals inspect'; or a passenger - 'inspecting individuals are bold'. Personality traits such as boldness, if drivers, are potentially genetically heritable characteristics. While it seems that predator inspection in ship rats is a learned behaviour, I cannot exclude the possibility that there may be a heritable element to its individual expression.

Ship rats are a highly adaptable and successful species and thus individual variability in terms of behaviours is likely a pre-requisite to this species success and survival (Burwash et al. 1998). As a species ship rats are not as well studied as a number of other rodent species, particularly in predator-prey research where laboratory rodents, predominantly the domestic variant of *Rattus norvegicus* and wild captured voles dominate the literature (see Apfelbach et al. 2005; Kats & Dill 1998 for comprehensive reviews). However, where ship rats have been used, 'high individual variability' has been cited when interpretingthe resultant statistics (Bramley & Waas 2001)

The data generated by the two experiments reported here is in many respects highly variable, and whilst this is of statistical concern it may be ecologically expected if predator inspection is being exhibited. In terms of learned predator inspection, the experience of an individual will affect how they respond behaviourally to a perceived threat, and as all my subject animals were wild captured I have no absolute knowledge of their individual life histories. I expected that not all ship rats, regardless of previous experience, would exhibit predator inspection behaviour. For example it may be that only those individuals with a predisposition for a 'bold' personality will, with experience, learn to exhibit predator inspection behaviour.

I predicted that if the investigatory behaviours, particularly mouthing and its apparently elevated behaviour, chew or remove, were evidence of predator inspection, they should be generalised to predators other than stoats. Because I considered predator inspection to be a learned behaviour in ship rats, cats were selected as an alternative predator because this is a species which is present at low levels throughout the wider study area, and in known populations (house and feral) in close proximity to two of the specific source and experimental locations. Thus at least two of the source populations (assigned as near cat) were known to be living under conditions where encounters with cats on a regular basis were highly probable and thus these rats should have had the opportunity to have learned from experience to generalise predator inspection behaviour towards this predator in addition to stoats.

Under both captive and field conditions male stoat odour generated a greater mouthing frequency, than either male rat (con-specific threat) or female stoat (predator). In captivity there was no significant difference in the frequency of mouthing responses towards any of the treatments. However the repeated mouthing analyses support the assertion that male stoat, and to a lesser extent cat odours, are the two odours most significantly responded to, thus mouthing is seen as a generalised behaviour in captivity. Under field conditions male stoat odour consistently elicited greater mouthing frequencies than any of the other treatment odours including cat odour. I suggest that the difference in response to treatment odours indicated by the G-test of the repeated mouthing data is representative of the rat behaviour. Bonferroni correction caused a loss of significance between male stoat and rat / cat / female stoat odours in the field experiment. I suggest that this is largely due the additive effect of a conservative correction being applied to the already conservative non-parametric tests on a small and highly variable dataset.

The chew or remove behaviour in captive rats, which I consider to be an escalation of the mouthing response, was most commonly directed towards cat odour, equally towards male and female stoat odour, and least towards male rat odour. The data showed that the two chew or remove responses towards male rat odour were elicited in rats which had both been previously exposed to male stoat and cat odour in the trials, and who subsequently exhibited chew or remove towards female stoat odour. Thus it might be suggested that the previous exposure to predator odour predisposed these rats to treat the male rat odour, or simply the presence of the treatment sample as an object, as a threat.

I suggest the chew or remove behaviour may be analogous to the defensive burying behaviour, a phenomenon which has been previously reported in laboratory rodents. Some authors consider defensive burying to be a response to a ‘threatening’ object, whereby the subject animal attempts to remove, by burying, the object, thus making safe their environment (De Boer & Koolhaas 2003; Londei, Valentini & Leone 1998; Pinel & Treit 1983). It may be that in this experiment the predator odour initially drives the rats' desire to remove the object, but in subsequent exposures even the non-predator odour is linked by association (presentation form and location) and thus similarly treated by some rats.

In the captive rats I studied some individuals did not simply remove the sample odour source from the foraging tray location, they carried the material back to the nest box and incorporated it into their bedding (Figure 5.7). This behaviour might be likened to prey animals attempting to disguise their own whereabouts by anointing themselves or their nest or burrow with predator odour, although evidence for the effectiveness of this tactic appears to be limited (Conover 2007). In terms of predator inspection this behaviour could represent a facet of the learning process, by keeping the "threatening" predator odours in their nest material the rats are able to constantly hone their recognition and detection of the predator odour. Under field conditions where all four treatment odours were presented simultaneously the chew or remove behaviour was almost exclusively directed towards the male stoat odour with only rare (one event each) exhibition towards cat and female stoat odour, and no exhibition towards male rat odour. I suggest this supports my assertion that the exhibition of this behaviour towards male rat was largely an artefact of the captive experimental protocol (see discussion below).

Figure 5.7 Odour treated blanket and zip-lock presentation bag, incorporated into the bedding of the ship rat who accumulated the most material.

<u>'Experience' as a mitigating factor?:</u> I used cat odour as my alternative predator because in the New Zealand forest ecosystem there are limited predators of rats. This may be

inappropriate because predator-naive laboratory rats show an innate avoidance of cat odour (Adamec & Shallow 1993; Blanchard et al. 2001; Dielenberg & McGregor 1999; Hubbard et al. 2004; McGregor et al. 2002). Furthermore the founder ship rat populations in New Zealand would have had to survive an extended sea voyage most likely in the presence of cats, thus the survivors were possibly those best equipped to deal with cat predation. I consider that there is a strong possibility that an innate or other genetic predisposition to recognise cat odour exists in New Zealand ship rats. If this were the case I would expect that most rats would exhibit an avoidance response towards cat odour, most particularly those with less cat exposure and therefore with less opportunity for behavioural modification via experience to develop. There was no evidence of any avoidance behaviour in either the captive or field experiments. Overall rats assigned as ‘far cat’ consumed significantly less sunflower seeds in captivity than those assigned ‘near cat’, but this significance was not maintained when seed consumption in the presence of cat odour was tested independently of the other three treatments, nor was there any evidence for any avoidance in terms of approach data that could be attributable to cat experience or innate factors.

I predicted that the management background or proximity to inferred cat populations would not affect the investigatory response of ship rats and overall this was upheld because neither mouthing nor chew or remove behaviour was affected by background. When the response variables were measured against the influence of individual odours for each site pairing, there were no significant differences for either captive or field situations except ship rats from possum-unmanaged areas were more likely to approach female stoat odour in captivity. This result was driven largely by a single individual (59 approaches). In the field repeated mouthing by ship rats from possum-unmanaged areas was also more likely to be directed to male stoat odour than any other odour. Rats from the possum-managed area did not exhibit any mouthing behaviour. This reflects the lack of mouthing response in possum-managed rats in the previous season, although as with the previous season these rats did exhibit mouthing in captivity. Why rats from this particular location should fail to exhibit in the field a behaviour that can be induced in captivity is unclear, however it is reasonable to suggest that while the rats have the behaviour within their repertoire it is some component of the captive experiment or habitat that is encouraging expression, a factor that appears to be absent in the field presentation.

There is some evidence that the hunger state of a prey animal can affect it’s disposition to exhibit predator inspection or other risk taking behaviours (Brown & Chivers 2005). Under captive conditions the rats were fed to satiation, as reflected in their hoarding of food stuffs (pers. obs.), and in captivity the possum-managed rats did exhibit the mouthing behaviour. It seems unlikely that satiated individuals would exhibit greater risk-taking behaviour than hungry ones: if satiety were a factor in decreasing risk-taking then I would expect to see greater evidence of mouthing in the field experiment populations. This was not observed. The rats from possum-unmanaged areas and stoat-unmanaged areas exhibited the highest mean frequency of mouthing responses both in captivity and in the field, indicating that hunger was unlikely to be a factor. Furthermore at both these locations evidence of stoats was seen during the trapping phase. This observation, combined with the mouthing behaviour supports my assertion that the rats responded most strongly to the predator of greatest imminent threat.

Comparison of experiments and wider research: Under captive conditions male stoat and cat odour elicited the most repeated investigations, whilst in the field only male stoat odour elicited a similar level of response with very occasional repeated investigation of cat and male rat odours. Chew or remove data suggests some investigatory behaviour directed at female stoat odour but there was no video evidence of this. I suggest that there are experimental explanations for these differences: under captive conditions the rats are (1) enclosed within a limited physical space, albeit more generous than many captive arrangements; (2) enticed into the presence of the treatment odour by the known presence of a food resource; (3) presented with only one treatment odour on any one night. Under field conditions the rats are (1) entirely unconstrained; (2) no food enticement is present; (3) all four treatments are presented simultaneously. Thus under captive conditions the rats are unable to escape the presence of the potentially threatening odour, and if they choose to approach in order to gain access to the known food resource they are confronted with that odour. Because they are likely to repeatedly enter the foraging tray area, especially if they are engaging in a carry and consume strategy (c.f. Chapter 4), they are likely to exhibit more than one investigation of the treatment odour presented.

Under field conditions whilst the rats were, during trapping, attracted to the sites by a food resource, food was not used as an enticement during the experimental phase so I can be confident that food was not a motivator for repeated approaches by any one individual. The rats were free to approach or completely remove themselves from the area at will, thus only those with the greatest propensity to investigate would do so. As all four treatment odours were presented simultaneously it is possible that investigating rats focussed upon the one that was of the most interest to them, male stoat odour. In terms of approaches in captivity, cat odour elicits the most frequent approach events, but in the wild, cat odour approaches remained secondary to male stoat odour. This raises another possible experimental factor;

the captive enclosure was located in an outdoor setting in a semi-rural environment where a known feral cat population exists.

Feral cats are populous around the Massey University environs (pers obs) and whilst there was no evidence recorded on camera, in hindsight it is possible that they may have been drawn to the enclosure. Stoats are also likely to live in the general area but no direct (visual or scat) evidence was seen indicating that they were in close proximity to the enclosure location. Thus under captive conditions, the most imminent predatory threat may have come from cats. Similarly, the immediate proximity of a dairy farm raises the possibility of wild rat populations (albeit more likely Norway than ship rats) which may have heightened the captive subjects sensitivity to 'foreign' rat odour. This suggestion is potentially supported by the highest frequency of mouthing occurring under field conditions at the possum-unmanaged and stoat-unmanaged sites. At both sites multiple stoats were known to have been present during the preceding trapping phase (pers. obs.). Chew or remove was most commonly observed at these two sites, thus at the time of the field experiments the predator of greatest risk was likely stoats, hence the overwhelming response to male stoat odour.

Recent research (Gsell, Shapira and colleagues) has suggested that wild Norway rats can be attracted by the presence of an unfamiliar con-specific (Anon 2008, 2012). This does not appear to be true of the wild ship rats I used. This may be because: (1) I used male con-specifics. The intention was to present a threat cue i.e. an 'intruder' male. Had I used an oestrous female the results may have been different in terms of male attraction. Although by that reasoning the 'intruder' male should have attracted resident females in the field experiments where the response to this odour cue was less than that in captivity; (2)odour may be only one factor of many that rats use to make decisions, Gsell, Shapira and colleagues (Anon 2008, 2012) used a live 'Judas rat', thus it is possible that acoustics, olfaction and vision play a role in the attraction of wild Norway rats; (3) ship rats although capable of living at high densities are possibly not as obligatorily social as Norway rats which seem to be more predisposed to colony living, thus ship rats may not be drawn to the presence of a con-specific in the same way as Norway rats are. Further research would be required to establish whether a con-specific could be used as a lure for ship rats specifically.

<u>General discussion:</u> There is strong evidence that ship rats generalize their responses to predators in terms of mouthing and chew or remove behaviour. The strongest response appears to be restricted to the male stoat odour and the mixed sex cat odour. Female stoat odour generated a lower response which was largely indiscernible from that of foreign rat. Results were consistent in captivity and in the field. Any attempt to explain this is currently

purely speculative but possibilities include: (1) female stoat odour is not as pungent (pers. obs.) as male stoat odour and thus under field conditions especially, it is the stronger male stoat odour that attracts the most interest; (2) female stoats are less of a threat to rats by virtue of different hunting strategies. Alternatively they may pose a greater threat by virtue of a less detectable odour; (3) the causal mechanism for the investigatory behaviour is not predator inspection but rather a chemical cue overlap that is present in male stoat and possibly cat odour. This is not necessarily a 'male' chemical such as testosterone because male rat odour was not strongly responded to, nor is it likely to be dietary as both the male and female stoats were being fed the same captive diet. Sulphide compounds are thought to act as attractants to carnivores and omnivores (Mason, Epple & Nolte 1994). While this possibly explains the apparent attraction to male stoat and cat odour, if this were the only or predominant factor I would have expected to see a similar level of attraction across all four treatment odours presented in this experiment as female stoats and male rats should also exude sulphide odours.

The two experimental protocols produced broadly similar results, with both male stoat and cat odours eliciting inspection responses, and female stoat and male rat eliciting significantly less inspection responses. Under field conditions male stoat was the primary focus of the mouthing behaviour. I have suggested that there are potentially experimental artefacts which explain the more generalised response to predator odours in the enclosure: e.g. the individual presentation and proximity to a food resource; and speculated that the captive enclosure experiments may have been affected by feral cats. This suggestion may have some support in the response of the free-living rats where stoats were known to be present at the experimental locations. The investigatory response shown by the ship rats continues to be at odds with much rodent predator-prey literature, but this may be because ship rats are rarely researched and the experimental methods used may have been inappropriate.

5.6 CONCLUSION

The results of these two experiments have supported my prediction that ship rats exhibit a generalized inspection response (mouthing) to predator odours, including stoat and cat odours under both captive and wild conditions. These observations are consistent with the predator inspection interpretation (thesis objectives A and B). There were some anomalies between captive and field experiment results, such as the more generalised response in the enclosure and markedly more male-stoat-specific response in the field. These I attribute to experimental conditions such as the presentation method in the enclosure and *in-situ* predation threats in the field. My second prediction, that predator inspection, as a learned behaviour requiring minimal exposure, would be evident in all populations regardless of potential stoat or cat exposure conditions, was largely supported (thesis objective C). The major anomaly was the lack of mouthing evident at the possum-managed field location, although rats sourced from this location exhibited mouthing under captive conditions.

The results of the enclosure experiment appear in many respects to exaggerate the behaviours witnessed in the field, for example no mouthing behaviour was seen in rats from the possum-managed area in the field but mouthing behaviour was commonly seen in the enclosure. More mouthing in response to cat and male stoat odour was seen in the enclosure, and there was also more male stoat odour focus in the field. I suggest that while the enclosure continues to generate results that are broadly reflective of those that will occur in free-living populations, captive behaviours will be somewhat exaggerated as a result of the experimental protocol and confined nature of captive research. Furthermore there were circumstances that were very temporally and spatially specific to the field experiments: i.e. the presence of stoats in the immediate vicinity of the experimental apparatus during the trapping phase; that may have affected the behavioural response of the rats. This very real and current stoat predation threat may have led to a heightened response to this predator specifically.

The results of this pair of experiments cannot be claimed to conclusively support predator inspection as the best interpretation of the mouthing and chew or remove behaviours, for example the low level of response to female stoat and occasional response towards male rat odours require further investigation. However, overall I suggest that predator inspection remains the most plausible explanation for the investigatory behaviours.

The mouthing and chew or remove behaviours are clearly elicited in response to cat odour and stoat odour, particularly male stoat odour, thus they cannot be said to be behaviours specific to one predator. I posited that predator inspection in ship rats is a learned behaviour, albeit one that may be linked to heritable characteristics such as 'boldness', and I previously (Chapter 4) suggested that only a very few encounters may be sufficient to develop the behaviour. I suggest that the evidence continues to support this with rats from all management and cat proximity backgrounds exhibiting the non-avoidance behaviours, i.e. they are generalised across populations even where exposure to a given predator is limited. Similarly there was no evidence of very young animals interacting with the apparatus, despite their known presence (pers obs.).

Further research is required to add support to the predator inspection interpretation of the behaviours observed, and similarly further consideration of the possibility of other explanations such as chemical cues and parasite infection is required. Chapter 6 will begin to address some of these issues as I attempt to demonstrate that male stoat odour, rather than the presentation form *per se*, attracts ship rats; and that the attraction is an adult phenomenon: i.e. a learned rather than innate behaviour.

5.7 REFERENCES

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Chapter 6 DO FREE-LIVING SHIP RATS TRULY FIND STOAT ODOUR IRRESISTIBLE?

6.1 ABSTRACT

The preceding research (Chapters 3-5) revealed an investigatory behaviour exhibited by both the captive and free-living ship rats (*Rattus rattus*) towards predator odours represented by stoats (*Mustela erminea*) and cats (*Felis catus*). The investigatory behaviours ranged from approaching and sniffing responses to mouthing, and at its most extreme a chew or remove response to the predator odour. I interpreted this behaviour as evidence of predator inspection in ship rats, a behaviour widely acknowledged in the piscean literature in particular, but less so in mammalian and particularly rodent literature, despite the plethora of predator-prey research utilising rodent subject species.

This snap trapping experiment was conducted to determine (1) whether it was the odour or the novel presentation that the rats were attracted to i.e. the observed behaviour represented a form of neophilia; and (2) assess the demographics of the captured rats in light of predator inspection versus neophilia hypotheses. The results showed that when presented with paired traps adult ship rats were overwhelmingly more inclined to closely inspect the stoat lure traps than the control traps. Juveniles on the other hand were more inclined to inspect the control lure trap than the stoat odour tainted trap. The evidence does not support neophilia as an explanatory mechanism for the investigatory behaviour but is consistent with predator inspection.

The results of this experiment have implications for predator-prey research because my results were consistent with my prediction that more predator-experienced individuals develop more investigatory responses to predator cues which ultimately over-ride the default innate predator avoidance. The results suggest that predator (stoat) odour should not be applied to areas of high conservation or economic sensitivity. The corollary of that is that while odours are probably less effective as a lure than the more conventional peanut butter, there may be a role for its development as a 'mop-up' lure.

6.2 INTRODUCTION

My previous research (Chapters 3-5) indicated that the response of free-living ship rats to stoat odour may be very different to that intuitively expected i.e. avoidance. This research had presented wild rats with the smell of stoats, initially along with both familiar (possum, *Trichosurus vulpecula*) and unfamiliar (chinchilla, *Chinchilla lanigera*) non-predators, and subsequently with a presumably less familiar predator, cat (*Felis catus*); and an unfamiliar male con-specific under both field and captive conditions. The results of these experiments clearly demonstrated a consistent behavioural investigatory response ranging from sniffing to mouthing behaviour, and at its most extreme a chew or remove response directed primarily at the stoat odour, and more specifically male stoat odour.

I found no evidence in the preceding experiments of the expected avoidance response of the rats towards the predator cues and suggest that predator inspection behaviour might be the causal mechanism responsible for the investigatory behaviour. Predator inspection has been extensively researched in several aquatic species (Godin and Davis 1995; Brown and Godin 1999; Brown and Cowan 2000; Brown and Dreier 2002; Gomez-Laplaza 2002; Brosnan, Earley & Dugatkin 2003; Walling et al. 2004; Dugatkin et al. 2005; Haberli, Aeschlimann & Milinski 2005; Thomas et al. 2008), and reported to a lesser extent in terrestrial mammals, including Thomson's gazelle (*Eudorcas thomsonii*) and red-necked pademelon (a macropod; *Thylogale thetis*), and as an investigatory behaviour in black-tailed prairie dogs (*Cynomys ludovicianus*) (Owings and Owings 1979; FitzGibbon 1994; Ramp, Russell & Croft 2005).

Predator inspection can be considered to be an adaptive learning behaviour through which prey animals are information gathering for the purpose of predator recognition (Brown and Chivers 2005; Lima and Steury 2005). Such information gathering behaviour, may, in future encounters, allow an individual an advantage in escaping detection by the predator, and / or allow the individual to make more accurate or faster decisions as to when it is safe to resume foraging and other routine but essential behaviours (Fishman 1999; Lima and Steury 2005). Most of the examples cited in the current literature focus on group living prey species where there is safety in numbers, potential for social learning and co-operative pay-offs to inspectors.

Ship rats, with the exception of females with young at-foot, and co-exploring sibs or mating pairs are largely solitary in their behaviour in New Zealand forest (pers. obs.; Innes 2005). This may be more a reflection of the habitat they occupy and their ability to spread out than it is of ship rat social habits because they can and do live at very high densities in some forest habitats (Innes 2005). Ship rats were also observed living in high densities in houses within ca. 1km of the research site (pers. obs.) and thus it is reasonable to assume that rats in the vicinity had maintained the group living characteristics that may predispose them to exhibit predator inspection behaviour.

Body odour, as used in my research, is an ephemeral cue intended to convey the likely presence of a predator. It could indicate that the predator is very close by and thereby pose an imminent threat, or as illustrated by the ability of predators such as wolves to track prey using hours old scent trails, the predator could be miles away (Conover 2007). In such cases the prey species would benefit from being able to assess rapidly the imminence of the threat, and if found not to be immediate, the source of the odour (nest or den site for example) could serve to provide useful information to the prey animal in honing it's future recognition and judgement skills, and gaining information specific to that individual predator. Thus I have suggested that as ship rats gain experience in terms of predator recognition, they will be more inclined to inspect the source of a predator odour as part of a safe information gathering exercise.

Whilst I have posited that it is the odour that is specifically eliciting the investigatory responses in the ship rats, there remains a possibility that neophilia i.e. curiosity about the presentation as a novel object, might play a role in the apparent attraction. This experiment aimed in part to address this suggestion by using the paired presentation of the stoat odour lure traps and control, un-lured traps, both novel to the experimental subject rats. Thus any difference in capture rate would be attributable to the lure, not the trap itself.

The preceding experimentation was live behavioural research and as such the captive populations were subject to certain selection criteria which I imposed and that may have inadvertently affected the subjects responses. Furthermore the field populations were both small and difficult to assess in terms of demography (see Chapter 2-5 for details). I previously suggested that predator inspection behaviour in the ship rat is a largely learned response that is exhibited by predator-experienced individuals, whereas avoidance, as witnessed in much rodent predator-prey research (see Kats and Dill 1998) is the default innate response of predator-naive individuals.

In order to test my hypotheses I undertook a kill-trapping experiment at the conclusion of my live behavioural research, thus targeting many of the same animals for which I had previously obtained behavioural video records. If my interpretation of the non-avoidance or investigatory response of ship rats towards a stoat odour as an experience based (learned) predator inspection is correct then I hypothesized that in this experiment:

(1) stoat tainted traps would have a greater capture rate than control (no odour) traps;

(2) stoat tainted traps would capture more adults than juveniles / sub adults.

If the behaviour was due to a neophillic response I expected that:

(1) the control traps would capture equal or more rats than the stoat tainted traps;

(2) there would be an even demographic spread across the capture traps as the traps themselves represent a novel object in this habitat.

Thus the aims of this experiment were to:

(1) determine whether it is the stoat odour or the experimental presentation *per se* that attracted the ship rats in my earlier experiments;

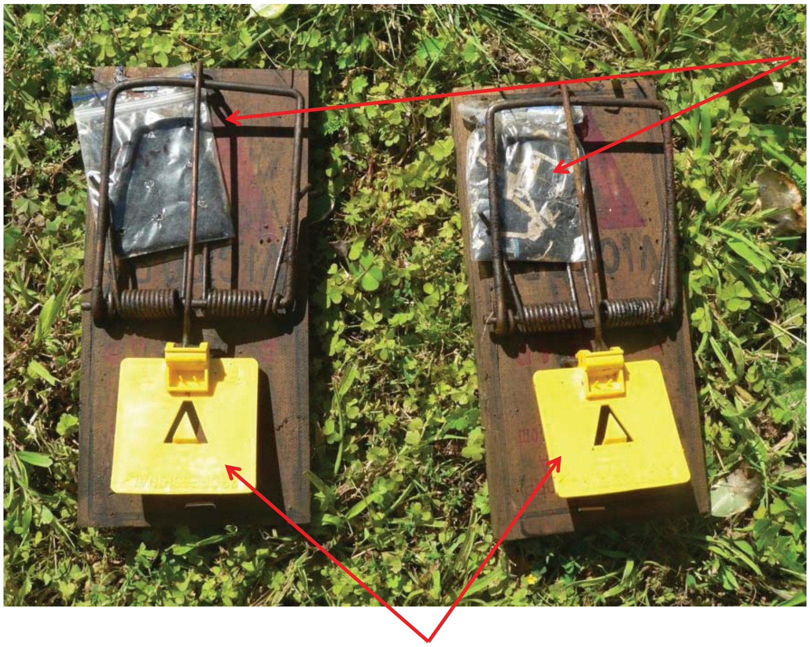
(2) assess the demographics of the captured population with respect to my interpretation of the investigatory responses as predator inspection.

In terms of thesis aims this chapter addresses thesis objective (A): the analyses of the behavioural response of prey (ship rat) to the odour of a familiar predator (stoat).

6.3 METHODS

Location & timing: The study took place at the Kaitawa Lakes loop track, Lake Waikaremoana, Te Urewera National Park, New Zealand (Latitude: 38° 46' 0 S, Longitude: 177° 4' 60 E). This possum-unmanaged and inferred near cat site was the most accessible, and based upon rat trappability in the preceding live capture phase, probably most rat dense of the four behavioural research sites that had been used in the preceding field research (Chapter 5, Table 5.8). A total of 16 rats had been captured and micro-chipped in January 2011 for that years' behavioural research (site A n= 9; site B n=7). The behavioural experiments conducted in February 2011 at this location revealed high levels of investigatory behaviour in the resident population (Chapter 5). Trapping for this experiment occurred during 16 March to 28 March 2011, thus at the end of the New Zealand summer.

Lures: Stoat tainted fleece blanket samples (ca. 5 x 5 cm) as used in the previous research were used as treatment lures, non odour-tainted fleece blanket samples were used as control lures. The stoat lures comprised one piece each from male and female stoat tainted blankets, although preceding research (Chapter 5) suggested it was the male stoat component that would be the most effective. The blanket samples were presented in zip-lock bags, pierced with a small nail nine times on each side to allow odour dissemination on the first day of deployment, and stapled to the trap beyond the trigger mechanism as shown in Figure 6.1. This positioning meant that the rats had to cross the trigger mechanism to investigate the odour rather than be able to investigate it without triggering the trap. No food lure such as peanut butter was used. Because the traps were deployed within wire mesh exclusion cages (Department of Conservation trapping protocols), the rats entry was directed across the trigger mechanism. An investigating rat could get within approximately 5cm of the samples from outside the mesh cages, but any closer inspection required entry through the 'front' of the cage and across the trap.



Treatment and control lures stapled to the rear of the snap trap

Trigger mechanism across which the rat must cross to investigate the odour source when the trap is deployed in the protective cage.

Figure 6.1 Snap traps with lures attached. When deployed the traps are positioned within a wire mesh cage to avoid accidental by-catch, thus guiding the approaching rats across the trigger mechanism in order to investigate the odour source.

<u>Trapping array:</u> 80 new, unused snap traps (Victor Professional rat traps; Pest Management Services, Paraparaumu, N.Z.) were placed in pairs 25 paces apart (approximately 15m ground distance) around the entire track. Because the traps were new, the inevitable manufacturing and any other odours associated with them were assumed to be equal. Home ranges were estimated to be in the order of 100m for females and >150m for males (Innes 2005), even allowing for the 3-dimensional use of space the trap spacing should have ensured at least one pair of traps would be encountered by any given rat foraging within the confines of its own

territory. Each pair of traps was placed with one on either side of the track and within no more than 2m of each other. To have them any closer risked having the odour sources interfering with each other; placing them too far apart I risked only one of the pair being encountered. While no pilot trial was conducted specifically to test this, a similar spacing had been used in the preceding field odour presentations and video footage confirmed that rats encountering one odour source in those experiments would regularly come within close range of the others. Treatment and control odours were attached to the traps in alternating fashion (Figure 6.2). All traps were placed within wire mesh cages to prevent capture / harm to non-target avifauna as per Department of Conservation, New Zealand requirements.

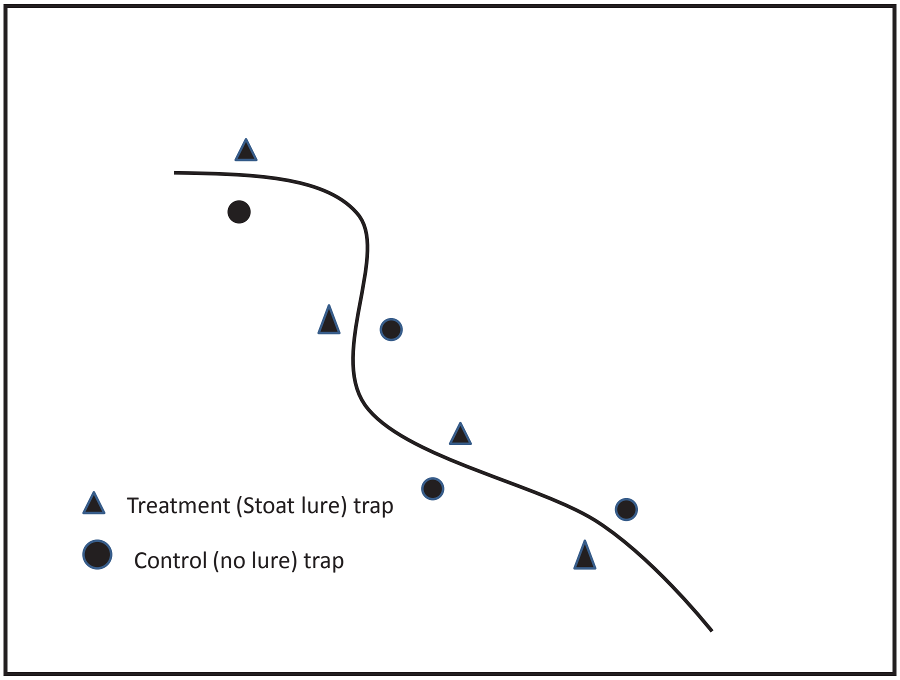


Figure 6.2 Layout of treatment and control traps as deployed along the track. Trap intervals approximately 15m; trap pair (control : treatment) spacing approximately 2m.

Trap deployment: Traps were deployed and activated on the same day, i.e. there was no pre-feeding phase.. Trapping ran for 12 consecutive nights.

Trapping was undertaken under MUAEC 11/12.

Data recorded: Suitable storage for rat bodies was not available hence the following variables were recorded and bodies discarded.

1. Trap number & assigned lure (treatment or control)
2. Rat sex

3. Rat body length (mm)
4. Rat weight (g)
5. Rat age class (assessed from body-length and weight)
6. Rat reproductive status (testes descent, nipple status, vaginal perforation)
7. Rat colour morph ('rattus' = black / grey with grey belly; or 'frugivorous' = brown / grey with cream belly)
8. Micro-chip number (if present)

Traps that were triggered with no capture or non-target capture (mice) were also recorded.

Traps were checked daily when deployed, with checks completed prior to mid-morning so as to reduce the risk of data loss due to scavenging by diurnally active predators including cats, stoats or wandering dogs.

Data analyses: Due to the small sample size, non-parametric statistical analyses were undertaken using SPSS 16 (student version). These tests were: binomial test for treatment versus control and adult versus juvenile/subadult rat captures; Chi square goodness of fit testing for sex bias in captured rats and Kendall's Tau testing for any correlation between captured rats' physical attributes (sexual status, size and weight) and night of capture; and correlations between body length and weight.

6.4 RESULTS

A total of 16 rats were captured over the 12 nights (Figure 6.3) of which eight were female, seven were male and one unidentifiable due to scavenging (head only remaining) (Table 6.1). Four traps (33a, 31a, 12a and 9a) captured rats on two separate nights. No triggered traps showed any indication that there had been a rat captured which had subsequently escaped or been scavenged, except that previously indicated where only the head remained. One trap (23a) captured an adult mouse on night 1.

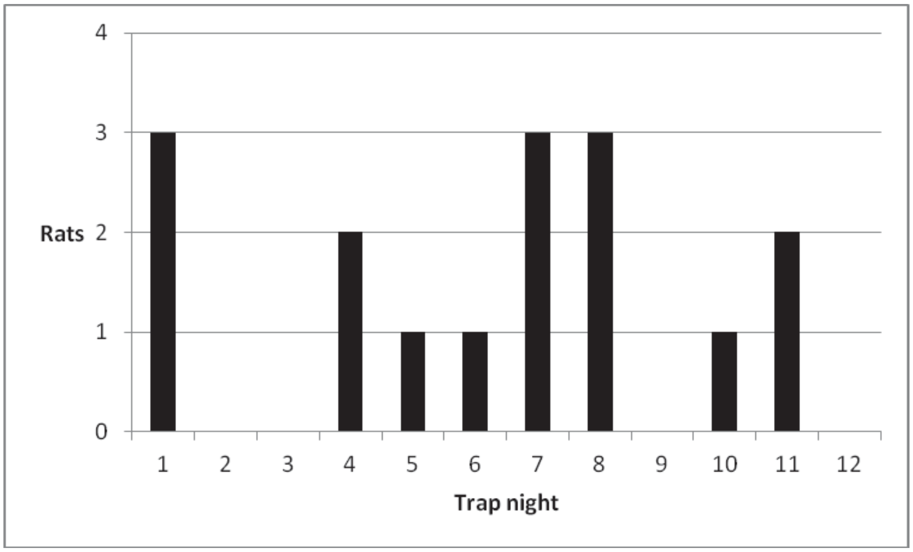


Figure 6.3 Distribution of rat captures during the twelve night trapping period.

In total 13 rats were captured in treatment traps and three in control traps. All 13 treatment captures were classified as adults (Females (n= 5) – 92 – 114gm; 145 – 160mm; Males (n=7) – 94 – 172gm; 154 – 204mm), and all three control captures as juveniles / sub-adults (40 – 45gm; 110 – 123mm), all three sub-adults were clearly female from external examination. A binomial test (SPSS 16) returned a significant result ($P< 0.05$) for both treatment versus control (13/16), and adult versus sub-adult / juvenile (13/16) captures.

Of the mature adult females three were vaginally perforate with discrete nipples and two as vaginally perforate with raised nipples, one of these may have been pregnant. Of the mature males, five had descended testes and two had abdominal testes. There were eleven rats of the 'frugivorous' colour morph and five of the 'rattus' morph. Three snap trapped rats were found to carry micro-chips (two rats in trap 9a and one rat in trap 11a), all three were males. None of these males were recorded in any of the preceding field experiments.

Of the adults captured there was no significant difference in capture rate between males and females (Chi Sq Goodness of Fit, $x^2=0.333$, d.f.=1, n=12, P=0.564). There did not appear to be any bias in terms of sexual status or body size / weight, nor was there any correlation noted between these variables and the night of capture (Kendall's Tau=-0.32, n=12, P=0.889). As expected individual's weight and body length were significantly correlated (Kendall's Tau=0.708, n=12, P=0.002).

The three rats assigned as juvenile / sub-adult were captured on nights 1 (two rats) and 4 (one rat). The two juvenile / sub-adult rats captured on night 1 may have been siblings based on (1) similarity in body length, weight and morph; (2) proximity of capture traps (4b and

5b); (3) visual confirmation from behavioural video the preceding nights where two young rattus morph individuals interacted with each other and an adult in this specific localion (i.e. the immediate vicinity of trap pairs 4-6; Figure 6.4). Analysis of the micro-chip data associated with the video footage identified the apparent mother of these two young rats as a micro-chipped individual that was not captured during this experiment, unless she was the scavenged individual and the tag was lost during that event. The third juvenile / sub-adult is considered to be of another litter due to size / weight differences; trap location and colour morph. Aside from the scavenged animal whose sex could not be ascertained, no clear maternal candidate for this third juvenile / subadult emerges from the trapping data. The importance of no identified maternal candidates is that while these individuals were in the vicinity they were not captured in the snap-trapping and thus indicating that there was a proportion of the resident population that were not trappable by this method.

Table 6.1 Trapping record for Kaitawa Lakes snap trap experiment.

Night	Trap	Treatment*	Micro-chip	Sex	Age/stage	Reproductive Status	Weight gm	Body length Mm	Colour morph
1	4b	0		f	j/s	Vup	40	110	r
1	5b	0		f	j/s	Vup	40	110	r
1	17a	1		f	A	vp nd	114	160	r
1	23a	1	mouse						
4	2b	0		f	j/s	Vup	45	123	fr
4	33a	1		m	A	Td	94	154	fr
5	33a	1		m	A	Td	144	189	fr
6	11a	1	34781	m	A	Td	172	192	fr
7	31a	1		f	A	nr p	94	155	fr
7	12a	1		m	A	Ta	112	170	r
7	1a	1	predated		A				
8	1a	1		m	A	Td	168	204	fr
8	9a	1	37580	m	A	Ta	140	175	fr
8	31a	1		f	A	fvp nd	90	145	fr
10	13a	1		f	A	fvp nd	92	155	fr
11	9a	1	72201	m	A	Td	170	195	fr
11	12a	1		f	A	fvp nr	108	153	fr

***Treatment 1 = stoat 0= control; Sex f = female m= male; Age/stage j/s = juvenile / sub-adult a = adult; Reproductive status vup = vaginally unperforate vp = vaginally perforate nd = nipples discrete nr = nipples raised p = pregnant ta = testes abdominal td testes descended; Colour morph r = rattus fr = frugivorous**

Figure 6.4 Micro-chipped adult female and two juveniles prior to the snap trapping experiment in which it is believed these two juveniles, but not the adult female were captured.

6.5 Discussion

The most important finding from this experiment is the overwhelming adult capture rate in stoat lure traps, compared to the juvenile / sub-adult captures in the control traps.

I predicted either a random distribution of adult captures across both treatment and control traps if no preference was observed, or a bias towards control traps if the neophilia hypothesis was supported. What I found does not support this prediction. All the adults were captured in stoat tainted traps. If neophilia were the predominant behaviour then I might further expect to have seen some indication that resident rats were most likely to be captured, and that captures would occur across both treatment and control traps. Younger, but still independently dispersing rats, should avoid capture altogether and thus be excluded from my dataset. It is fair to say that the dataset is too small to accurately assess whether I lack such a class of rat, however the overwhelming adult capture success in stoat tainted traps, argues against neophilia of residents being the primary behavioural motivation in force. With respect to the three juvenile /sub-adult captures, I suggest that their natural inquisitiveness drove them to inspect the 'safer' of the two novel objects, the trap associated with the control blankets.

I deliberately positioned the traps in such a way as to maximise the chance that upon encountering one trap the rat would be within visual or olfactory range of both and thus able to decide which to approach closest. The capture trap cannot be interpreted as the first trap approached, only as the first trap approached closely enough to result in triggering (rats were captured by the head in all cases). This response probably best sits between the sniff approach and mouthing responses previously reported (chapters 3-5). This may go some way toward explaining the near even ratio of male to female snap trap captures, whilst the mouthing behaviour appears to be predominantly though not exclusively a male behaviour (Chapter 4, Experiment 1 & 3; Chapter 5, Experiment 2). The sniff behaviour was found to be more equally observed across both sexes in the captive experiments (Chapter 4, Experiment 1). As the snap traps were triggered at a distance more similar to a close sniff approach, a number of individuals, particularly females, that may have engaged in sniffing behaviour, but not necessarily escalated to the mouthing behaviour, would be captured by the snap trap.

It cannot be assumed that night of capture represents the first night of encounter, indeed some animals may have paid repeated visits over repeated nights prior to approaching closely enough for capture. The highest per night capture rate (four rats) occurred on night 1 (including two juveniles / sub-adults) indicating that a close approach on the first nights' encounter is not totally avoided, hence neo-phobia is not a sustained response even on first encounter. Nights 7 and 8 generated three rats each, the remaining nights 0 – 2 rats. The actual trap night capture data, i.e. the propensity of rats to approach or avoid novel objects within a familiar setting, does not of itself strongly support either neophilia or neophobia in the rats studied.

Three of the adult rats had previously been live captured and micro-chipped, two in the vicinity of site A (snap traps 4-6), and one in the vicinity of site B (snap traps 16-18). The two site A rats were both captured in snap trap 9a (ca. 60m track distance), whilst the site B rat was captured in snap trap 11a (ca. 90m track distance), thus all three rats had either migrated away from the original capture sites between 7th / 8th Jan 2011 and 22nd - 27th March 2011; or had territories that encompassed both live capture and snap trap capture areas. All three were mature adult males at point of original capture (176 – 195mm; 140 – 172 gm) and two had grown in length and / or weight since micro-chipping whilst the third remained at a similar size and weight. This would indicate that they were not all young dispersing males at the time of first capture. Since January is probably the height of the New Zealand rat breeding season (Sept – April) (Innes 2005), these rats may have been at the greatest extent of their home range at the time of live capture and micro-chipping. That none of these three rats were recorded interacting with the experimental apparatus suggests that they were no longer routinely visiting the Site A and B experimental locations where they were originally captured, either having been scared off by the capture experience, or simply as a consequence of a gradual retraction of their home range as the breeding season wound down.

Much rodent research has been conducted into the response to predator threat, the overwhelming consensus of which is that there is an innate recognition which often manifests as an avoidance or fear response, particularly in predator-naive individuals (see Kats and Dill 1998; Apfelbach et al. 2005 for comprehensive reviews). Research into innate recognition has not been deliberately undertaken (to the authors' knowledge) in either captive or free-living ship rats, however predator-naive laboratory rats will exhibit an innate 'fear' response to predators, particularly cats (*Felis catus*) (McGregor et al. 2002; Hubbard et al. 2004). In this experiment I captured three juvenile / sub-adult animals, all in the control traps. This is consistent with the theory that in predator inexperienced or naive prey individuals, such as juveniles and young sub-adults; avoidance of the predator odour is the default setting. That all the adults were captured in the stoat traps is consistent with my prediction that more predator-experienced individuals develop more investigatory responses to predator cues which ultimately over-ride the default innate predator avoidance.

Some research suggests that neophilia or neophobia may be related to the resident or nomadic lifestyle of a species, with residents being more inclined to neophilia than nomads (Mettke-Hofman et al. 2004). Thus within a territorial species such as ship rats where juvenile / sub-adult dispersal is believed to occur, resident rats will be both more familiar with objects within their territory, thus more likely to recognise and investigate them; and have a greater 'vested interest' in ascertaining whether a novel object poses threat or resource potential. Dispersers however will be unfamiliar with the territory through which they are passing, thus less able to identify 'novel' objects, and have little motivation to investigate for threat or potential resource value.

Contrary to this hypothesis, ship rats are generally considered to be cautious in approaching novel objects (i.e. neophobic) (Innes 2005), thus the routine practice of pre-feeding in New Zealand trapping and poisoning management strategies. However, as with the neophilia hypothesis, it may be more accurate to suggest that such responses may be context specific with the familiarity of the environment in which the object is encountered playing a larger role than the object itself. For example a novel object appearing in a familiar environment elicits a greater phobic response in captive ship rats than a novel object in an unfamiliar environment (Cowan 1976; Hughes 2007). It has also been reported that Norway rats, generally considered to be notoriously neophobic, from the historically uninhabited Hawea and Breaksea Islands, New Zealand, exhibited no neophobia (Taylor and Thomas 1993) and that non-(human) commensal species in general, as the subject ship rats were, show only weak neophobia (Cowan 1977). Thus the apparent lack of neophobia in the study population may be more typical of forest dwelling ship rats than is currently recognised.

<u>Management implications:</u> I would suggest that as a deterrent either for the protection of economic or ecological resources, predator odour will not be effective against ship rats, rather it may have quite the opposite effect of luring them away from more traditional traps or control measures although this remains to be substantiated. Whilst stoat odour seems to have proven a more effective lure than no odour, a number of tagged individuals who succumbed to peanut butter and carrot lure in previous live capture were not attracted by the stoat odour (n=13), including the micro-chipped mother of at least two juveniles who was logged in the immediate vicinity of traps 4-6 a night prior to the snap trapping commencing.

Similarly, given the snap trap location of the three tagged individuals in relation to their original live capture location, there were a number of untagged rats captured that may not have been lured by peanut butter, but did investigate the stoat odour. I suggest this is evidence that not all rats within a given population will exhibit the investigatory behaviour, which is consistent with predator inspection behaviour within other species studied (e.g. FitzGibbon 1994; Godin and Davis 1995; Dugatkin et al. 2005), and that different individuals within a given population will respond to different lures. As Gsell, Shapira and colleagues recently found using the domestic variant of Norway rats to attract wild Norway rats into trapping apparatus (Anon 2012), stoat odour may have a management role as a lure in specific circumstances such as where peanut butter cannot be used, where a greater target (ship rat) specificity is needed, or as part of a 'mop-up' operation after more conventional lures have been deployed.

6.6 CONCLUSION

In the light of the results of this experiment I suggest that the phenomenon broadly described as predator inspection behaviour does occur in free-living ship rats within the New Zealand forest ecosystem, demonstrably between stoats and ship rats but potentially encompassing other common terrestrial predators such as cats (Chapter 5). I consider that predator inspection behaviour in ship rats and its evolution within an individual animal can go some way towards explaining apparent disparities between captive laboratory experiments and field experiments in some instances. I suggest that in the predator-naive or inexperienced individual regardless of chronological age, the default response is that of avoidance, often reported as a 'fear' response in captive research. As an individual becomes more experienced in its risk assessment a drive towards inspection behaviour develops and over-rides the innate fear or avoidance behaviour.

Such a shift with maturity is ecologically adaptive in a relatively short-lived species such as the ship rat for which predation threat is probably a constant background reality. Such species may have only one or two breeding seasons in which to maximise reproductive success, individuals who are most successful at risk assessment will spend more time foraging, defending their territories and finding mates. Inspection behaviour I suggest serves to enhance risk assessment, and thus life history success in forest dwelling ship rats.

Finally, predator (stoat) odour perhaps should not be applied as a deterrent to areas of high conservation or economic sensitivity as it may prove more effective as a lure than a deterrent to predatory ship rats.

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Chapter 7 PARASITE INFECTION OR CHEMICAL CUE OVERLAP AS BEHAVIOURAL DRIVERS: A BRIEF DISCUSSION

7.1 ABSTRACT

The preceding chapters (4-6) have reported the results of research which I have interpreted as evidence for predator inspection behaviour in ship rats (*Rattus rattus*). However, there are three other hypotheses identified that would, at least potentially, explain the behaviours observed. The first, a neophilic response to the odour presentations, was addressed in Chapter 6. Two other alternatives, parasite infection and chemical cue overlap hypotheses were less easily tested with the resources available and are addressed here, citing both experimental and literary evidence.

Parasite infection, specifically toxoplasmosis, is addressed via laboratory analyses, behavioural experiment results and evidence from the literature with no strong support forthcoming for toxoplasmosis specifically as the behavioural modifier in this instance. Chemical cue overlap is more difficult to address, largely due to the immense breadth of possible odour sources, however three likely chemical overlaps are discussed: dietary or sulphide chemicals; stoat specific chemical; and a male specific chemical. In the light of the results of the behavioural experiments, there was no compelling evidence to support of any of these possibilities.

The following discussion is not exhaustive; rather it deals with the possibilities that the results of the preceding experiments lent themselves to investigating. To fully investigate and eliminate these hypotheses would require resources beyond the scope of the current research.

7.2 INTRODUCTION

The research presented in the preceding chapters has demonstrated that ship rats (*Rattus rattus*) exhibit a non-avoidance response that I have interpreted as an investigatory reaction to the odour of two familiar predators, stoat and cat. The response was significantly less common in response to non-predators including possum, chinchilla, unfamiliar male con-specific and 'blank' odour. My favoured interpretation for these investigatory responses, particularly the more extreme examples which I have termed mouthing and chew or remove, is that of predator inspection behaviour. However, there were at least three alternative hypotheses that needed to be considered. The first alternative hypothesis, neophilia, was addressed experimentally (Chapter 6) with no support found. Two additional hypotheses that have proven more difficult to discount are a behaviour modifying parasite infection or chemical cue overlap causing the rats to behave boldly.

Parasite –host relationships can be exceedingly complex, particularly where the parasite undergoes several life stages and specifically requires transmission into a particular end-host for its own reproductive stage, such is the case with *Toxoplasma gondii*. Parasitic infections, including those by *T. gondii*, can cause often fatal behavioural symptoms in their host (Moore 2002). This parasite is widely reported to cause behavioural changes in infected rodents in response to cues, particularly the urine of cats (*Felis catus*) (Berdoy, Webster & MacDonald 2000; Vyas et al. 2007a; Vyas, Kim & Sapolsky 2007b). The behavioural change commonly reported is the depression or inhibition of normal avoidance responses, causing the infected rodent to engage in highly risky behaviour in the presence of a predator. It has also been suggested that sexual arousal may be implicated in the behavioural change of infected laboratory rats in response to cat odour (House, Vyas & Sapolsky 2011). Since the cat is the definitive host of the parasite it requires transmission to a cat host in order to complete its lifecycle. The abnormal behaviour of the infected rodent makes it easier prey for the cat, thus the *T. gondii* parasite is transmitted to its final host ensuring the completion of the life cycle and reproductive success of the parasitic individuals.

Feral cats are often present in low to moderate levels in New Zealand forests, including the source locations for this research, and while the presence of *T. gondii* infection in ship rats may be generally accepted, there is no data indicating the prevalence of infection in rats captured in New Zealand. Thus the possibility that this parasite may be causing the investigatory rat response to stoats was considered plausible enough to warrant further investigation. However, it was not clear, and remains uncertain, whether *T. gondii* infects stoats in the New Zealand forest. Evidence from other locations indicates that infection of this species is theoretically possible because a number of other mustelid species have tested positive for the infection (Smith & Frenkel 1995; Frank 2001; Burns et al. 2003; Smielewska-Los & Waldemar 2004; Jones et al. 2006).

As it was not within the scope of my research to investigate the prevalence and symptomatic response of *T. gondii* infection in rats and stoats, I opted to: (1) have a small sample of euthanased rats that had been used in phase 1 captive research tested for the parasite; (2) undertake further behavioural research incorporating a cat odour cue, a response to which would support the possibility of toxoplasmosis infection being the causal mechanism. From the results of these two test approaches I hoped to determine whether *T. gondii* infection could be eliminated as a probable causative mechanism for the observed investigative behaviour.

Chemical mimicry is the ability of one organism to either imitate components of odours released by another, for example non-toxic plants which may smell or taste like toxic ones (Batesian mimicry) (Muller-Schwarze 2006) or the imitation of a pheromone complex in order to avoid detection by a host species (Stoeffler et al. 2007; Strohm et al. 2008; Silveira, Oliviera & Trigo 2010). Chemical crypsis is the ability to hide by using subtle changes in pheromone chemistry (Raffa et al. 2007; Silveira et al. 2010). Both phenomena have often been reported in the invertebrate predator / prey literature (see Ruxton 2010 for a review of non-visual crypsis research). Whilst much of this research focused on prey avoiding predators, or parasites avoiding host detection, there is also evidence of predators imitating prey via chemical cues (Zhang, Sun & Novotny 2007). Thus the possibility arises that the apparent attraction witnessed between ship rats, and stoat and cat odour is related to a similar phenomenon, i.e. that stoats are mimicking rat odours

There is some precedent for this theory in terms of interactions between rodents and mustelids, since it has been shown that male mice may be attracted to specific components of ferret urine when mixed with estrous female mouse urine (Zhang et al. 2007). However other chemical ecology research suggests that while many pheromones are widespread across disparate species such as elephants and moths, animals recognize and respond to a cocktail of chemicals at specific concentrations which together create an odour rather than any one of the individual components alone (Wyatt 2003; Muller-Schwarze 2006).

The chemical cue hypothesis is possibly the most difficult to prove or disprove, in large part because of the breadth of possibilities it encompasses: sex pheromone overlap, con-specific or prey alarm pheromone overlap or mimicry, dietary (sulphide) compounds, con-specific territory or individual marker to name but a few. Thus I hoped that via my behavioural experiments I might be able to elucidate: (1) whether the rats would exhibit the behaviour to a predator other than stoat; implying that any chemical cue must be shared beyond stoat and rat; (2) whether the rats responded differentially to either male or female stoat; thus isolating those chemicals exclusive to one or other sex as candidates; (3) whether male subject rats responded to a 'stranger' male con-specific; thus implying that any chemical cue may be male rat or dominance related.

In my research it was evident that the strongest ship rat response was towards male stoat odour followed by the mixed sex cat odour. Responses to the other odours presented; female stoat, unfamiliar male con-specific, possum and chinchilla were uniformly low for the more extreme mouthing and chew or remove responses as discussed in Chapters 4 and 5. This sexually differentiated response to male and female stoat odour is difficult to explain under the predator inspection hypothesis, indicating that greater consideration needs to be paid to the possibility of some form of chemical overlap being the driving mechanism for the behaviours witnessed. If the inspection behaviour could be shown to occur towards other non-mustelid predators then either the chemical cue is shared between ship rats, stoats and e.g. cats; or some other explanatory mechanism must be in action.

7.3 METHODS

7.3.1 TOXOPLASMA GONDII

TEST 1: PCR analyses of euthanized rat subsample: A sub-sample of 12 animals was randomly selected from the total captive sample available (31 animals). The sub-sample comprised of six 'responders', i.e. animals for which repeated mouthing responses were recorded towards stoat odour; and six 'non-responders' i.e. animals exhibiting no mouthing responses towards stoat odour. Of the responder group four were males and two were females, which is reflective of the bias in the mouthing behaviour overall. In the non-responder group two were males and four were females. Overall six males and six females were selected. The animals were sourced from both Egmont National Park (n=6) and Lake Waikaremoana (n=6) sites. Eight rats were sourced from stoat-managed populations and four from non-stoat-managed populations. The subjects had all been euthanized by CO_2 at least 6 months previously and storeed frozen and intact.

Selected animals were thawed and the brain was extracted for PCR analysis. Analysis was undertaken by Dr Laryssa Howe, IVABS, Massey University. Tests undertaken were (1) Apicomplexan PCR testing for *Toxoplasma, Neospora* and *Sarcocystis*; (2) *Toxoplasma* specific PCR; and (3) *Toxoplasma* nested PCR (a more sensitive test than the Toxoplasma specific PCR). *Neospora* and *Sarcocystis* are other parasite infections which may have been present and also needed to be considered if found.

TEST 2: Cat odour presentation in behavioural experiments: In phase 1 of my research stoat odour was presented to ship rats under both captive and free-living conditions. Non-predatory odours including possum, chinchilla and a blank control treatment were also presented. The full experimental procedure is described elsewhere (Chapter 2 General Methods; Chapters 3, 4 and 5).

If *T. gondii* were a causal factor I expected to see the investigative behaviour previously seen in response to stoat odour, towards the cat cue presented in phase 2. Although not urine based, if infection caused a response to stoat body odour, cat body odour should also be effective at eliciting a response.

7.3.2 CHEMICAL CUE OVERLAP

During both phases 1 and 2, as briefly outlined above, the rats were exposed to a range of predator and non-predator odours and a significant mouthing behaviour reported in response to stoat odour (Chapter 4 and Appendix 3). Phase 2 incorporated cat odour as a second familiar predator to assess the specificity of the mouthing response (Chapter 5). At the conclusion of the live behavioural research a snap (kill) trap experiment was undertaken where paired treatment (stoat odour) and non-treatment (untainted) lured traps were deployed. The capture rates and morphological data including sex, body length and weight of the captured rats were recorded and analysed using binomial testing (Chapter 6).

7.4 RESULTS

7.4.1 *TOXOPLASMA GONDII:*

TEST 1: PCR analyses of euthanized rat subsample

- The Apicomplexan PCR test returned no positive results on any of the 12 brains submitted.

- The Toxoplasma specific PCR also returned no positive results on any of the 12 brains submitted.

- The more sensitive Toxoplasma nested PCR returned weakly positive results on 5/12 rat brain samples (rat numbers 106, 107, 203, 204, 308).

Rats 106, 203 and 308 were from the non-responder group, rat 204 and 107 were from the responder group.

Four of the positive rats were sourced from stoat-managed backgrounds (2 x LW; 2 x ENP), and one from the non stoat-managed LW site.

TEST 2: Cat odour presentation in behavioural experiments: Cat odour was tested against male stoat, female stoat and 'unfamiliar' male rat odour. Under both captive and field conditions male stoat odour elicited the greatest mouthing response, with cat odour eliciting a lower level of response than male stoat but greater than either female stoat or unfamiliar male rat odour (Chapter 5). Under neither captive nor field conditions was the frequency of cat odour mouthing not significantly different to any other odour. Under field conditions the physical evidence (chew or remove) of the cat odour mouthing response was significantly less than male stoat odour (G-test, G=22.11, d.f.=3, P=0.00006) and similar to that of female stoat and male rat odour.

7.4.2 CHEMICAL CUE

The results of the phase 1 experiments generated no evidence of an avoidance response in the form of either approach rate or seed consumption in the presence of stoat odour (Chapter 3). There was a significantly greater mouthing response towards stoat odour than any of the other treatment odours (possum, chinchilla or control) reported in the captive enclosure experiment (Chapter 4 Experiment 1)(G-test, G=20.09, d.f. = 3, P<0.0001; GLM, n = 31, z= 2.617; p=0.0104). This was reflected in the field experiment where mouthing was significantly more often directed towards stoat odour (Wilcoxon signed-rank test, Z= -1.997, n= 16, P<0.05), and repeated mouthing and chew or remove behaviours were exclusively directed at stoat odour (Chapter 4).

In the phase 2 captive experiments mouthing was significantly greater in response to male stoat than either female stoat (P= 0.014) or male con-specific odour (P= 0.012)(Table 5.4). There was no significant difference in mouthing frequency between male stoat and cat odour. Similarly when repeated mouthing events were assessed male stoat odour elicited a significantly greater response than either female stoat or male con-specific odours (G-test, (G= 13.03, d.f.=2, P=0.0014).

The snap trap experiment captured significantly more rats (13/16,) in the stoat tainted traps (n = 16, P<0.05).

7.5 DISCUSSION

On the face of the PCR analyses carried out on euthanased rats subsample, the overall level of infection is moderate, and given the distribution of infection across both responders and non-responders, it seems unlikely that *T. gondii* can be considered a likely explanation for the observed behaviour. However, Dr Howe cautions that rats testing negative may have since cleared the infection or that damage to the brain caused by cysts or foci of gliolisys, could have caused the behaviour observed and that further testing on fresh brains would be required to totally rule out this parasite as a causal mechanism (Appendix 4). Toxoplasmosis was found to be weakly present in rats from three of the four geographically disparate source sites submitted, suggesting it is probably prevalent throughout the North Island ship rat population. Furthermore it is present in rats from both stoat-managed and unmanaged populations. Whilst further investigation would be required to substantiate this, it appears that infection with toxoplasmosis is not differentiated between unmanaged and reduced (stoat-managed) predator exposed ship rat poplations.

While there were some behavioural responses to cat odour in both the wild and captive rat samples it was always less than that of the response to male stoat odour. The mouthing response to female stoat odour however was consistently less than that shown towards cat. This suggests that while the rats are exhibiting some response to the cat odour it is not a strong response as might be expected if *T. gondii* were the causal mechanism. Vyas et al. (2007b) found that infected rodent response to cat odour was dose dependant, suggesting too little cue would fail to elicit a response, while too much would elicit the innate avoidance response shown in laboratory rats. If *T. gondii* were the causal mechanism of the investigatory behaviour by ship rats towards stoats then a similar level of response should be observed towards both male and female stoats since the sex of the predator should not affect the non-avoidance behaviour as reported in infected rats towards cats.

In the toxoplasmosis research carried out to date the focus has primarily been on the response of infected rodents to cat urine with little testing to assess the response to other predators, and the usual response has been depressed avoidance. However, it has been reported that there is no depression of the avoidance response in infected rodents to dog (*Canis familiaris*) or mink (species unspecified) urine (Vyas 2007a; Lamberton, Donnelly & Webster 2008). Similarly Vyas et al. (2007a) reported no difference in locomotion or anxiety between infected and uninfected laboratory rats in an open-field arena test. This suggests that the response, even if not exclusive to cats, is certainly not generalised to all potential predators. Currently it seems there is insufficient published evidence to predict the response of ship rats to stoat odour under the influence of toxoplasmosis, and the effect of infection on rats towards non-felid predators is disputable.

Lamberton et al. (2008) and House et al. (2011) have argued that from an evolutionary perspective, if cats are the only known definitive host of the *T. gondii* parasite, it would not be an evolutionarily advantageous strategy for the parasite to render rodent hosts susceptible to all predators, rather selective pressures would drive towards a specificity of response towards the definitive host. Thus it should be expected that any response might be limited, if not to cats, then at least to felids, or only those animals with an overlap of the specific stimulatory components to their urine or other excreta. However, whether the likelihood of predation by a predator other than cat is sufficient to drive a differential response towards predators by infected rodents remains open to debate.

Chemical mimicry or crypsis occurs between co-evolved predators (or parasites) and prey (or hosts) allowing organisms to hide among predators or evade the defences of a prey or host species; and is commonly reported among invertebrates e.g. (Strohm et al. 2008; Hojo et al. 2010; Martin et al. 2010; Pfeiffer, Huttenlocher & Ayasse 2010; Ruxton 2010; Silveira et al. 2010). As stoats and ship rats did not co-evolve, unless the chemical in question is mustelid-wide, co-evolution cannot be cited as the origin of the relationship. It is possible that some component in stoat odour mimics an alarm cue in either ship rats or a ship rat prey species, however since the rats also responded to a non-mustelid predator (cat), this chemical must be more generally exuded by predatory species.

The ship rat response to cat odour suggests that the chemical overlap must be shared beyond a rat and stoat (or mustelid) relationship, extending also to cats (or felids). Mixed sex colonies were used to source the cat odour sample and it may be that it is either the male or female cat component that is causing the response as was seen with the stoat odour (Chapter 5). Further testing would be required, but if it were found that it was the male cat odour that was generating the response it may be that the chemical overlap is not species -related, but rather is sex specific.

The subject male rats exhibited little response to ‘foreign’ male rat suggesting that although sex specific chemicals may be implicated, this doesn’t appear to extend to con-specifics. Recent Norway rat research (Gsell, Shapira and colleagues) has indicated that in that rat species ‘Judas rats’ can be used as a lure for wild (Anon 2008; Anon 2012). This success has been interpreted in terms of the social nature of Norway rats and may be as much or more as a result of acoustic cues than odour alone as I have used.

Dietary factors are often implicated in prey response to predators, particularly the avoidance responses where prey may be detecting sulphides in the faeces of carnivores (Muller-Schwarze 2006) or con-specific alarm cues, particularly in aquatic environments (Muller-Schwarze 2006). Sulphur-containing semio-chemicals have been found to be highly attractive to both carnivores and omnivores, especially in feeding contexts, with herbivores generally avoiding such compounds (Mason, Epple & Nolte 1994).

While the presence of such chemicals in faeces and urine may explain the attractant properties of male stoat and cat odour, it cannot explain the reduced attraction to female stoat odour observed since presumably both stoat sexes exude sulphides, and as omnivores so too do male rats and possums. That said, captive male stoats are apparently more inclined to defecate in their sleeping area (S. Brown, personal communication, 2009) and the male stoat tainted blankets had a stronger aroma to the human nose (pers obs). Thus it is possible that the level of sulphur-containing semio-chemical was greater in male stoat bedding than female. If the exuding of sulphide chemicals is diet (meat) related, the possum odour used may have been affected by the vegetarian diet of the captive possums (S. Brown, personal communication, April 19, 2012), however, the rodent nuts that formed a component of the captive rat diet do contain meat proteins (D. Chesterfield, personal communication, April 26, 2012).Thus captive diet alone cannot be solely implicated in the lack of response towards male con-specifics and possums.

While most responding rats did exhibit investigatory behaviours towards both cat and male stoat in the multi-predator captive enclosure experiment (Chapter 5, Experiment 1), if there was a chemical cue driving the response why does it not affect 100% of the population as all were mature adult males and thus presumably affected by the same physiological drives? As with the predator inspection hypothesis it may be valid to suggest that, for example, the hormonal state or personality traits of the individual will determine its propensity to respond, but experimentally demonstrating this would require large sample sizes (I. Castro, personal communication, 2011).

Other rat species, notably Norway rat, are widely demonstrated to exhibit an avoidance response to predator odours (Kats and Dill 1998; Apfelbach et al. 2005), including those that presumably contain sulphide chemicals such as faeces and anal gland compounds (Masonet al. 1994). However, this avoidance response seems less apparent in predator-experienced individuals (Bramley, Waas & Henderson 2000). If the causal mechanism for the investigative behaviours witnessed in my experiments is sulphide-containing semio-chemicals, why do these same chemicals not have an attractant effect in predator naive Norway rats? These rats have had no opportunity for negative associative learning and thus should be more inclined than predator-experienced individuals to investigate sulphide-containing semio-chemicals.

7.6 CONCLUSION

Both parasite infection and chemical cue overlap remain valid alternative hypotheses in explanation of the non-avoidance behaviours, particularly the mouthing and chew or remove responses reported in Chapters 4 and 5. While the investigation of toxoplasmosis as a causal mechanism has yielded no support for this hypothesis, it suggests several pertinent questions regarding the current state of understanding of this parasite and how it may affect rodent interactions towards predatory species other than cat:

- what is the prevalence of this parasite in mustelid, specifically stoat, populations in the New Zealand forest?;
- how specific or otherwise is the behavioural modification of infected rodents towards other predators such as stoats? And what are the implications specifically for the New Zealand forest ecosystem in terms of prey availability to stoats and prey-switching?

Although not experimentally tested, toxoplasmosis infection may have been a factor in the response towards cat odour reported in the captive multi-predator experiment (Chapter 5; Experiment 1). There also remains the possibility that parasite infection is implicated, but that it is not *T. gondii* , but rather an as yet unrecognised parasite. Further research would be required to assess this possibility. As the evidence stands, parasite infection, and toxoplasmosis specifically seems an unlikely causal mechanism for the investigatory behaviours reported.

Chemical cue overlap is more difficult to test for, largely because of the vast array of forms that this might take. Based upon the results presented, a specific dietary cue is unlikely, a sex pheromone is possible but not strongly supported, and while a male specific cue such as testosterone may be implicated it does not appear to apply to male con-specifics. The most likely chemical overlap may be one explained by a sulphur-containing semio-chemical that for reasons yet to be ascertained was most strongly evident in the male stoat and mixed sex cat treatment samples. Any chemical cue which acts as a behavioural motivator does not appear to have a generalised effect across all the ship rats sampled, but may be more attractive to adults than sub-adults / juveniles (Chapter 6), and males than females (Chapter 4).

On balance the evidence does not offer convincing support for either parasite infection or chemical cue hypotheses as behavioural modifiers in this instance, however both, particularly the sulphur-containing semio-chemical hypothesis, would require considerable research investment to definitively refute.

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Chapter 8 GENERAL CONCLUSIONS

8.1 MAJOR FINDINGS

This thesis presents a study of the behavioural responses of wild caught ship rats towards the odours of stoats and cats, in both a captive and free-living environment. Overall the aims of this thesis were to determine how prey animals respond behaviourally to familiar predator odours, to evaluate the role of predator experience in prey behaviour, and to test whether captive derived results are applicable in wild populations.

Based upon the mainstream predator-prey research, particularly the captive rodent and other small mammal literature (reviewed by Kats & Dill 1998; Apfelbach et al. 2005), I had expected to observe an avoidance type response elicited towards the predator odours I used. My studies show that, contrary to this expectation, the wild-captured ship rats exhibited no evidence of avoidance in response to a predator odour cue under captive conditions. This reflects the findings of Bramley and Waas (2001) where wild captured ship rats under captive conditions failed to consistently demonstrate an avoidance response to a range of natural and artificial predator odours. These authors suggested that individual variability in the rat subjects may have largely contributed to the lack of statistical support for an avoidance response; furthermore they suggested that predator experience in their wild-caught subjects of three rat species (*R.norvegicus, R. rattus* and *R. exulans*) may account for some of this apparent variability.

Under both captive enclosure and free-living conditions, the ship rats I studied exhibited several distinctly investigatory responses. An investigatory response exhibited by prey animals in response to the odour of predators is not unprecedented: in piscean, avian and a number of mammal species, behaviours described as maternal aggression, mobbing, defensive behaviours and predator inspection are routinely reported (Vieth, Curio & Ernst 1980; FitzGibbon 1994; Godin & Davis 1995; Hein 1996; Swaisgood, Owings & Rowe 1999; Owings et al. 2001; Brosnan, Earley & Dugatkin 2003; Caro et al. 2004; Dugatkin et al. 2005).

Within rodentia the evidence for non-avoidance behaviour is largely limited to aggressive interactions under field conditions by prairie dogs, rock and ground squirrels towards snake predators to which the prey has evolved some degree of venom immunity e.g. (Owings & Owings 1979; Swaisgood et al. 1999; Owings et al. 2001); and a defence or panic response by rodents confronted by a snake predator in a closed maze system (Guimaraes-Costa et al.

2007). The investigatory behaviour exhibited by the ship rats in my studies was exhibited by approximately 50% of any given subject group, and primarily, although not exclusively, exhibited by males. This is consistent with predator inspection behaviour wherein not all members of any given group or population exhibit the behaviour, instead it may be associated with particular activities such as mate selection; or personality traits such as boldness (Dugatkin & Alfieri 2003; Dugatkin et al. 2005; Quinn & Cresswell 2005; Pelligrini, Wisenden & Sorensen 2010). The investigatory behaviour exhibited by these ship rats represents the first report of such a response in this species (ship rats), and if correctly interpreted as predator inspection behaviour, is the first report of this specific behaviour in the mainstream rodent predator-prey literature.

Within the wider predator response literature there is considerable debate as to the roles of innate responses and learning, and this debate extends into the predator inspection literature. The avoidance response routinely elicited in predator naive laboratory rodents is often cited as evidence of innate predator recognition. Similarly in predator inspection studies, hatchery reared European sea bass (*Dicentrarchus labrax*) of wild parentage for example, were found to exhibit innate predator inspection behaviour (Malavasi et al. 2004). Other studies have indicated that predator recognition is learned, and that this learning can be achieved not only through direct experience, but also embryonic learning, and via con-specific or hetero-specific warning signals (Brown & Chivers 2005; Ferrari & Chivers 2010). In other instances prey species have successfully been taught to avoid exotic predators (McLean, Lundie-Jenkins & Jarman 1996; McLean et al. 2000).

There was no differentiation in the exhibition of non-avoidance behaviour in my subject groups according to their predator exposure background, thus if the behaviour is learnt it requires very little predator exposure to develop. Bramley and Waas (2001) suggested that predator avoidance in Norway rats is an innate response that is modified with experience such that avoidance behaviour is no longer expressed, and that this might also be true of ship rats. However Bramley and Waas do not interpret any observed behaviours as investigatory or inspection behaviour. My results support their behavioural modification suggestion and I would advance the suggestion that whilst avoidance is an innate response in predator naive individuals, this is rapidly modified by experience such that avoidance behaviours are no longer expressed within the free-living adult population. Further, in many adult individuals the loss of avoidance behaviour develops into an approach behaviour that might best be described as predator inspection.

Whilst my studies produced no evidence of explicit avoidance behaviour in response to predator odour, there was some indication that foraging strategies may differ between rats sourced from unmanaged versus reduced (managed) predator and competitor exposure, with those from a reduced exposure background more inclined to exhibit a 'carry and consume' strategy than a 'sit and eat' strategy when in the presence of stoat or possum odour. This behaviour has been previously reported in free-living Norway rats in response to con-specific competition (Whishaw & Whishaw 1996). Such a shift in foraging strategy might be driven by differing risk assessment skills between the different source populations.

Throughout my research I undertook a dual captive and field experimental approach in order to verify that the results I obtained in captivity were representative of the behaviours of free-living individuals. My captive enclosure protocol, including the choice of predator cue was designed to reflect a natural encounter and to allow the subject individuals as much temporal and spatial freedom to respond as was practicable. In all major findings the field experiments verified the results of the captive enclosure experiments, thus my enclosure experimental protocol has been demonstrated to produce reliable and replicable results for generalisation into free-living ship rat population behaviour.

8.2 IMPLICATIONS FOR BEHAVIOURAL PREDATOR-PREY RESEARCH

I have suggested that the investigatory behaviours exhibited by ship rats are evidence of predator inspection behaviour (Objective A). This is the first reporting of this response in ship rats specifically and *Rattus* spp more broadly. Given the extent to which rodent species and laboratory rats in particular are used in predator-prey research this is both surprising and potentially of crucial importance in recognising the general applicability, or otherwise, of laboratory rodent research to the advancement of predator-prey theory.

While there was no evidence in the enclosure experiment of any avoidance behaviours in response to the stoat odour, there was a clear escalating series of behaviours that could only be described as non-avoidance, even investigatory. These behaviours began with approach and sniffing behaviours, and escalated into what I termed 'mouthing' behaviour. In later experiments (Chapters 4 & 5) a rare but clearly definable 'chew or remove' behaviour also occurred. This behaviour was significantly more likely to be directed towards predator odour, and both mouthing and chew or remove behaviour were exhibited under both captive enclosure and field conditions (thesis objectives A & C). These behaviours, including the lack of avoidance response, were generalised across all the sample groups thus predator management background did not appear to affect the expression of the behaviours (Objective C).

Any investigative behaviour such as predator inspection is potentially a high risk behaviour for which there must be a strong motivating factor. Bramley and Waas (2001) had previously suggested that the lack of avoidance response witnessed in their predator-experienced rat subjects may be risk assessment related and I suggest that this would be sufficient motivation for a short-lived species such as the ship rat to engage in predator inspection behaviours. For my interpretation to be supported the behaviour would be expected to be exhibited in response to multiple predators. In Chapter 5 I tested this by presenting a second familiar predator odour, cat and found that the investigative behaviours were also directed at this predator odour (Objective A).

Other hypotheses were investigated as possible explanatory mechanisms. Neophilia by resident rats towards the odour presentation was tested in Chapter 6 via a snap trap experiment with no evidence in support of either the neophilia hypothesis, or the commonly assumed neophobic response of rats. Convincing evidence was found for a tendency by adult rats to investigate stoat odour. Infection by a behaviour modifying parasite, specifically toxoplasmosis was also investigated. No evidence in support of this was found despite evidence of the *Toxoplasma gondii* parasite being present in approximately 50% of rat brains submitted for PCR testing.

Finally a chemical overlap was considered; this hypothesis was only indirectly tested as the range of possible sources of overlap is vast. The results of my research indicate that any chemical cue that the rats are responding to is not stoat specific because it is shared by cats. Nor is it related to the specific predator diet because the cat and stoat odour donors were fed very different diets, neither of which featured rat. While the cue may be male specific it does not extend to male con-specifics. The most likely chemical explanation may be sulphide containing semio-chemicals which are recognised as attractants to both carnivores and omnivores (Mason, Epple & Nolte 1994). However the low level of response to female stoat, male ship rat and possum odours, all of which should also exude such semio-chemicals, and the apparent differentiation in attraction by age class and possibly sex requires explanation. Therefore whilst this hypothesis cannot be excluded there is no strong evidence found in its support.

Under captive conditions the wild-caught, i.e. presumably predator-experienced rats exhibited no avoidance behaviours towards the predator odours. This is contrary to expectation as many laboratory based predator – prey studies have reported clear evidence of ‘avoidance’, ‘aversive’ or ‘fear’ responses in their study populations; however captive research using wild sourced study subjects seems less likely to report these same responses (see Kats & Dill 1998; Apfelbach et al. 2005 for reviews). I have suggested that the reason for the discrepancy between laboratory and wild sourced animal studies lies with the subject species and the test environment. Laboratory animals are truly predator naive, and where individuals are exposed to predators it is done so under very artificial controlled and contrived conditions that may bear little resemblance to those under which predator experience in the wild may occur. Examples of studies that have attempted to utilise live predators include a more realistic visible burrow system for colony living Norway rats wherein a cat was allowed to walk across the surface (Blanchard & Blanchard 2003); and a more contrived and confined maze system in which rodents were confronted with live snakes (Guimaraes-Costa et al. 2007).

Not only are laboratory animals predator naive as individuals, but as domesticated variants they are captive bred, and thus predator naive over hundreds of generations. If predator responses, including predator inspection include potentially heritable traits as the results of hatchery reared sea bass experiments suggest (Malavasi et al. 2004), the process of domestication could conceivably have caused a loss or weakening of the potential for its expression. If character traits such as boldness are an integral component of the propensity to predator inspection as suggested by Dugatkin and Alfieri (2003), these traits may again have been unwittingly manipulated in the domestication process. However, if behaviours such as predator inspection are learned, as seems the consensus opinion (see Brown & Chivers 2005 for discussion), laboratory bred animals are incapable of expressing non-avoidance responses as they have not had the opportunity to learn them.

Methodologically, laboratory research is more controlled and contrived than field research, with the subject animals being tested under conditions of both spatial and temporal constraint. This has lead to criticism that there is little or no consideration of the ethology of the subject species, scope for the expression of a full range of potential behaviours within the experimental design, or scale of experimentation (Genaro & Schmidek 2000; Jonsson, Koskela & Mappes 2000; Blanchard & Blanchard 2003). The wild Norway rat had characteristics, including colony dwelling which made it amenable to domestication: specifically laboratory housing and captive breeding systems. Within laboratory rat research (at Massey University) the tendency has been that animals are housed in pairs unless specifically required otherwise (D. Chesterfield, personal communication, April 26, 2012), but most rodent behavioural research has experimented upon rats as individuals. This act of isolation from a familiar cage-mate may create a potentially stressful situation leading to stress motivated behaviour rather than strictly cue motivated behaviour in the presence of a predator cue. Similarly it can be argued, that the stress imposed on a wild subject at being captured and held in captivity may effect the responses of the animals under experimental conditions. I acknowledged and managed this risk in several ways: my initial selection for calmer animals at trapping; my largely 'hands-off' approach to husbandry; my development of the enclosure experiments and housing; and crucially, my use of free-living rats to verify the results from the captive research.

8.3 IMPLICATIONS FOR CONSERVATION

In Chapter 3 I reported that while there was no evidence of avoidance behaviour in response to predator odour, animals sourced from predator or competitor managed versus unmanaged locations may have been adopting different foraging strategies (Objective C). While this requires substantiating, if correct it may have implications for control. Poison baits or trapping are undertaken in areas of different pest management and the different foraging strategies of rats may impact the effectiveness of the rat control operation. Where stoats and possums are more common the rats appeared more confident to sit and consume food in-situ, possibly through better developed risk assessment strategies thus they might potentially consume greater quantities of toxic bait at source. Those rats sourced from managed locations, where stoat and possum numbers were reduced, seemed more inclined to carry and consume the food away from the odour source, thus these animals may take small quantities of toxic baits away from the source location to consume in a 'safe' place. Such a strategy might:

1. reduce the quantity of toxin consumed;
2. increase the likelihood that dependent offspring or co-habiting individuals may also share consumption of the toxin;
3. increase the likelihood of toxic material being discarded 'en-route';
4. reduce the bait availability to conspecifics and other target species.

All four of these possibilities have negative implications: 1 & 2 may result in sub-lethal amounts being consumed, leading to either behavioural or physiological resistance developing; 3 may result in the inadvertent poisoning of endemic species in particular, high wastage of toxin, and the increased availability of sub-lethal doses within the environment; and 4 would reduce the effectiveness of the poisoning strategy.

Chapters 4 and 5 discuss the investigatorybehaviour exhibited by the ship rats. In the context of the development of repellents for the protection of vulnerable resources, either ecological or economic, previous research has produced mixed results when predator 'odour' (urine, faeces, anal gland etc) has been applied to crops or locations to deter rodents and other pest species e.g. (Coulston, Stoddart & Crump 1993; Heale & Vanderwolf 1994; Ward, MacDonald

& Doncaster 1997; Borowski 1998; Burwash et al. 1998a; Burwash et al. 1998b; Bramley & Waas 2001; Monclus, Rodel & von Holst 2006). While some of these experiments have demonstrated a degree of avoidance, even in free-living populations, how long-lived this avoidance remains is not always clear. My research suggests that when an animal is familiar with a particular predator, the odour of that predator not only has no long term deterrent properties but may in fact have attractant properties. This raises serious concerns for the development and application of any such deterrent product. However it does raise the possibility that familiar predator odour may act as an attractant or lure for pest control.

In Chapter 6 I tested the attractiveness of stoat odour-tainted traps against non odour-tainted traps and found that adult ship rats were overwhelmingly attracted to the stoat-odour tainted traps, thus as a lure for stoat experienced ship rats, stoat odour may have some potential. How stoat odour would fare against the current favoured lure, peanut-butter is untested. The role of sulphide semio-chemicals should also be considered here, because while such substances have been found to have a repellent effect on herbivores, the effect on omnivores is often that of an attractant (Mason et al. 1994). Thus while a predator cue such as faeces, urine or gland scent may elicit an avoidance response in herbivorous mammals such as rabbit, hare, deer species, sheep and cattle for example (as reviewed by Kats & Dill 1998; Apfelbach et al. 2005), it may have the opposite effect on some omnivorous species, particularly wild, predator-experienced, variants including for example rats, mice or possums.

The majority of the rat research involving response to predator odours, with four notable exceptions (Burwash et al. 1998a; Burwash et al. 1998b; Bramley, Waas & Henderson 2000; Bramley & Waas 2001), has been undertaken on the domesticated variant of the Norway rat. It should be considered that the non-avoidance response I report in ship rats may not represent a generalised rodent response, but is ship rat specific. This would neatly explain why non-avoidance responses have not been reported in the rodent predator-prey literature to date and why ship rats, despite being the smaller species and later arriving have managed to displace Norway rats through much mainland New Zealand forest habitat. While the superior climbing skills of ship rats over Norway rats may be sufficient explanation for this displacement (J. Innes, personal communication, May 2012), if the ship rat also has superior risk assessment skills, manifesting as investigative behaviour, this may have given the extant ship rat populations an even greater competitive advantage when predators, cats and later stoats, began to spread further into the forest habitat.

8.4 FUTURE STUDIES

As is almost inevitable in any major research undertaking, for every question addressed several more are raised, my research has proved no different. The preceding discussion has alluded to several areas in which further research is required, here I outline those that I consider are of most urgency and / or interest.

1/ *Is predator response learned or innate?*

I have suggested that the way in which a prey animal responds to a predation threat has both a genetic (or innate) component, and a learned component. I have suggested that the innate avoidance frequently reported in predator naive laboratory rodents is in effect a default setting which the prey animal is born with, but which in a free-living animal is very rapidly over-ridden or modified by learned responses. Free-living animals must learn risk assessment skills in order to succeed, survive and reproduce, an individual whose over-riding response is to exhibit avoidance behaviour at every cue is unlikely to achieve these, rather they must make behavioural risk / benefit trade-off decisions (Lima & Dill 1989; Lima & Steury 2005). Defensive behaviours such as freezing probably do occur in wild animals, and probably did occur in my study populations, albeit out of camera-shot, however that is likely to be only part of a sequence of responses, which for many of my study animals culminated in a vigorous investigation of the predator odour.

If my hypothesis is correct, then the application of much laboratory rodent research in particular needs to be carefully reconsidered before it is accepted into more generalised predator-prey theory. The extent to which the behavioural repertoire of domesticated rodent variants has been affected by the domestication process is poorly represented in the current literature. Therefore the behavioural implications of the predator naive and domesticated status of most laboratory rodents needs to be carefully considered when interpreting the effects of treatments such as fear inducing cues and parasite infections.

2/ Predator inspection

I have maintained that the most likely explanation for the investigatory response witnessed is a form of predator inspection. I have considered several alternative hypotheses for the behaviour and although I cannot totally exclude some role for either parasite infection or a chemical cue overlap, I have found no convincing evidence in support of either of these hypotheses. If my interpretation is correct, this represents the first report of predator inspection in ship rats in the mainstream literature. Further research is required to confirm this, and to investigate whether it is a behaviour that can be elicited in other rodent species, most notably the wild and domesticated variants of the common laboratory rodent species.

To date predator inspection research has largely focussed upon group living piscean species, thus many of the motivators for the behaviour focus upon the group living aspect of the species' behaviours. This seems somewhat of a 'chicken and egg' situation – is this behaviour truly derived from group living, or is it easiest seen in group living species, hence the apparent exclusively group living reporting? Many rodents live in colonies, even if they are not obligate colony or group living, thus if group living is a pre-requisite to the behaviour the rodents are 'qualified'. Ship rats appear to exhibit the inspection behaviour in the absence of any obvious audience, thus many of the proposed motivators for predator inspection such as information sharing, reciprocation, mate selection and social status are unlikely. Furthering our understanding of the development and motivators of predator inspection behaviour may be significantly advanced by studying it in rodents, particularly in species such as ship rats that are in many environments solitary.

3/ *Ship rat specific or more generalised behaviour*

Throughout this thesis I have been obliged to draw my comparisons and references from non-ship rat specific research, and whilst this puts my results in a broader research perspective it does leave open the possibility that the reason this investigatorybehaviour has not been previously reported in the rodent, specifically rat, predator-prey literature; is simply because it is a ship rat specific behaviour. Clarifying this detail would be a crucial step in understanding the importance of my research in the broader predator-prey field. If found to be present in, for example wild -captured Norway rats, i.e. generalised beyond the ship rat, the possibility that non-avoidance or predator inspection is more widespread amongst wild rodents at least, requires greater consideration. If it is found to be ship rat specific this may help explain why the ship rat is such a successful pest in many places including New Zealand. Any advances in control methods that utilise this investigatorybehaviour are potentially very ship rat specific: a disadvantage in a system where there are multiple rodent pests requiring management, but a major advantage in systems where there are endemic rodents that require protection, or are non-target species.

4/ *Predator odour as a lure*

I have suggested that while stoat odour does not appear to have any deterrent effects for adult ship rats, it may have a role in the development of an alternative lure to the commonly used peanut butter. I consider that my own experience indicates that peanut butter is more effective in the first instance, although this requires specific testing. Predator odour might make a useful lure in some applications:

- as a 'mop-up' lure after peanut butter has been used in trapping regimes;

- as a non food-based lure, for example where peanut butter cannot be used for health and safety reasons (such as hospitals and schools);
- in situations where a greater specificity of target species is required (although testing for attraction by non-target species would be required).

5/ *Toxoplasma gondii: predator specificity & predator naivity*

The *Toxoplasma gondii* parasite is known to cause behavioural modification in infected rodents in response to cats, reducing their fear and in some cases increasing their locomotion levels. I was unable to ascertain through the published literature several key pieces of information which would have informed my hypothesis. Specifically, there was no data available as to the prevalence of toxoplasmosis in the wild ship rat population in New Zealand, nor in the wild stoat population. There was research indicating that toxoplasmosis does occur in other mustelid species elsewhere in the world (Smith & Frenkel 1995; Frank 2001; Burns et al. 2003; Smielewska-Los & Waldemar 2004; Jones et al. 2006); thus I can only assume it does occur in New Zealand wild stoats although to what extent is mere speculation.

There is much research investigating the effect of cat urine (or other felid odour cues) on infected rodents, but there are very few studies investigating the effect of other predator species cues on infected rodents, and none investigating stoat odours specifically. The few studies that have looked at the response of infected rodents to non-felid predators conclude that the fear behaviour of infected rodents is normal in response to non-felid predators, including a mustelid predator (Vyas et al. 2007; Lamberton, Donnelly & Webster. 2008). This is an area of toxoplasmosis research that appears to require substantiation, especially for stoat : rodent interactions.

Much of the *T. gondii* research to date has been undertaken comparing laboratory and / or predator naive; infected versus uninfected rodents in response to cat cues e.g. (Berdoy, Webster & MacDonald 2000; Gonzalez et al. 2007; Vyas et al. 2007; Webster 2007). If however predator experience causes a non-avoidance behavioural response to develop, how much do the behaviours of infected versus uninfected predator-experienced individuals then differ? I suggest that the profound changes in infected rodent behaviour in comparison to uninfected individuals, may be far less apparent when tested in predator-experienced individuals rather than predator naive individuals. The effect of *T. gondii* infection in wild-captured and wild / laboratory hybrid Norway rats has been investigated under natural conditions and both increased locomotion and decreased cat aversion and neophobia were reported by Webster and colleagues (Webster, Brunton & MacDonald 1994; Berdoy, Webster & MacDonald 1995; Webster 2001; Webster 2007). While this suggests that predator experience may not affect the extent of behavioural change in infected animals, this was not specifically tested in those experiments. The effect of *T. gondii* infection on ship rat behaviour has not been specifically tested either, despite evidence that laboratory mice and laboratory rats exhibit different levels of behavioural modification after infection (Piekarski, Zippelius et al. 1978 as cited in Webster, 2001).

Finally, while the neo-phobic response of the Norway rats was reported to greatly decrease with *T. gondii* infection, I did not witness any strong evidence of neophobia in ship rats throughout my research. The rats readily explored their new environments and consumed new foods when brought into captivity (pers obs.), they readily approached foreign odour (chinchilla) under both captive and field conditions, including foraging in the presence of chinchilla odour in captivity (Chapters 3 and 4). They readily approached foreign objects (teaspoons, pers obs., and snap traps, Chapter 6) introduced to familiar captive and field environments respectively. Thus there are sufficient grounds to suggest that the responses of laboratory variant rodents, or even wild type Norway rats to *T. gondii* infection should not be applied to wild, predator-experienced ship rats without validation. In particular the generality of the behavioural modifications caused by the *T. gondii* parasite in ship rats and the induction of neophobic behaviour need to be critically assessed.

8.5 SUMMARY STATEMENT

The aims of this research were to:

- determine how prey respond behaviourally to a familiar predator odour: I found that contrary to expectation ship rats do not exhibit an avoidance response to stoat or cat odour, but do exhibit investigatory behaviours.
- evaluate the role of predator experience in prey behaviour: I found that neither avoidance nor investigatorybehaviours differed according to predator exposure background, but that ship rats with reduced predator exposure may utilise a ‘carry and consume’ rather than a ‘sit and eat’ foraging strategy.
- test whether captive derived results are applicable in wild populations: I found that my captive enclosure protocol produced results that were replicated under field conditions thus validating the enclosure protocol as a reliable form of captive experimentation, but Y-maze results proved inconsistent and difficult to interpret.

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Chapter 9 APPENDICES

The Reader is referred to the accompanying CD-R for the supporting appendices in full and the supporting image (video and stills) files.

9.1 APPENDIX 1

Maintaining wild caught ship rats (*Rattus rattus*) under captive conditions and generating captive experimental results relevant to free-living animals.

Abstract

Ship rats are a major conservation pest species in New Zealand and despite significant research, their behaviours in the wild remain poorly understood. Behavioural field research into this species can be time-consuming, expensive and offer little in terms of substantive results. An alternative is to carry out captive research; however this approach to ‘choice-type’ behavioural questions seems to rarely generate data which can then be replicated in field research. I aimed to develop an inexpensive and low maintenance method of husbandry and experimentation that would allow wild-caught captive ship rats to exhibit behavioural responses that were genuinely reflective of behaviours exhibited in the wild. I designed and had built an outdoor housing and experimental enclosure in which the animals could be housed individually in modest sized runs that allowed the animals’ social interaction without allowing direct access to each other. Experimental enclosures were designed such that the animals were provided with a three-dimensional habitat enhanced with branches and hides. The experiments were designed in such a way that all observations were obtained via overnight video-recording. Direct handling of the animals was kept to bare minimum by utilising removable ‘nest-boxes’ and providing fresh bedding within runs for the animals to use at will. Preliminary analyses of the enclosure trials generated behaviours highly reflective of observations previously obtained from wild populations. Indications from subsequent field trials showed that the same behaviours observed in the captive populations are exhibited in wild populations. My approach to captive research into ‘choice-type’ behavioural questions for wild caught ship rats has generated behavioural responses that are replicable in wild populations and thus relevant to field management strategies. My low cost, low maintenance approach could easily be replicated by students and other agency researchers offering a reliable alternative / compliment to costly field research.

9.2 APPENDIX 2

Developing and testing a presentation method for predator 'body odour' under field conditions.

Abstract:

Field conditions often dictate that materials cannot be maintained in optimal conditions. In predator odour experiments the relative efficacy of freshly thawed versus aged thawed odour samples may be a crucial component to the success or interpretation of the experiment. Thus, the efficacy of odour samples stored under pseudo-field conditions was tested against those stored under ideal conditions.

The technique involved the preparation of odour samples (donor predator bedding), storage under freezer conditions and a comparison of freshly thawed samples (ideal conditions) with aged thawed samples (pseudo-field conditions). In a laboratory trial I tested whether predator naive laboratory rats (*Rattus norvegicus*) could be seen to exhibit different behavioural responses when presented with fresh thawed cat odour samples and aged thawed cat odour samples.

The results indicate that in terms of defensive (head-outs and concealment), avoidance, and grooming behaviours, the rats are able to detect and respond to both the fresh thawed and aged thawed treatment odours in an in-differentiable way.

This suggests that the thawing of the predator odour samples in the field should not detrimentally affect the efficacy of the sample. Therefore the responses of the subject prey species (rats) should not be impacted by the aged nature of the samples presented in field trials.

9.3 APPENDIX 3

3.a Leiyan Wang Statistical Modelling

3.b Tian Mao Statistical report

Analysis of Wild Rat Behaviour

Tian Mao

November 9, 2010

Contents

1 Abstract

The study considered rats trapped at Lake Waikaremoana and Taranaki New Zealand, which were used in a experiment to determine their reaction to other animals odor. Several sets of rats were trapped, each set being formed a block in the experiment. The design a repeated-measures split-plot analysis of variance (ANOVA) design. There are four blocks of rats and there are eight rats within each block with measures for each rat for the scent of, chinchilla, stoat, possum and one block (which is the control). The same rat may display different reaction for different treatments. For the dependent variable of interest, which is behavior toward the scents, a repeated-measures split-plot design will partition the variation due to (1) a treatment, (2) sampling blocks, (3) the temporal correlation that may occur among repeated measurements, and (4) the potential interactions among these variables. The aim is to provide with new insights on the responses of rat populations to these scents.

9.4 APPENDIX 4

Toxoplasma Report

December 9, 2010

Attention: Gaylynne Carter

Regarding: Suspected Toxoplasma in Rats.

Samples:
12 Brains submitted: 101, 105, 106, 107, 202, 203, 204, 205, 206, 207, 305, 308

Results:
Apicomplexan PCR: Used to detect Toxoplasma, Neospora, or Sacrocystis in samples. 0/12 rats brain samples were positive in this PCR.

Toxoplasma specific PCR:
0/12 rat brain samples were positive in this PCR.

Toxoplasma nested PCR:
5/12 rat brain samples were weakly positive in this PCR. Positive samples in numbers 106, 107, 203, 204, 308.

Comments:
Although most of these results are negative for Toxoplasma DNA, there is still a possibility that the rats had been infected but have cleared the infection. However, damage to the brain (particularly cysts or foci of gliosis) may still be present and possibly be causing the observed behaviour modifications.

I would suggest the following in order to clear the rats of Toxoplasma infection before ruling out this parasite:

1. Using non-frozen brain tissue, submit it to IVABS pathology for examination. They will be able to detect changes in the tissue which might be consistent with current or past Toxoplasma infection.

2. Serology testing on living animals or heart blood of freshly dead rats. Even if the pathology is negative for Toxoplasma, positive serology would rule out any previous exposure.

Please don't hesitate to contact us if you have any further questions.

Kind regards,

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