THE FUNCTIONS AND MECHANISMS OF PREDATOR-ASSOCIATED VOCALISATIONS IN NORTH AMERICAN RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*).

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ABSTRACT

North American red squirrels are a solitary, territorial species that confront various predators. Previous research proposed that squirrels produce predator-specific, referential 'seet' and 'bark' alarm calls to aerial and ground predators, respectively. To test this hypothesis, I examined alarm call production during natural encounters with predators, conspecific intruders and in a series of predator simulation experiments. Call production patterns were consistent across all types of disturbance and involved protracted bouts where both call types were inter-mixed. Hence, the call types were not predator-specific but rather their patterning reflected the persistence of disturbances of any type. Tests of alternative call functions further indicated that calls were not actually directed at conspecifics, but rather at predators and intruders and might function to deter or repel them directly. These outcomes are consistent with life-history details of red squirrels and contradict the proposal that this species produces predator-specific, referential alarm calls.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

1.1 OVERVIEW

Predation is one of the largest components of natural mortality for all animals, and the persistent threat of predation thus exerts a major influence on behaviour. Different species respond to predatory threats in different ways. Some species adopt cryptic strategies (i.e., nocturnal activity cycle, or cryptic behaviour) to reduce detection by predators, while others focus on strategies to better detect the predators themselves and then either elude or confront them (Caro 2005). One of the most common behavioural responses to predators involves the production of loud, conspicuous alarm vocalisations by prey species. Such calls are produced by many species of mammals and birds when they encounter predators and they can function to alert conspecifics (offspring and extended kin) to the danger and prompt appropriate escape responses in them that improves their survival (Sherman 1980; Searcy & Nowicki 2005). They can also communicate to the predator itself that it has been detected and that the probability of a successful hunt is requisitely diminished (Caro 2005).

Antipredator behaviour and communication has thus become a central focus of animal behavioural biology both for the functional insights it offers into adaptive behavioural plasticity and for the mechanistic insights it can provide into how animals perceive, categorise and communicate about the world around them. For example, research on group-living primates and ground squirrels indicates that conspicuous vocal signals function, at least in part, to warn offspring and collateral kin about impending

danger (e.g., Sherman 1977; Cheney & Seyfarth 1981). It also shows that prey species

sometimes evolve multiple, acoustically-distinct types of calls that may communicate

different, informationally-specific messages to listeners either about the category of

predator faced (e.g., a raptor hunting from above, or a terrestrial predator approaching on

the ground), or about the imminency of the danger and thus the urgency of escape that is

required (Owings & Hennessy 1984; MacWhirter 1992; Manser 2001). Alarm calls like

this that communicate additional specific information about predator encounters might

facilitate even more adaptive responding by listeners. They also provide some insight into

the salient dimensions of the external environment along which prey species recognise

important distinctions.

North American red squirrels, *Tamiasciurus hudsonicus*, are also reported to

produce acoustically distinct alarm vocalisations to different types of predators, namely

aerial predators such as goshawks and owls, and terrestrial predators such as coyotes and

pine martens (Smith 1968, 1978; Embry 1970; Nodler 1973; Lair 1990; Greene &

Meagher 1998). Hence, they are potentially similar to some other species of primates, as

well as some other birds and mammals, for which predator-specific, referential alarm

signals have been reported that appear to promote discrete adaptive responding to

different predators (e.g., Seyfarth et al. 1980). In the case of red squirrels, however, the

same alarm vocalisations have been reported to be produced also in non-predator contexts

such as when a squirrel encounters a neighbouring squirrel intruding on its territory, or

when an entirely non-threatening species, such as a white-tailed deer or a raven, is

encountered (Smith 1968; Embry 1970; Price et al. 1990). This kind of variation in the

contexts eliciting alarm vocalisations raises some question about the potential specificity of the messages that the calls might convey in any of the contexts in which they are used.

At the same time, there are some important differences in the socioecology of red squirrels compared to the primate and other mammalian species previously reported to produce predator-specific, referential alarm messages. For example, while these other primates and mammals are often relatively social species surrounded by a variety of direct and collateral kin, red squirrels are solitary and highly territorial with comparatively little social engagement with conspecifics. As a result, it is not entirely clear that their alarm vocalisations are even directed at, or for the benefit of, other squirrels. So, it is not entirely clear then what might be the value of discrete, predator-specific vocalisations in red squirrels. Taken together, these observations suggest that the predator-associated vocalisations of red squirrels might function somewhat differently than the alarm vocalisations of some other species and that the circumstances motivating calling may also differ.

To address these ambiguities in what is one of the most conspicuous features of red squirrel behaviour, I report here a systematic programme of research on the predator-associated vocalisations and behaviours in this species. Research entailed naturalistic behavioural observations, and recording and analysis of vocalisations produced during encounters with predators, with other non-predatory species, and with conspecific territorial intruders. I also report a series of experiments to examine further the squirrels' predator-associated vocalisations and behaviour in controlled encounters with different

simulated predators. Finally, I report the results of an experiment assessing squirrels'

responses to hearing the different species-specific alarm vocalisations produced by other

squirrels during encounters with predators. The goals of this work were to test alternative

hypotheses arising from existing theory about both the functions of alarm vocalisations

and about the mechanistic factors that motivate their production.

1.2 THEORETICAL BACKGROUND

1.2.1 The Functions of Alarm Signals

Alarm vocalisations tend to be among the loudest and most conspicuous calls in species'

vocal repertories. As a result, they represent a potentially risky behaviour, because

producing loud, conspicuous calls when a predator is detected almost certainly draws the

predator's attention towards the caller itself. Therefore, those who call (as opposed to

those who might remain silent) almost certainly increase their own risk of being preyed

upon. In fact, among Belding's ground squirrels, individuals that produced alarm calls

were more likely to be attacked than those who did not produce calls (Sherman 1977).

Nevertheless, alarm calling is very common among birds and mammals; therefore it

seems there must be some important benefits to calling that more than compensate for

these costs. It is critical to ask, then, "What does an individual gain by producing

conspicuous predator-associated calls?"

Individuals that live in groups composed primarily of closely related kin might

acquire inclusive fitness benefits from warning and potentially protecting relatives from

predators (Hamilton 1964; Sherman 1980). If true, individuals with kin nearby should

call more than those without. In fact, research on several mammalian species supports this prediction (Da Silva et al. 2002; Blumstein 2007; Wheeler 2008). For example, among thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*), adult females with offspring and collateral kin nearby produce more alarm calls to terrestrial predators than do adult males with fewer direct and collateral kin nearby. Importantly, alarm calling is correlated with increased survival of offspring and close relatives (Schwagmeyer 1980). Similar results have been reported in black-tailed prairie dogs (*Cynomys ludovicianus*) and Columbian ground squirrels (*Spermophilus columbianus)*, where parous or breeding females that are surrounded by kin produce alarm calls at higher rates than do males or non-parous females (Hoogland 1983; MacWhirter 1992).

Alarm calling to warn kin may occur more widely. For example, among nonhuman primates, vervet monkeys are noted for producing a variety of conspicuous alarm vocalisations when they detect predators nearby. Like female ground squirrels, female vervet monkeys are also the philopatric sex; hence, groups of vervet monkeys are comprised of related females (and their offspring) and unrelated, immigrant males (Cheney & Seyfarth 1990). Female vervets also tend to maintain close social bonds with their female relatives, while males are socially peripheral and interact predominantly only with adult females in estrus and with their own offspring (Struhsaker 1971; Cheney & Seyfarth 1990). If the alarm calls of vervet monkeys function to warn kin, as they seem to do in ground squirrels, adult female vervet monkeys should produce calls at higher rates than do males. Results indicate that, in fact, there is no difference in the calling rates of males and females (Cheney & Seyfarth 1981). However, high-ranking females with

more kin in the group produce more calls than lower-ranking females with fewer kin. Furthermore, high-ranking males with more offspring were also found to produce more calls than low-ranking males with fewer offspring (Cheney & Seyfarth 1981). Although these results indicate that alarm calls are produced by both males and females in vervet monkeys, they nevertheless suggest that the production of alarm calls during predator encounters may have evolved through the kin-selected benefits associated with warning relatives about potential dangers.

Warning kin is not the only possible selected benefit associated with producing alarm calls, however. When survival of a mate is important to an individual's fitness, individuals may call to warn mates (Witkin & Fricken 1979; Krams et al. 2006). For example, Carolina wrens form pair bonds throughout the year and these pair-bond relationships are critical in maintaining and defending a breeding territory (Morton & Shalter 1977). When a female loses a mate, she can no longer maintain the territory and is quickly driven out by another pair. Because a female cannot defend a territory alone, it might benefit her to protect her mate from potential predators by producing alarm calls. In fact, results using a tethered hawk confirmed that female Carolina wrens produce significantly more 'chirt' calls than do males (Morton & Shalter 1977). In contrast, in great tits (*Parus major*) it seems to be the males who produce alarm calls to warn mates. Males produce alarm calls both within and outside their breeding ranges if a female is nearby, but do not call when alone. This pattern suggests that male alarm calls function to warn potential mates of predators (Krams et al. 2006). Warning mates is proposed primarily for pair-bonded species; however, if the fitness of the signaller relies on the

6

presence or continuing survival of a previous mate, then solitary individuals may also benefit by producing alarm calls to warn mates in the wider area.

It is also possible that alarm calling serves other, more diffuse, social functions. For example, among solitary, territorial species, alarm calling may represent a form of 'cooperation' among neighbours (Smith 1986). After establishing territorial relationships, often through protracted aggressive interactions, individuals in many territorial species subsequently respond less aggressively to intrusions by neighbours than to intrusions by strangers, a phenomenon that has been termed the 'dear-enemy' effect (Fisher 1954; Ydenberg et al. 1988). In many cases, the phenomenon appears to hinge on individually distinctive vocalisations that allow for vocal recognition of neighbours (and discrimination of strangers) at a distance. Individually distinctive territorial vocalisations and the 'dear-enemy' effect have been well documented in many bird species (for review see, Stoddard 1996). For example, individual Carolina wrens (*Thryothorus ludoviciaus*) can reliably discriminate the territory advertisement calls of neighbours from those of strangers. Playback experiments also indicate that individual discrimination of these calls results in reduced aggressive responses to the calls of neighbours compared to those of strangers (Hyman 2005).

The 'dear enemy' effect has been explored predominantly in territorial bird species. However, some mammal species are also more tolerant of neighbours than strangers. For example, male deer mice (*Peromyscus maniculatus*) fight significantly less with familiar male neighbours than with new or unfamiliar neighbours (Healy 1967).

Banner-tailed kangaroo rats (*Dipodomys spectabilis*) also tend to tolerate territorial neighbours more than strangers. Neighbour pairs were observed to interact more peaceably than were stranger pairs (Randall 1989).

Even more relevant to the present thesis is research conducted on neighbour interactions in red squirrels (*Tamiasciurus hudsonicus*). Results of a playback experiment indicate that individuals produce more intense responses to playback of the species-specific territory advertisement call (termed a 'rattle') when produced by strangers than when produced by familiar neighbours. This pattern of reduced aggressive response to established neighbours suggests that there may be benefits to solitary squirrels that tolerate and maintain relationships with current neighbours (Price et al. 1990). Specifically, such tolerance in the context of an established territorial relationship may be less costly than re-establishing territorial relationships with new neighbours (Healy 1967; Randall 1989). If true, then there might be a benefit to producing alarm calls that could warn an existing neighbour of predatory threats, thereby preserving established territorial relationships and avoiding the time, energy, and aggression required to establish a new relationship with the squirrel that would inevitably enter to replace it.

Alarm signals can function as warnings to kin, mates or neighbours, but it's also possible that these signals can function to manipulate and potentially deter predators themselves (Woodland et al. 1980; Hasson 1991; Caro 1995; Blumstein 2007). Somewhat counterintuitively, prey species sometimes produce conspicuous vocal or visual signals that actually draw a predator's attention towards them. For example, when

they spot a predator, Thomson's gazelles sometimes stop and make repeated vertical jumps in the air (a phenomenon called 'stotting') rather than run directly away from the predator (Caro 1986). This conspicuous stotting almost certainly draws the attention of the predator; however, that result may in fact be commensurate with the selected function of the behaviour if the stotting effectively announces to the predator that it has been detected and may not succeed in preying on a gazelle who can afford to squander time and energy stotting rather than running away. In other words, the stotting may effectively signal to the predator the gazelle's high quality and athletic ability that would allow it to escape easily if pursued. Caro (1986) has shown that, in fact, cheetahs preying on gazelle are more likely to chase and kill those individuals that do not stot compared to those that do.

The majority of research conducted on predator deterrence has focused on the conspicuous signals produced by ungulate species such as gazelles (Caro 1986, 1995; Tilson & Norton 1981). However, primates also produce loud alarm calls that may function to deter predators, particularly those that rely on stealth (Zuberbühler et al. 1997, 1999). For example, research on six different species of monkey in the Tai forest, Ivory Coast, shows that the monkeys are more likely to produce conspicuous alarm calls to predators, like the leopard, that rely on stealth (or ambush) to capture prey, and remain silent when they detect pursuit predators (e.g., chimpanzees) that are comparatively undeterred by having been detected and instead rely on outpacing their prey. Furthermore, through observations of a radio-collared leopard, Zuberbühler et al. (1999) have shown that the alarm calls of the monkeys do have an effect on the predator;

following natural vocalisations by the monkeys, or experimental playback of their calls, a radio-collared leopard was more likely to give up its hunt and leave the area.

1.2.2 The Mechanisms Underlying Alarm Signals

An additional important focus of research on alarm signalling behaviour in animals concerns the underlying mechanisms that motivate the production of these conspicuous signals and the associated issue of the potential messages that alarm signals convey to listeners. Here, a long-standing view is that alarm vocalisations often reflect the underlying emotional or motivational state (e.g., fear) of the individual encountering a predator (Morton 1977, 1982). Morton (1977) developed this view into a broader theoretical framework that relates the detailed acoustic structure of signals given in a variety of circumstances – including encounters with predators – to the different states of arousal or motivation that they reflect, what he termed 'Motivation-Structural rules'. Morton (1977) predicted, for example, that harsh, low-frequency sounds indicate aggressive motivations, while tonal, high-frequency sounds indicate appeasement or fear. Additionally, if a signal rises in frequency, whether it is harsh or tonal, it represents a decrease in hostility and an increase in appeasement or fear; while a signal that decreases in frequency represents an increase in hostility. Morton also proposed that most calls are structurally graded and are used in a variety of circumstances such that the specific message content of any particular signal can only be reliably interpreted with the aid of additional contextual information (Morton 1982; see also Smith 1977).

There is general support for these Motivation-Structural rules of vocal communication. However, at the same time, additional research has demonstrated that, at least for some species, alarm vocalisations may also convey more specific information about other details of predator encounters (Marler 1985; Hauser 1996). For example, the structurally distinct alarm signals produced by vervet monkeys appear to track the category of predator encountered (i.e., eagle, leopard, snake). Given their apparent predator-specificity, such calls have been labeled 'referential' because they appear to function as rudimentary symbols of the predators themselves akin to our human words for these animals. In order for a vocalisation to be considered a referential signal, it needs to meet two specific criteria (Evans 1997): 1. it must be produced only in circumscribed contexts, such as in encounters with a specific category of predator, and; 2. it's effect on listeners must be context-independent in that the call alone must allow listeners to engage in functionally appropriate responses without the need for additional disambiguating contextual information (Marler et al. 1992; Macedonia & Evans 1993).

A number of primate and other mammalian taxa have been reported to produce alarm signals that meet these criteria for functional reference (Seyfarth et al. 1980; Greene & Meagher 1998; Manser 2001; Zuberbühler 2001; Digweed et al. 2005; Kirchlof & Hammerschmidt 2006). The best-documented example comes from the alarm calls of vervet monkeys (Struhsaker 1967; Seyfarth et al. 1980; Cheney & Seyfarth 1990). Vervet monkeys are small-bodied monkeys that tend to move both on the ground and in the trees and are thus exposed to a wide variety of predators, including large cats, raptorial birds, and snakes. Observational and experimental research has shown that vervet monkeys

produce three acoustically distinct alarm calls to these three different classes of predator, and that each predator induces a distinct behavioural response that is appropriate to it (Seyfarth et al. 1980). For example, upon spotting a leopard, vervets give one type of alarm call and immediately scramble up a tree and out onto its terminal branches where leopards are too large to go. On spotting an aerial predator, the monkeys give a different alarm call and immediately drop out of the tree where they are most vulnerable to raptors. On encountering a snake, the monkeys give a third alarm call type and stand bipedally to scan the ground around them. Importantly, animals that hear an alarm call engage the appropriate response even if they themselves have not actually seen the predator. Hence, the calls seem to 'refer to' or 'symbolise' the predators in a way at least crudely similar to our own words for these animals (Seyfarth et al. 1980).

Subsequent research has documented a similar system of acoustically distinct, predator-specific alarm calls in a handful of other primate species including ring-tailed lemurs, Diana monkeys, and Campbell's monkeys (Macedonia 1990; Zuberbühler 2000, Zuberbühler 2001). The word-like properties of such alarm calls have inspired considerable research to explore the selective pressures favouring the evolution of referential signalling systems (for review see Evans 1997; Zuberbühler 2003). Some hypotheses stress the importance of a social organisation comprised of cohesive kin groups, others stress a threshold of cognitive ability that might be required to represent objects or events in the world conceptually, and still others stress specific ecological factors such as the diversity of predators faced and the associated diversity of responses that the habitat provides (Macedonia & Evans 1993; Evans 1997). The primate species in

12

which referential signals have been so far documented are characterised by all three traits, which has raised the possibility that word-like systems of referential communication may arise primarily only in cognitively complex, social species that face a diversity of predator types in a structurally complex habitat that also permits multiple escape responses.

However, this possibility appears to be contradicted by the behaviour of domestic chickens that also produce two acoustically distinct alarm calls, one for ground predators and one for aerial predators (Evans et al. 1993). Upon seeing an aerial predator, or hearing an aerial predator alarm call, a chicken will turn an eye to the sky (as though searching for it) and/or crouch and run for cover. In contrast, when they spot a ground predator (e.g., raccoon) or hear the ground predator alarm call, chickens assume an erect vigilant posture as the prelude to rapid escape (running or flying away). Systematic experiments revealed that caged chickens produce an aerial alarm call when videos of eagles are shown from monitors positioned above their cages and produce terrestrial alarm calls to videotape of raccoons on a monitor positioned at ground level (Evans et al. 1993). Playback experiments revealed that the same responses were elicited by playback of the alarm calls in the absence of any associated predator or model of it, suggesting that the behavioural responses do not require additional contextual information. As a result, these alarm calls of chickens appear to be functionally referential signals.

However, further experiments revealed that when video of a raccoon was presented from a monitor above the cage, the chickens produced the aerial alarm call and

crouched low to the ground, behaviour more appropriate to raptors flying overhead

(Evans & Marler 1995). Similarly, when video of an eagle was played from a monitor

positioned on the ground, the chickens gave the terrestrial predator alarm call and stood

erect and vigilant, behaviour more appropriate to terrestrial predators encountered on the

ground (Evans & Marler 1995). These findings suggest that the alarm calls and

associated behavioural responses of chickens might be driven more by the position of the

predator (in the air, or on the ground) than by the class of predator *per se*. Nevertheless,

they do seem to put the lie to the hypothesis that predator-specific, or referential, alarm

communication necessarily hinges on the kind of intense sociality and complex cognitive

abilities exemplified only by primates.

At the same time, research on other primate and mammal species suggests that

alarm calls do not necessarily represent the predators at all, but instead reflect the relative

threat involved or the urgency of the response that is required (Manser 2001; Le Roux et

al. 2001; Fichtel & Kappeler 2002; Randall & Rogovin 2002). One of the clearest

documented examples of these types of calls comes from California ground squirrels,

Spermophilus beecheyi. Ground squirrels were found to produce 'whistle' vocalisations

primarily in response to large raptors and 'chatter' vocalisations primarily in response to

terrestrial predators (Owings & Virginia 1978). On the surface, then, the calls appeared to

represent the different predator types much the way the alarm vocalisations of vervet

monkeys represent the different predators they encounter. However, additional research

on California ground squirrels revealed additional important variation.

Ground squirrels typically produce a broadband 'chatter' vocalisation when they detect terrestrial predators at a distance and then stand bipedally to monitor the predator's movements to see if they need to escape to their burrows. In contrast, when they spot an aerial predator, typically only at close range and stooping, the squirrels produce a high-frequency, tonal 'whistle' and bolt immediately for their burrows (Owings & Virginia 1978). However, when a terrestrial predator is detected only at the last minute and at close range, the ground squirrels emit the tonal whistle and run immediately for their burrows; whereas, when a raptor is spotted in the distance, they emit the chatter call and remain vigilant to see what the raptor will do (Owings & Leger 1980). As a result, although the whistle and chatter alarm calls correlate with the distinction between aerial and terrestrial predators, this correlation seems to reflect a coincidence in how these different predators are typically encountered because, ultimately, the production of the calls is most sensitive to the immediacy of the risk and therefore the urgency of response that is required independent of the type of predator involved (Leger et al. 1980; Owings & Leger 1980; Owings & Hennessy 1984).

Alarm call systems in other species appear to reflect neither strictly referential categorisations of predator types nor response urgency. For example, white-faced capuchin monkeys (*Cebus capucinus*) of Central America produce two subtly distinct alarm calls, one given relatively exclusively to aerial predators and the other given to terrestrial predators but also produced in a range of other circumstances as well (Digweed et al. 2005). The loud bark call used in terrestrial predator encounters is sometimes also given to non-predatory species and can be used in broader mobbing contexts, such as

when confronting and surrounding potential food competitors or species on which the capuchins themselves prey. It appears then that capuchins have a mixed system of alarm calling, with one call given fairly specifically to aerial predators and a subtly different call given in a wider range of contexts that involve threat or aggression on the part of the monkeys (Digweed et al. 2005).

Such mixed alarm call systems are not without precedent in primates. Paralleling the capuchins, saddleback tamarins (*Saguinus fuscicollis*) use the same call that they produce to terrestrial predators in other assertive contexts such as inter-group encounters (Kirchhof & Hammerschmidt 2006). Similarly, white sifakas (*Propithecus verreauxi*), a prosimian primate from Madagascar, also display a mixed alarm call system (Fichtel & Kappeler 2002). Like capuchins, sifaka produce one call specifically for aerial predators and another for terrestrial predators and in other situations of high arousal, including aggressive interactions with conspecifics.

These mixed systems of calling may highlight other important ecological or social distinctions among species. For example, both the white sifaka and saddleback tamarin are almost exclusively arboreal and so, by definition, may have a more limited range of escape options and thus a less focused need for multiple predator-specific alarm messages (Fichtel & Kappeler 2002; Kirchlof & Hammerschmidt 2006). White-faced capuchins are semi-arboreal and semi-terrestrial and so, like vervet monkeys, have a diversity of possible escape responses to the various predators that prey on them. However, unlike vervet monkeys, capuchins are, as a species, more assertive and pugnacious generally.

They are known to prey on other small mammals (e.g., coati pups) and birds; and they

aggressively confront other fruit competitors (e.g., peccary). They also sometimes

confront and mob potential predators like tayra and some snake species. Hence, their

approach to many species may be more confrontational generally. As a result, they may

have evolved a mixed alarm call system that can alert conspecifics to some specific

dangers (e.g., stooping raptor) but that can also function more generally to attract others

attention to the source of a disturbance, whether it is a predator or a competitor, or even a

potential prey animal, to facilitate active engagement or mobbing (Digweed et al. 2005).

In sum, there are a variety of possible functions for the conspicuous alarm

vocalisations produced by prey species encountering predators and a similar variety of

possible underlying mechanisms that motivate calling, and there is, as yet, no clear

framework for predicting which will apply in a given species. The research reported in

this thesis is designed to contribute to this literature by systematically testing the

applicability of alternative functional and mechanistic hypotheses for alarm calling in

North American red squirrels and thereby providing additional useful data for theorising

about the selective factors influencing species diversity in anti-predator behaviour.

1.2.3 Alarm Signals in Red Squirrels

Red squirrels (*Tamiasciurus hudsonicus*) are an ideal species in which to test the various

functional and mechanistic hypotheses for alarm calling behaviour outlined above.

Although there have been only a handful of studies on the vocal communication of red

squirrels (Smith 1968; 1978; Embry 1970; Nodler 1973; Searing 1977; Lair 1990; Price

et al. 1990; Greene & Meagher 1998), they are a notoriously vocal species well-known for confronting predators, intruders and other squirrels with protracted sequences of loud vocalisations. Early research suggested that the vocalisations produced in such encounters involved a variety of different calls and that these calls were not used in any context-specific fashion (Smith 1978; Searing 1977; Lair 1990). However, a more recent study by Greene & Meagher (1998) has proposed that, in fact, squirrels produce two structurally distinct vocalisations – a tonal 'seet' call and a broadband noisy 'bark' call – in response to aerial and terrestrial predators, respectively. This report raises the possibility that red squirrels, like some species of primate, and some other mammals and birds, might also manifest a system of predator-specific alarm calls.

On the surface, this possibility might seem to be undermined by the fact that red squirrels are a solitary species in the sense that they do not live in cohesive, highly social groups like many of the primates and ground squirrels for which kin-selected, referential alarm calls have been documented. As a result, it is not clear whether the conspicuous alarm vocalisations of red squirrels are even directed at, or would benefit, conspecifics. However, red squirrels do interact regularly with descendant kin (offspring) before they disperse, as well as with neighbours (Gurnell 1987; Price et al. 1990; Price & Boutin 1993), and either or both features of their social organisation might favour alarm calling to warn kin, mates, or neighbours.

Red squirrels are also small-bodied, diurnal mammals that spend time in a variety of locations in their habitat including in the trees and on the ground (Smith 1968; Gurnell

1987). As a result, squirrels are exposed to predatory threat from many species of both ground and aerial predators (e.g., coyote, lynx, marten, owl, hawk and eagle). The forest habitats they occupy also provide a diversity of escape responses, both on and under the ground and in the trees. Hence, several of the critical functional pressures that appear to have selected for differentiated, predator-specific alarm calls in primates and some other taxa are present also in squirrels – namely a diverse predator complement with variable hunting strategies and a variety of escape options (Macedonia & Evans 1993).

At the same time, although red squirrels are rodents and therefore are not as encephalised and perhaps as cognitively sophisticated as many primates are thought to be, they are relatively encephalised for their taxon and more encephalised, for example, than comparably sized terrestrial squirrels (Meier 1983). Furthermore, as the alarm calling behaviour of chickens described above appears to show, cognitive ability, by itself, might not be a necessary (or sufficient) condition for referential communication.

Taken together, this combination of life-history characteristics in red squirrels affords a unique opportunity to separate the possible effects on the evolution of predator-specific referential communication systems due to sociality and cognitive complexity *per se* from those due to specific ecological pressures and general life-history patterns.

1.3 THESIS OUTLINE

In the next chapter (Chapter Two), I report on squirrels' behavioural and vocal responses during natural encounters with predators, non-predatory species and conspecific intruders

as well as in response to predator models presented in a series of controlled experiments. The results of this chapter specifically address the proposal by Greene & Meagher (1998) that red squirrels produce predator-specific, referential alarm vocalisations to different classes of predator that prey on them in a fashion similar to the referentially specific alarm vocalisations previously reported for some species of nonhuman primates and some other mammals and birds.

Chapter Three examines patterns of call production during these natural and simulated predator encounters for evidence in support of one or more of the traditional hypotheses for the function of alarm calling. Specifically, I ask whether the predator-associated vocalisations of red squirrels function as warnings to kin, neighbours, or mates? Based on the results, which do not cleanly support any of these possibilities, I outline an additional possible functional alternative to account for the details of calling in red squirrels.

In Chapter Four, I report the results of acoustic analyses and a playback experiment that together provide additional evidence bearing on the potential functions and mechanisms of red squirrel alarm calls.

Finally, in Chapter Five, I provide a summary of major findings and I entertain some additional novel interpretations of alarm calling in red squirrels that are suggested by results reported in the preceding chapters. I also consider some limitations of the current work and consider some possible future research directions to address them.

CHAPTER TWO

PREDATOR-ASSOCIATED VOCALISATIONS IN NORTH AMERICAN RED SQUIRRELS: ARE ALARM CALLS PREDATOR-SPECIFIC?

2.1 INTRODUCTION

Studies of communication are often thought to offer some insight into the way animals perceive the world around them and the proximate internal mechanisms that support adaptive responses to it (Cheney & Seyfarth 1990; Griffin 1992). Alarm calls have been an especially common and productive focus of this kind of research because the calls are often among the most conspicuous in species' repertoires and they can be connected comparatively unambiguously to discrete and dramatic events in the animals' lives, namely encounters with predators (reviewed in Blumstein 2007). One long-standing interpretation is that alarm calls reflect the underlying state of arousal or motivation experienced by callers when they encounter a predator, and that this motivational information is reflected in, and communicated to listeners through, structural details of the calls that are transmitted. Morton elaborated this interpretation of animal experience into a set of motivation-structural rules to account for structural variation in alarm calls and in other kinds of vocalisation as well (Morton 1977, 1982). For example, harsh low-frequency calls were proposed to reflect a caller's aggressive state, whereas tonal high-frequency calls were proposed to reflect fear or appeasement. Motivational states intermediate between aggressive and fearful endpoints yield calls with intermediate acoustic structures (Morton 1982). This framework emphasises an animal's emotional

engagement with events in the world both as the mechanistic catalyst to vocal production and as the content of the vocal messages that are thus transmitted to others.

An important shift in interpretations of animal communication was precipitated by landmark studies of the alarm vocalisations of vervet monkeys (Seyfarth et al. 1980). Vervet monkeys are small terrestrial monkeys subject to heavy predation by a variety of predators, including large raptors, cats, and snakes. Seyfarth et al. (1980) documented a small repertoire of discrete alarm calls that were produced quite specifically in response to these different predator classes and the calls alone were sufficient to induce functionally discrete escape responses in listeners. In combination, the predator-specific production of alarm calls and listeners' discrete responses to them suggested a capacity for language-like referential communication. While this framework does not exclude emotional processes, it does highlight the importance of some additional perceptual or evaluative categorisation of predators in both the mechanistic processes that influence signalling and in the content of vocal messages that are then transmitted to listeners (Evans 1997).

Subsequent research has confirmed the potential for categorical classifications of predators in some other mammalian and bird species (e.g., chickens: Evans & Marler 1997; Diana monkeys: Zuberbühler 2000; meerkats: Manser 2001; chickadees: Templeton et al. 2005; tamarins: Kirchlof & Hammerschmidt 2006). At the same time, studies of an equal variety of species have shown that what is most salient about predator encounters is not always the identity of the predator *per se* but other dimensions of such

encounters such as the imminency of the threat that it represents or the urgency of the response that is required (e.g., Blumstein 1995; Mateo 1996; Mateo et al. 1997; Le Roux et al. 2001; Fichtel & Kappeler 2002; Randall & Rogovin 2002; Digweed et al. 2005). For example, California ground squirrels (*Spermophilus beecheyi*) generally produce one kind of alarm call (the 'whistle') when they encounter large raptors and a structurally different alarm call (the 'chatter') when they encounter terrestrial predators (Owings & Virginia 1978). Production of the two call variants maps closely onto the categorical distinction between aerial and terrestrial predators. However, the apparent predator-specificity of the alarm calls proves to be a coincidence of how squirrels typically encounter aerial versus terrestrial predators because production of the two alarm calls actually tracks variation in the imminence of the threat represented by either class of predator and thus how urgently vulnerable squirrels must respond to them (Leger et al. 1980; Owings & Leger 1980). Functionally, an alarm call system like this based on a distinction in response urgency rather than predator class makes sense for ground squirrels which are a ground-dwelling species with comparatively few different escape options.

North American red squirrels (*Tamiasciurus hudsonicus*) offer an additional natural opportunity to examine potential variety in predator perception and response. Red squirrels are exposed to a diversity of predator types (e.g., coyotes, lynx, marten, owls, hawks) and the forest environments they inhabit offer a diversity of escape responses from those predators. There have been relatively few studies on the vocal communication of red squirrels (Smith 1968, 1978; Embry 1970; Searing 1977; Price et al. 1990; Lair

1990; Greene & Meagher 1998). However, they are widely known for being extremely vocal and for confronting predators and other intruders with conspicuous vocalisations. One experimental study reported that red squirrels produce one kind of vocalisation (a tonal 'seet') in response to aerial threats and a structurally distinct vocalisation (a broadband 'bark') in response to terrestrial threats (Greene & Meagher 1998), suggesting that, like vervet monkeys, red squirrels might also manifest a system of acoustically distinct, referential alarm calls based on some discrete perception and categorisation of predator types. At the same time, other studies of red squirrels have described the production of these call types in other, non-predatory contexts (e.g., deer, raven; Smith 1978; Lair 1990) suggesting that the calls might not be entirely predator-specific.

In this chapter, I report results of a multi-year study of red squirrels to address these ambiguities in the predator-specificity of this species' alarm call production. In the first part of the chapter, I report patterns of behaviour and alarm call production during natural encounters with predators. In the second part, I report a series of follow-up experiments that probe the predator-specific production of alarm calls more systematically. Finally, the third part of the chapter reports on patterns of production of the same calls during other, non-predatory disturbances.

PART I. NATURAL PREDATOR ENCOUNTERS

2.2 METHODS

2.2.1 Study Site and Subjects

Research was conducted at the R.B. Miller Field Station in the Sheep River Valley of Kananaskis Provincial Park, Alberta (50°39′ N, 114°39′ W), which is situated in the foothills of the Canadian Rockies. The habitat in the Sheep River Valley is a mix of aspen (*Populus tremuloides)* parkland and montane (sub-alpine) coniferous forest composed primarily of lodgepole pine (*Pinus contorta*) and white-spruce (*Picea glauca*). Red squirrels are more common in the latter forest types in North America where they hoard and feed on the seeds of conifers (Smith 1968; Hurly & Lourie 1997). Research focused on a population of 47 individually marked squirrels in a single, contiguous forest patch approximately 60 ha in size. Each individual maintained an exclusive territory (0.5 – 1.0 ha) containing at least one central midden with a supply of stored cones, which was actively defended against intruders. In order to provide positive individual identifications, each squirrel was uniquely marked. To apply unique marks, each squirrel was captured in its territory using a live-trap baited with peanut butter (Tomahawk Live Trap Company, Wisconsin). Once captured, the squirrel was transferred to a funnel-shaped cloth handling bag and was weighed on a spring scale and examined for general health. While in the bag, a unique dye mark was applied through a central mesh portion. Dye marks were visible at distances up to 20 meters facilitating positive identification under most conditions. The squirrel was then shunted to the funnel end of the cloth bag where their head region was exposed to allow a small numbered tag to be placed in one or both ears

(National Band and Tag Company, Kentucky). The placement of the ear tags also facilitated recognition of marked animals at a distance and the unique numbers on the tags allowed certain identification across seasons and across years when animals were re-trapped. These trapping and handling techniques and the research protocols explained below were approved by the Animal Welfare Committee of the University of Lethbridge (Protocol #0809) and by Alberta Sustainable Resource Development, Fish and Wildlife Division (Research Permit GP 30031; Collection License CN 30046).

2.2.2 The Predator Community

Kananaskis Provincial Park is part of a network of protected foothills and mountain habitats that extends virtually uninterrupted from Yellowstone National Park, Wyoming (USA) in the south to the Yukon Territory (Canada) in the north. This network preserves a largely pristine montane ecosystem with continuous natural habitat and migratory corridors for many species, including many predators. In the Kananaskis study area, the predator community contains various raptors that could prey on red squirrels, including large eagles (golden eagle and bald eagle), broad-winged hawks (red-tailed hawk), agile forest accipters (sharp-shinned hawk, Cooper's hawk, northern goshawk), falcons (prairie falcon) and large owls (great horned owl, great grey owl). It also includes several mammals that are frequent or occasional predators on red squirrels including grizzly and black bears, bobcats, cougars, pine martens, weasels, and coyotes. During the study, all of these species were confirmed in the study area, although I did not witness squirrels encountering every one of these species during focal observations.

2.2.3 Data Collection and Analyses

Research was conducted in three consecutive years (2005-2007) primarily between May

and November, representing the late spring, summer, and fall seasons at this latitude.

Data collection focused on all natural encounters with known predators. For each

encounter, I noted the species involved and details of the squirrel's response to it which

included either freezing and remaining immobile, retreating up a tree or into their

midden, or approaching and inspecting from a safe distance. In addition, a continuous

recording was made of all types of vocalisations produced from which tallies were later

constructed for each call type. Vocalisations were recorded using a digital Marantz

PMD660 recorder and a Sennheiser ME66 shotgun microphone with a K6 powering

module and a Sennheiser MZH60-1 windscreen. The data available for analysis thus

consisted of squirrels' behavioural responses to specific classes of predators as well as

the number and types of vocalisations they produced in encounters with them.

2.3 RESULTS

2.3.1 General Responses to Predators

I witnessed 21 cases where a squirrel encountered a known predator (Table 2.1). This

sample involved 16 different squirrels and entailed seven encounters with coyotes (*Canis

latrans*), six encounters with great grey owls (*Strix nebulosa*), four encounters with

northern goshawks (*Accipiter gentilis*), and four encounters with pine marten (*Martes

americana*). In each case, the squirrels who spotted a predator from a position in a tree

tended to respond by immediately stopping their current activity and freezing

momentarily while oriented toward the predator. They then typically remained in the

same position staring at the predator and, after a short interval, began to stomp their hind feet and flick their tail back-and-forth over their back while producing a steady stream of vocalisations; or they first scurried a short distance up or down the tree they were in before stopping, orienting again toward the predator, and then stomping their feet, flicking their tail and vocalising. Alternatively, if the squirrel was on the ground to begin with, it tended to bolt immediately to the nearest tree, climb to a safe height and then commenced foot-stomping, tail-flicking and vocalising. In each encounter, squirrels produced vocalisations in extended bouts, which could be quite protracted, lasting up to 11 minutes ($X = 65.35$ seconds), involving hundreds of calls, and sometimes continuing well after the predator had left the area. In all cases, vocal bouts were composed of a mix of two different call types, a tonal 'seet' call and a composite 'seet-bark' call composed of a tonal seet component appended to a broadband 'bark' call. Spectrograms of the two call types are shown in Figure 2.1.

I observed an additional 10 cases where squirrels encountered another species that would seem to pose no predatory threat to them but to which the squirrels responded as though they did (Table 2.1). That is, squirrels showed behavioural responses and produced bouts of vocalisations that paralleled their reactions to confirmed predators. This sample involved eight different squirrels and entailed six encounters with ravens (*Corvus corax*), three with white-tailed deer (*Odocoileus virginianus*), and one with a short-tailed weasel (*Mustela erminea*). Although none of these species are reported to be predators on squirrels, it is certainly possible that weasels, who can easily access underground burrows and arboreal nests, might represent a threat to infant squirrels. It is

also possible that ravens pose some threat to infant squirrels in the nest, or that they could

be mistaken for a raptor when flying overhead. It is unlikely that deer pose any real threat

to squirrels but it is possible that they too could be mistaken for a mammalian predator if

spotted at a distance and moving through the forest.

This sample of natural disturbances was analysed for evidence that the squirrels'

behavioural or vocal responses differed as a function of the species encountered as would

be predicted by the referential hypothesis.

2.3.2 Predator-Specific Behavioural Responses

The potential association between specific behavioural responses (freeze, move up tree,

move down tree) and specific predator types was tested using contingency analyses.

Because the sample was small when divided among the various different species

encountered, I first collapsed the species encountered into two predator classes: aerial and

terrestrial. The resulting test revealed no significant association between the squirrels'

behavioural response and either of these predator classes (Pearson's Chi-Square: χ_1=1.3,

P=0.51, N=31). Squirrels were as likely to move up or down the tree they were in, or to

remain immobile in a tree, after detecting a predator whether that predator represented an

aerial or a terrestrial treat. I then eliminated from the sample encounters with seemingly

non-predatory species and limited the test to only known predators. This precaution did

not change the outcome. There was still no association between behavioural response and

predator type (χ_2=1.55, P=0.45, N=21). Finally, I broke the terrestrial predator category

into two different categories, one for coyotes and one for marten, in case my original

lumping of these two predators into a single category served to blur important variation in the squirrels' responses to them. Marten are certainly distinct from coyote in the sense that they are highly arboreal and are capable of pursuing squirrels through the trees as well as on the ground. However, this re-categorisation of predator types did not change the outcome. There was still no significant variation in the squirrels' behavioural responses to coyote, marten or aerial predators (χ_4=6.49, *P*=0.16, N=21).

2.3.3 Predator-Specific Vocal Responses

The manifest mixing of both seet and seet-barks within all bouts of calling indicated from the outset that there was no strict association between call type and predator class. Nevertheless, one or other call type might still predominate in encounters with particular predators. To test this possibility, the relative production of seets and seet-barks to aerial and terrestrial predators was tested using a Repeated Measures Analysis of Variance (rmANOVA) with both call type and predator type as within subject factors. Because the duration of calling bouts varied tremendously, I focused these and subsequent tests on the squirrels call production in the first 30-seconds of their encounters with a predator. The first test compared call production in response to the two predator categories of aerial and terrestrial, where both threatening and non-threatening species were included. Results indicated a main effect for call type (ANOVA: $F_{1,16}$=14.9, *P*=0.0014, N=31) with more seet-barks than seets produced overall. However, there was no main effect of predator type ($F_{1,16}$=0.90, *P*=0.355), nor was their a significant interaction term ($F_{1,16}$=1.21, *P*=0.288).

In a follow-up analysis, I again tested for variation in call production to aerial and terrestrial predators but after restricting the sample to known predators so that I could be sure that the non-predators were not blurring potential predator specific usage of the two call types. Results again indicated a main effect for call type ($F_{1,14}=24.61$, $P=0.0002$, N=21), with more seet-barks than seets produced overall. There was no main effect of predator type ($F_{1,14}=0.75$, $P=0.402$) but there was now a significant interaction between call type and predator type ($F_{1,14}=4.84$, $P=0.045$), which reflected greater production of seet-barks to terrestrial predators.

Finally, I again sub-divided the terrestrial predator category into separate categories for coyote and marten. Results based on this re-categorisation of known predators into three classes were similar in that there was again a main effect for call type ($F_{1,13}=32.27$, $P=0.000075$, N=21), with more seet-barks than seets produced overall but still no main effect of predator type ($F_{2,13}=0.35$, $P=0.709$). There was again a significant interaction between call type and predator type ($F_{2,13}=7.40$, $P=0.0071$), which appeared to be driven most by the fact that coyotes elicited more seet-barks and fewer seets by comparison to the other classes of predator (Fig. 2.2).

2.3.4 Call Patterning within Encounters

To examine the additional possibility that the mixed bouts of seets and seet-barks produced in all predator encounters might nevertheless show some predator-specific patterns of internal structuring, I divided the initial 30-seconds of each calling bout into three successive 10-second time bins (i.e., 0-10 seconds; 10-20 seconds; and 20-30

31

seconds). I then quantified the production of seets and seet-barks within each time bin, and used a rmANOVA to test these call tallies for variation as a function of predator type after limiting the data to encounters with known predators. Results indicated a main effect of call type (ANOVA: $F_{1,13}$=34.21, P=0.000057, N=21), with more seet-barks than seets produced overall. There was no main effect of predator type ($F_{2,13}$=0.33 P=0.726), but there was a significant interaction between call type and predator type ($F_{2,13}$=8.04, P=0.0053), with the coyote eliciting more seet-barks and fewer seets compared to the other predators. There was no main effect of time bin ($F_{2,26}$=0.18, P=0.998), but there was an interaction between call type and time bin ($F_{2,26}$=7.39, P=0.00288) with seet calls produced more in the first time bin and seet-barks produced more in the later time bins (Fig. 2.3a). There was no significant three-way interaction between predator type, call type and time bin ($F_{4,26}$=0.73, P=0.576).

I also tested the degree to which calls of each type were clumped together or intermixed with each other during a calling bout. Here, I used contingency analysis to compare the transitional probabilities within and between call types. A separate Pearson's contingency analysis was conducted for each calling bout that contained 10 or more calls of each type to explore the relative clumping of calls within bouts. 76% of calling bouts (16 of 21) showed statistically significant (p-value >0.05) clumping of calls, meaning that seets followed seets and seet-barks followed seet-barks more than would be expected by chance alone (Fig. 2.4a).

2.4 DISCUSSION

Squirrels in my study population in the foothills of the Canadian Rockies were exposed to a full complement of predators and their behavioural and vocal responses to them agreed well with previous descriptive reports for this species (Smith 1968, 1978; Embry 1970; Lair 1990). Thus, squirrels displayed the two broad patterns of response commonly described: the first being to remain silent and immobile initially and to monitor the predator from a safe location; and the other being conversely to confront predators with a stream of loud vocalisations, while stomping their hind feet and flicking their tail over their back.

The more detailed pattern of call production that I observed during predator encounters also agreed in broad outline with that described in a more focused experimental study of alarm calls conducted by Greene & Meagher (1998). In that study, squirrels tended to produce two different calls in encounters with predators, a relatively low amplitude, high-frequency and tonal seet call that resembled in structure the ventriloquial seet alarm vocalisations of many passerine birds; and a broadband bark call that resembled the more localisable calls used by passerine birds when mobbing a predator (Marler 1955). Squirrels in my study likewise produced two different calls during predator encounters, one being the same tonal seet call and the other being a broadband call composed of a combination of a tonal seet note appended to a broadband bark, yielding a composite 'seet-bark' call. Greene & Meagher (1998) also reported use of this combination seet-bark call by their squirrels but it was produced less frequently than either the seet or the bark calls alone. In contrast, although squirrels in my study

population also occasionally produced bark calls by themselves, they did not do so in encounters with predators.

It is possible that this variation in call usage represents a real difference in call production patterns by squirrels in my southern Alberta population compared to those studied by Greene & Meagher (1998) in western Montana. However, it is also possible that the differences are largely definitional. While seets are structurally quite distinct and are easily discriminated from the other two call types spectrographically, the delineation of barks and seet-barks is less straightforward because the structure of these call variants grades continuously. It is possible, therefore, that some of the differences in call production can be attributed simply to a difference in the criteria used to label the broadband call variants (i.e., barks and seet-barks) in the two studies.

At the same time, Greene & Meagher (1998) reported that seets were produced primarily in response to aerial threats and barks were produced primarily in response to terrestrial threats and that this categorical mapping of call types and predator classes was the basis for a system of referential communication about predators. My results were again similar in as much as terrestrial threats (coyotes) also elicited more of the broadband type of call (seet-bark); however, I did not find anything like exclusive use of either the tonal or the broadband call types in encounters with particular classes of predator. Instead, squirrels in my population used the tonal and broadband call types in a far more mixed fashion: both types of call were produced to each of the different classes of predator; and, in fact, most calling bouts to any type of predator were composed of a

mixture of the two call types, with the tonal seet call predominating in the early stages of

a calling about and giving way to the more broadband seet-bark call as a calling bout

continued. Greene & Meagher (1998) do not say whether this kind of mixing of tonal and

broadband call types occurred within single bouts of calling in their study. However, they

do report some mixing of tonal and broadband calls to predators of the same type. For

example, they found that both tonal seets and the more broadband seet-bark calls were

produced to aerial threats, and that, although terrestrial threats elicited primarily bark

calls, they also elicited a small number of seet calls as well (see Fig. 2 in Greene &

Meagher 1998).

Taken together, these two focused studies of alarm call production in red squirrels

are entirely consistent with each other and with previous descriptive reports in finding

that squirrels produce both a tonal seet call and some more broadband call variant (either

a bark or a seet-bark) in response to predators. They are also consistent in finding some

degree of mixing of these two call variants in encounters with at least some types of

predator. Finally, they are consistent as well in finding a bias toward the production of a

more broadband call type in encounters with terrestrial predators, which bias might well

reflect some categorical labeling of predators by the squirrels. Some of the remaining

ambiguities in call type usage and their variable predator-specificity may be largely

definitional as noted above. It is also possible that they reflect qualitative differences in

research design and predator sampling. Whereas the results that I report and that form the

basis of earlier descriptive studies are based on naturalistic predator encounters, the

results reported by Greene & Meagher (1998) are from an experimental study involving

simulated predators. It is possible that this difference in predator exposure influenced squirrels' vocal responses. To address this possibility and try to resolve outstanding ambiguities in the predator-specificity of alarm call production in red squirrels, I also conducted a set of experiments using simulated predators.

PART II. EXPERIMENTS USING SIMULATED PREDATORS

2.5 METHODS

I conducted a series of three experiments involving simulated predators. The first involved using taxidermied models of known predators. The second and third experiments incorporated movement into the predator simulations and replicated as closely as possible the experimental treatments used originally by Greene & Meagher (1998).

2.5.1 Taxidermied Predators

This experiment involved taxidermied models of known predators that were also present in the study area and that focal squirrels were known to have encountered during my research: coyote, marten, and great horned owl. The coyote and marten were composed of a pliable foam body (Van Dyke's Taxidermy, South Dakota) to which were attached professionally tanned and treated pelts that included eyes and erect ears that improved their realism (Fig. 2.5). The great horned owl was a professionally constructed mount obtained from the Zoology Museum at the University of Lethbridge. It was attached to a five-foot pole that allowed varied placement in the forest. In each experimental trial, a

focal squirrel was exposed to one of these models. Each squirrel was the subject of one trial with each of the three models, with the order of presentation of the different models randomised across subjects.

The protocol for presenting predator models in experimental trials was designed to standardise as much as possible the way each squirrel encountered the predator mount. To this end, a focal squirrel was first trapped in its territory. The cage was then covered with a canvas cloth and moved to the central midden in the territory, while the predator model was set-up on or next to the midden approximately 5m from the trapped squirrel. The canvas cover was left in place for 60 seconds, after which it was lifted to expose the squirrel to the model from within the trap. The squirrel was then released in the direction of the predator model and its behavioural and vocal responses in the 10-minutes following release were audio- and video-recorded for later scoring.

Using this trapping protocol, I could control in my experiment for variation in many aspects of a squirrel's recent experience that might otherwise affect its detection of, or response to, the different predator models. I could also standardise how squirrels encountered each predator and where in their territory they encountered it, which could otherwise introduce additional uncontrolled variability into squirrels' responses. These squirrels were regularly trapped, handled and released in the course of other aspects of this research, such as to accommodate routine animal assessment, weighing and marking, or to facilitate other research protocols. As a result, they were accustomed to the trapping

regimen and readily entered traps making it an ideal way to present predators to them in a

controlled fashion.

2.5.2 Moving Terrestrial Predator

In their simulation of a terrestrial predator, Greene & Meagher (1998) used three dogs of

different breeds. In experimental trials, one of these dogs was released toward a squirrel

that was "foraging on the ground, collecting cones in trees, or resting on a branch." The

latter conditions included obvious variation in the squirrel's vulnerability, but this was

not controlled or analysed systematically. In my experiment, I attempted to replicate this

protocol but also to control for variation in how squirrels encountered the terrestrial

predator. I selected one dog (Billy) who was ideally suited for this experiment. Billy was

of mixed breed and similar in size to a coyote. He also had mottled charcoal-and-brown

fur similar in appearance to a coyote (Fig. 2.5).

Experimental trials were conducted opportunistically in the course of ongoing

behavioural sampling of focal squirrels. Trials were conducted only after a focal squirrel

had been followed for 10 minutes during which time it remained in its own territory and

had not encountered a neighbouring squirrel or any other disturbance. This precaution

provided some control for a squirrel's recent experience and minimised confounding

factors that might exaggerate or attenuate its response in an experimental trial. When

these conditions were met, Billy was introduced to focal squirrels in two different

conditions. The first condition was designed to simulate a threat in a context of high

vulnerability. In this condition, the focal squirrel was followed until it was located on or

near the ground, and Billy was held on-leash and maintained out of sight. On instruction, Billy was moved into the area and allowed to locate the squirrel on his own, at which point the leash-control was relaxed and Billy was allowed to pursue the squirrel naturally. However, I maintained control of the leash at all times to ensure that no harm could come to the squirrel, and to stop the trial if necessary.

The second condition was designed to simulate a threat in a context of low vulnerability. The protocol in this condition was the same except that the focal squirrel was followed until it was located in a tree a safe distance off the ground (>5m), at which point, Billy was moved into the area and to the base of the tree containing the squirrel. Thereafter, he was permitted to react naturally. In both conditions, Billy remained in the territory for 5-min after which he was led away and out of sight. I continued to follow the focal squirrel and record its behavioural and vocal responses for an additional 10-minutes.

2.5.3 Moving Aerial Predator

The simulated aerial predator used in experiments conducted by Greene & Meagher (1998) was a small model bird ('Amazing TIM': De Ruymbeke Co. Marseille, France). The model had an approximate wingspan of 30 cm and was described as similar in body size to a Cooper's hawk (*Accipter cooperii*). The main body of the model was clear plastic and the wings were multi-coloured with portions of blue, green, red and yellow. The model was made to fly by means of a wind-up rubber band that resulted in a sustained flapping flight. In an attempt to replicate this experiment, I obtained the same

bird model. In initial efforts to use this model, I found that it was difficult to control its flapping flight pattern and trajectory, which was also not at all natural for a stooping raptor. In flight, the unwinding elastic band also made considerable noise as did the flapping of the plastic wings. Both noises were conspicuous and unnatural. As a result, I abandoned the use of this model. In its place, I used a small frisbee, which has become a standard protocol for simulating a low-flying aerial predator and has been used successfully in previous studies on ground squirrels (e.g. MacWhirter 1992; Sloan et al. 2005; Wilson & Hare 2006).

Once again, experimental conditions were conducted opportunistically in the course of ongoing behavioural sampling of focal squirrels with the same precautions used in the terrestrial predator experiment just described. When during the course of such a focal follow these conditions were met and the focal squirrel was stationary on the ground, or moving slowly across the ground, and thus in a vulnerable position, the frisbee was projected over it's head at a height of approximately 1.5m to mimic the low-flight of a stooping raptor.

Experimental trials were conducted in two different conditions. The first condition was designed to simulate a fleeting predator threat. In this condition, the frisbee was projected over the squirrel's head and out of sight. The frisbee was small (25 cm in diameter) and forest-green in colour to limit the squirrel's ability to localise it after it passed over-head. The second condition was designed to simulate a more persistent aerial threat. In this condition, the frisbee was projected over the squirrel's head and in the

direction of, and landing near, the taxidermied model of a great horned owl. This combination was designed to simulate both the rapid movement of an aerial predator followed by its persistence in the area. In both conditions, data were collected on the squirrel's behavioural and vocal responses in the 10-min following stimulus presentation.

2.6 RESULTS

2.6.1 Taxidermied Predators

Eleven different squirrels were tested in this experiment, each one receiving one trial with each of the three predator types (N=33 trials total). The squirrels' responses in these trials were similar to those they displayed in natural encounters with predators. After release from the trap, squirrels tended to bolt immediately to the nearest tree and retreat to a safe height, where they then either remained silent and immobile while focused on the predator, or began foot-stomping, tail-flicking and vocalising. Occasionally, they bolted directly to their central midden instead, and disappeared into one of its burrows, or they bolted across the ground and out of sight. These behavioural responses were not differentiated by predator type (Pearson's Chi-square: χ_4=4.38, P=0.35, N=84).

Squirrels vocalised in 20 of the trials, in each case in bouts containing both seets and seet-barks. As in the natural predator encounters, there was a main effect of call type (ANOVA: $F_{1,10}$=11.4, P=0.0070), with more seet-barks than seets produced overall. There was some indication that predator type affected the number of calls given, with more calls produced to the coyote mount than to other mounts, however this effect was not statistically significant ($F_{2,10}$=2.72, P=0.090). There was no interaction of call type

and predator type ($F_{2,20}=1.11$, $P=0.349$). There was also no main effect of time bin ($F_{2,10}=0.17$, $P=0.842$) but there was a significant interaction between call type and time bin ($F_{2,10}=16.53$, $P=0.000058$) with seets produced more in the first time bin and seet-barks more in the latter two time bins (Fig. 2.3b). There was also some indication of a three-way interaction between call type, predator type and time bin, with more seet-barks produced to the coyote mount in later time bins, however this interaction term was not statistically significant ($F_{4,40}=2.46$, $P=0.060$).

Once again, I examined clumping of the two call types within bouts using contingency analysis. 75% of calling bouts (16 of 20) containing 10 or more calls of each type showed statistically significant clumping of calls, with seets following seets and seet-barks following seet-barks (Fig. 2.4b).

2.6.2 Moving Terrestrial Predator

A total of 12 different squirrels were tested in this experiment, each one receiving one trial in each of the two experimental conditions representing a terrestrial threat under conditions of high or low vulnerability (Table 2.1). In all trials in the high vulnerability context and most of those in the low vulnerability context, Billy easily detected the squirrel and ran after it. Billy was remarkably silent throughout, never growling or barking at the squirrel. However, he did remain fixated on it throughout each trial until led away, in many cases shifting position to get a better look at the squirrel when it moved and often lunging toward the squirrel or stretching up the base of the tree in an attempt to reach it. In the low vulnerability trials, when squirrels started from a safe

location in a tree, their initial response upon spotting the dog was to freeze and remain in the same position, while monitoring it. If Billy subsequently moved quickly or attempted to stretch up the tree, the squirrel typically retreated further up the tree. In the high vulnerability trials, when squirrels started on or near the ground, they tended to bolt to and up the nearest tree. If Billy remained motionless for a time at the base of the tree, squirrels would then often move down the tree to inspect Billy more closely. If he moved again, squirrels retreated back up the tree. There was a greater tendency for squirrels to retreat further up the tree in high vulnerability trials (32.1%) and to remain in the same position in low vulnerability trials (21.4%). However, analyses of the more protracted responses in the two experimental conditions indicated that these differences were not statistically significant (Pearson's Chi-square: χ_2 =6.72, P=0.081, N=24).

In all 12 of the high vulnerability trials, but only five of the 12 low vulnerability trials, squirrels also vocalised. Once again, calling involved mixed bouts of both seet and seet-bark vocalisations and was accompanied by foot-stomping and tail-flicking. Results revealed no main effect of call type (ANOVA: $F_{1,11}$=0.25, P=0.623, N=24) but a main effect of experimental condition ($F_{1,11}$=8.57, P=0.013), with more calls of either type being produced in the high vulnerability condition. There was no main effect of time bin ($F_{2,11}$=0.25, P=0.782), but there was a significant interaction of call type and time bin. This interaction showed the same pattern as was observed in the natural predator encounters and the two previous experiments, with seet calls predominating in the early time bins and giving way to seet-barks in later time bins ($F_{2,11}$=6.29, P=0.006; Fig. 2.3c).

There was no effect in the three-way interaction between trial type, call type and time bin ($F_{2,22}$=1.97, P=0.163).

There were 17 trials in which squirrels vocalised across the two experimental conditions and calling bouts contained 10 or more calls of each type. Of these, 88% (15 of 17) showed significant clumping of the two call types with seets following seets and seet-barks following seet-barks (Fig. 2.4c).

2.6.3 Moving Aerial Predator

A total of 13 different squirrels were tested in this experiment, each one receiving one trial in each of the two experimental conditions representing fleeting and persistent aerial predators (Table 2.1). In all cases, squirrels were on or near the ground when tested and their initial responses to the frisbee passing over-head were the same in both experimental conditions: they bolted to and up the nearest tree and then oriented in the direction of the frisbee's flight path. In one trial, a squirrel froze momentarily on the ground as the frisbee passed overhead before bolting for the nearest tree. In none of these trials did squirrels run to their midden or for any extended distance across the ground and out of sight. In the fleeting condition, and once in a tree, they tended to remain focused on the frisbee if it remained in sight, or to scan the area if it did not. In the persistent condition, and once in a tree, they tended to remain fixated on the owl mount for an extended period. Their more protracted responses in the two conditions involved either remaining in place or moving some distance up or down the tree while scanning the area or fixating on the visible frisbee or the owl mount. These more protracted behavioural

responses were not differentiated by experimental condition (Pearson's Chi-square: χ_1 =0.722, P=0.395, N=26).

In 11 of 13 trials in the fleeting condition, and 12 of 13 trials in the persistent condition, squirrels produced vocalisations upon reaching the tree. Once again, calling involved mixed bouts of both seet and seet-bark vocalisations and was accompanied by foot-stomping and tail-flicking. Results revealed no main effect of call type (ANOVA: $F_{1,12}$=0.53, P=0.480, N=26) but a main effect of experimental condition ($F_{1,12}$=5.09, P=0.043), with more calls of either type being produced in the persistent condition. There was no main effect of time bin ($F_{2,12}$=2.08, P=0.146). The interaction of call type and time bin showed the same pattern as observed in the still mount experiment and natural predator encounters with more seet-barks than seets being produced in later time bins, but this pattern was not actually significant in this case ($F_{1,12}$=2.8, P=0.08, N=26). There was also no significant three-way interaction ($F_{2,24}$=0.38, P=0.689).

In this experiment, the nature of the threat represented by the frisbee passing overhead may have been ambiguous to squirrels initially and so I re-ran this analysis after extending the response window beyond the initial 30-second period to include two additional time bins. These two additional time bins were constructed by dividing the remaining portion of each calling bout into middle and end time bins of equal length. The number of calls of each type produced in these time bins was tallied. Because the absolute duration of these additional time bins could vary across individuals and calling bouts, I adjusted the call tallies in these time bins to generate a rate of calling expressed

per 10-second interval, making the values in these time bins equivalent to those for the initial three time bins and my previous tests.

Results of these more protracted calling patterns confirmed the former main effect of experimental condition (ANOVA: $F_{1,12}=10.73$, $P=0.0066$), with more calls of either type being produced in the persistent condition. They also revealed a main effect of call type ($F_{1,12}=7.82$, $P=0.016$, N=26), with more seet-barks than seets produced overall, and a main effect of time bin ($F_{4,48}=2.80$, $P=0.036$) with more calls produced in the final time bin. The interaction of call type and time bin was now significant ($F_{4,48}=5.10$, $P=0.0016$; Fig. 2.3d) with more seet-barks being produced in the later time bins. Finally, there was also a significant three-way interaction between call type, time bin and experimental condition ($F_{4,48}=2.65$, $P=0.044$), with more seet-barks produced in the latter time bins particularly in the persistent predator condition.

There were 23 experimental trials in which squirrels called across the two experimental conditions and calling bouts contained 10 or more calls of each type. Of these, 78% (18) showed significant clumping of the two call types, with the pattern once again showing that seets followed seets and seet-barks followed seet-barks (Fig. 2.4d).

2.7 DISCUSSION

Squirrels tested in the three experiments appeared to treat the taxidermied predator models and the simulated moving predators similarly to natural predators. They showed similar behavioural responses to the various predator models, including retreating to a

safe distance or freezing and remaining immobile in a safe position while monitoring the predator initially, and then confronting it with a stream of vocalisations while foot-stomping and tail-flicking, thereafter periodically shifting up or down the tree in order to retreat further away or to approach and inspect the predator. The specific patterns of call production to the predator models also mirrored those observed in response to real predators. Thus, the squirrels produced protracted bouts of calling in response to the predator models which were again composed of a mix of both seet and seet-bark vocalisations. Seet-bark calls were produced in greater numbers overall than were seets, as was true in natural predator encounters as well. Similarly, calls of the two types were clumped together within bouts with seet calls following other seet calls and seet-barks following other seet-barks. There was also some indication that seet-bark calls were produced more in response to the taxidermied coyote model. This association was not statistically significant, but it did parallel the pattern observed in response to real coyotes in Part I and it matched the strongest call-type association reported by Greene & Meagher (1988). However, there were actually no significant predator-type effects on patterns of alarm call production, and thus little additional evidence that alarm calls were used in a predator-specific fashion.

At the same time, there were a number of additional illuminating patterns. First, in experimental trials with Billy, there was a clear effect of a squirrel's initial vulnerability on their call production: squirrels called at much higher rates when they detected Billy at close range from a relatively vulnerable position on the ground (the high-vulnerability condition) compared to when they detected Billy from a relatively safe location in a tree (the low-vulnerability condition). This outcome suggests that relative vulnerability and

47

perhaps the naturally associated dimensions of relative risk, threat and response urgency are additional salient dimensions of predator encounters which can affecting calling behaviour in red squirrels, just as they do in some other rodent species (Owings & Hennessy 1984; Blumstein 1995).

Notably, the mixing of seet and seet-bark calls within call bouts did not change in high-vulnerability conditions, only overall vocal output. Indeed, this pattern of mixed bouts of seets and seet-barks was consistent across the three experiments as well as the natural predator encounters and showed an additional consistent temporal pattern: low-amplitude seet calls were produced early in a calling bout and gave way to louder, broadband seet-barks as calling bouts continued. The consistency in this calling pattern across all predator classes strongly suggests that predator identity by itself is not the most salient dimension of predator encounters influencing call production. Rather, it suggests that there might be something specifically about the temporal patterning of such encounters that is more salient. Indeed, that possibility is supported by results of the simulated aerial predator experiment. Squirrels' vocal responses in that experiment showed the same mixing of seet and seet-bark calls within calling bouts. They also showed higher overall levels of call production specifically in the persistent condition, which simulated an aerial predator remaining in the area after passing overhead. Furthermore, this increase in overall call production in the persistent condition was driven by greater production of seet-barks in the later time bins as calling bouts became more protracted.

Taken together, these patterns suggest that the more salient dimension of predator encounters, at least as they affect the production of alarm calls, might be the persistence of the threat that it represents rather than the specific type of predator involved. In the initial stages of an encounter with any predator, red squirrels produce the low-amplitude tonal seet call. These calls give way to louder broadband calls if the predator persists in the area. This patterned vocal response could sometimes create an incidental association between call type and predator class, if aerial predators often present themselves fleetingly (and thus elicit only seets), whereas terrestrial predators tend to remain in the area longer (and thus elicit seet-barks as well). And this account might help to explain the statistical association between seet-barks and coyotes observed in the natural predator encounters I report because that association emerged in the later stages of calling bouts. It might also explain why I did not find the same association in the subsequent experiment using taxidermied predator models. In that experiment, I controlled and standardised how long squirrels saw each of the different predator models and thereby removed any natural occurring variation in predator persistence among them. This account might also help to explain the apparent predator-specificity of seets and barks reported by Greene & Meagher (1998) if there was some difference in how long the bird model and live dogs were presented to squirrels in their experiment.

Of course, it could still be argued that, although predator persistence might be the more salient dimension of predator encounters to the squirrels, the incidental association between predator persistence and predator identity nevertheless yields an alarm call system that is functionally referential in the sense that the calls will often effectively

49

serve to pick out the predator class distinction between aerial versus terrestrial threats. I

acknowledge this possibility but it is weakened substantially by the observation that red

squirrels also produce the same calls to non-predators, a phenomenon that others have

described (Embry 1970; Smith 1978; Price et al. 1990; Price 1994) and that I also

observed and reported in Part I. It is possible, as noted earlier, that some of these other

non-predatory species elicit calling because they are mistaken for predators. However,

among the non-predators that elicit these same calls are other, conspecific squirrels and it

is doubtful that they too are routinely mistaken for predators. It is not clear, though,

whether call usage in encounters with other squirrels is similar to that observed in

predator encounters, and so to address this issue, I report in the next section on squirrels'

behavioural and vocal responses in encounters with other squirrels.

PART III. ENCOUNTERS WITH OTHER SQUIRRELS

2.8 METHODS

2.8.1 Data Collection and Analysis

Instances in which a focal squirrel encountered and engaged another squirrel intruding on

its territory were recorded during the course of behavioural sampling in the same way

that encounters with predators were recorded. For each such encounter, I noted the

identity of the other squirrel where possible and details of the focal squirrel's behavioural

and vocal response to it using the same methodologies and equipment described earlier.

2.9 RESULTS

2.9.1 General Responses to Squirrel Intruders

I observed 36 cases where the focal squirrel being sampled purposefully engaged a conspecific intruder detected on its territory. This sample involved 18 different focal squirrels and at least 11 different intruders. It was not always possible to confirm the identity of the intruder and so the total number of different intruders represented in this sample could be higher. In these interactions, focal squirrels (the residents) were typically in a tree when they detected the intruder moving along the ground across the resident's territory. Residents tended to stop their current activity and orient toward the intruder. They then vocalised and flicked their tail over their head (but tended not to foot-stomp). They then either remained in place while continuing to vocalise and tail-flick, or they bolted down the tree and gave chase while the intruders retreated. Chases sometimes involved physical contact if residents overtook retreating intruders. Encounters sometimes also involved repeated cycles of calling and chasing if intruders remained within on near the territory, or returned to it again shortly after being chased away. As a result, some encounters were relatively short, while others were quite protracted lasting up to 15.6 minutes and involving hundreds of calls.

In 29 of the encounters, residents produced seets, seet-barks, or more typically a combination of the two call types in protracted bouts like those produced in response to predators. In 10 of these encounters they also produced one or more 'rattle' calls. In an additional seven cases, they produced only rattle calls. In some encounters, intruders also vocalised. Although it was not possible to collect systematic data on the number and type

51

of calls produced by the intruder at the same time that these data were being collected for the resident, it was the case that intruders tended not to produce rattles, seets or seet-barks but rather only 'squeak' calls.

2.9.2 Call Patterning within Squirrel Encounters

Because rattles were produced in only a fraction of encounters, my analysis of call patterning during encounters with other squirrels focused on their production of seets and seet-barks and was conducted in exactly the same way as my tests of calling patterns in response to predators. Statistical tests revealed a main effect of call type (ANOVA: $F_{1,17}=15.15$, $P=0.0011$, N=29), with more seet-barks than seets being produced overall. There was also an effect of time bin ($F_{2,17}=3.40$, $P=0.045$), with the number of calls produced increasing over time. There was also an interaction between call type and time bin ($F_{2,17}=10.64$, $P=0.00025$) which paralleled the pattern observed in natural and simulated predator encounters: seets were produced more in the first 10-seconds of encounters with other squirrels and then decreased in frequency, while seet-barks increased in frequency over time and predominated in the 20 and 30 second time bins (Fig. 2.3e).

There were 25 trials in which squirrels vocalised in encounters with other squirrels and calling bouts also contained 10 or more calls of each type. Of these, 76% (19) showed significant clumping of the two call patterns. The pattern mirrored that observed in encounters with real and simulated predators with seets following seets and seet-barks following seet-barks (Fig. 2.4e).

2.10 DISCUSSION

Squirrels' responses to conspecific intruders were both similar to, and different from, their responses to predators. The principal difference lies in the fact that squirrels aggressively chased intruders, typically across the ground, which is something they never did to predators. In contrast, their vocal responses to intruding squirrels closely paralleled their vocal responses to predators. When confronting intruders, resident squirrels sometimes produced one or more 'rattle' calls which are often also produced spontaneously throughout the day when no intruders are present and are regarded as general territorial announcements (Smith 1978; Lair 1990; Price et al. 1990; Price 1994). However, the preponderance of their vocal responses were protracted and mixed bouts of seet and seet-bark calls paralleling those produced in encounters with predators. Once again, seet-bark calls were produced in greater numbers overall than were seet calls, and calling bouts showed the same internal structuring as observed in calling bouts to predators: seets were produced primarily in the initial stages of calling bouts and gave way to seet-barks as calling bouts continued.

This combination of responses indicates that the squirrels clearly do discriminate in important ways between conspecific intruders, who they call at and chase, and predators, who they call at but do not chase. As a result, the fact that their calling patterns to the two groups are so similar strongly suggests that the calls themselves are not 'about predators' *per se* but rather reflect some broader concern with disturbances of any kind.

In fact, this broader emphasis on the importance of disturbances of various kinds dovetails well with the general ecology and life-history of red squirrels. Red squirrels are solitary throughout the year, except during the short mating season. They actively defend a small territory year-round in which they harvest cones that are their principal food supply (Boutin & Schweiger 1988). They spend considerable time and energy caching these cones in a central midden and additional distributed cache sites in anticipation of winter (Hurly & Robertson 1987; Hurly & Lourie 1997) and this stored supply of cones proves to be critical to a squirrel's overwinter survival (Gurnell 1987). These cone caches are also subject to intense pilfering from neighbouring squirrels and other cone-eating species (e.g., chipmunks, mice and jays). Gerhardt (2005) found that, in any given year, 97% of all red squirrels stole cones from neighbouring caches and 92% of all squirrels lost some portion of their cache to pilferage. Losses for individual squirrels ran as high as 84% of their cone supply. On average, squirrels lost 25% of their cache to pilferage. Given the energetic constraints facing a small-bodied, non-hibernating mammal overwintering in boreal forests, cone loss might therefore represent as serious a threat to red squirrel survival as do many forms of predation.

These life-history factors might then help to account for the fact that red squirrels seem to call vigorously and relatively indiscriminately at a wide variety of predatory and non-predatory species, including conspecific intruders: as a small-bodied, territorial, food-caching species, disturbances of many kinds are salient and threatening. This modified account of alarm calling helps to resolve discrepancies in earlier reports which emphasised either the comparatively broad, or the comparatively specific, use of alarm

calls for various types of disturbance, and it is also consistent with important details of the species' ecology. However, it does not ultimately explain how the production of these 'alarm calls' is functional to the squirrels and to whom the calls are actually addressed. These issues are addressed in the next chapter.

Table 2.1. The squirrels involved in natural predator encounters (a) and encounters with intruder squirrels (e), or who served as subjects in the predator simulation experiments (b-d).

Individual (M/F)	(a) Natural Predator Encounters*	(b) Taxidermied Predator Models	(c) Moving Aerial Predator	(d) Moving Terrestrial Predator	(e) Intruder Squirrels
Ajax (F)		X	X		X
Aphrodite (F)	G, C	X			
Ares (F)			X	X	
Artemis (M)	D, W, M		X	X	X
Arthur (M)			X	X	
Athena (F)	C, O	X			
Calpurnia (F)					X
Cassie (F)	G				
Dionysus (M)					X
Eros (M)	D, M, R	X			X
Hades (F)		X			
Hermes (M)	O, R			X	
Holmes (M)			X	X	X
Homer (M)					X
Hudson (M)					X
Isadora (F)					X
Lestrade (M)		X			
Moriarty (M)	R				X
Mortimer (F)	O				X
Mycroft (M)				X	X
Negra (F)	M, G				X
Nibbles (F)		X			
Persaus (F)	C				
Rip (F)					X
Rowdy (M)			X	X	
Russel (M)	C, D				
Scar (F)	C, R	X			
Scratch (F)	R		X		X
Slip (F)			X		
Snap (F)	O, C	X			
Triton (M)	G		X	X	
Vesper (M)			X	X	X
Watson (F)	C, O	X	X		X
Winter (F)	O, R		X	X	
Zap (M)				X	
Zip (F)	M	X	X	X	X

* Natural predators: G=goshawk, O=owl, R=raven, C=coyote, M=marten, D=deer, W=weasel.
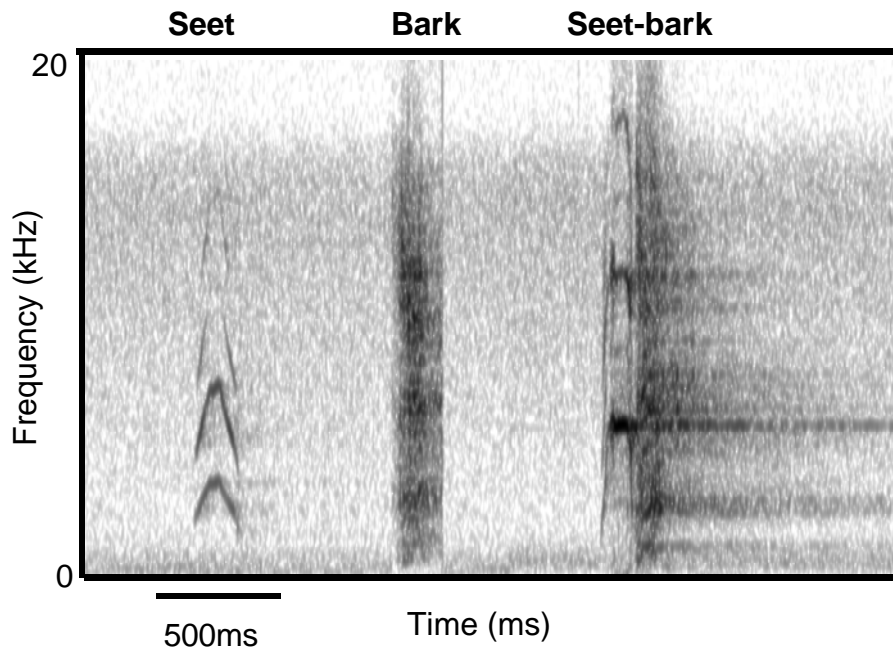
Figure 2.1. Spectrograms of the seet, bark and seet-bark vocalisations. Spectrograms were produced using overlapping 220-point fast-Fourier transforms with a 7.5ms time step and 44.3 Hz frequency step.
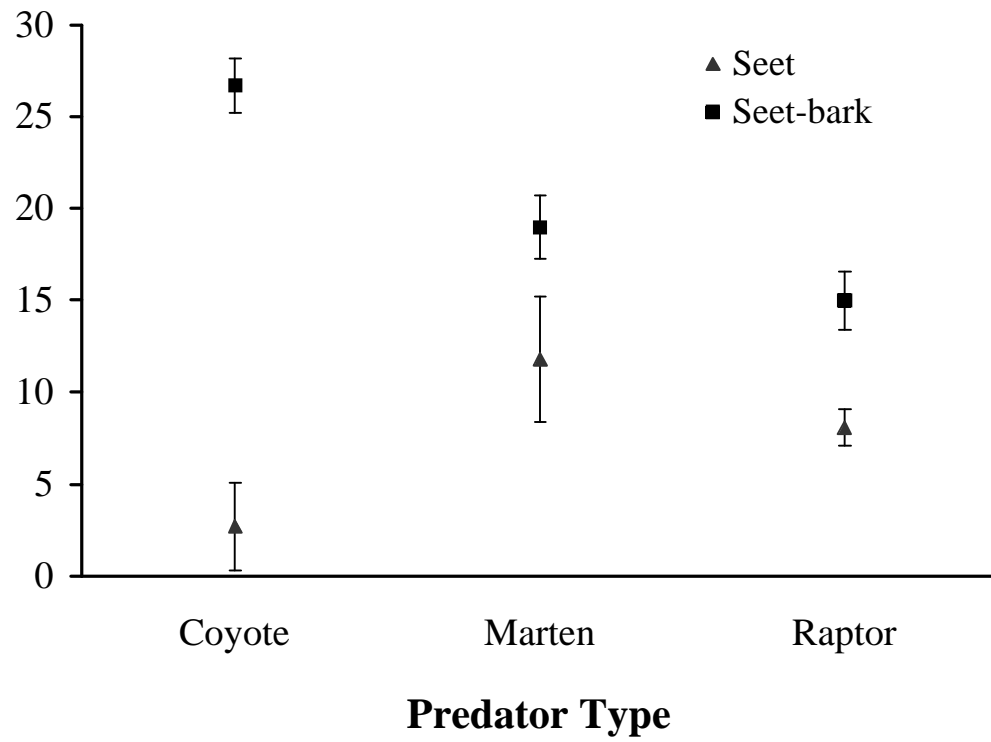
Figure 2.2. Variation in the average production of seet and seet-bark calls within a bout during natural encounters with coyote, marten and owl predators.
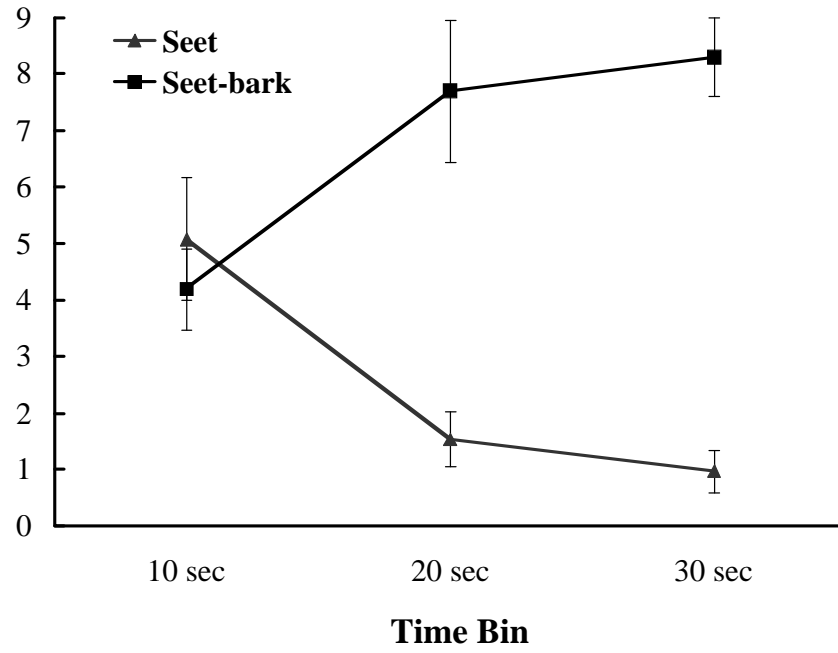
(a)
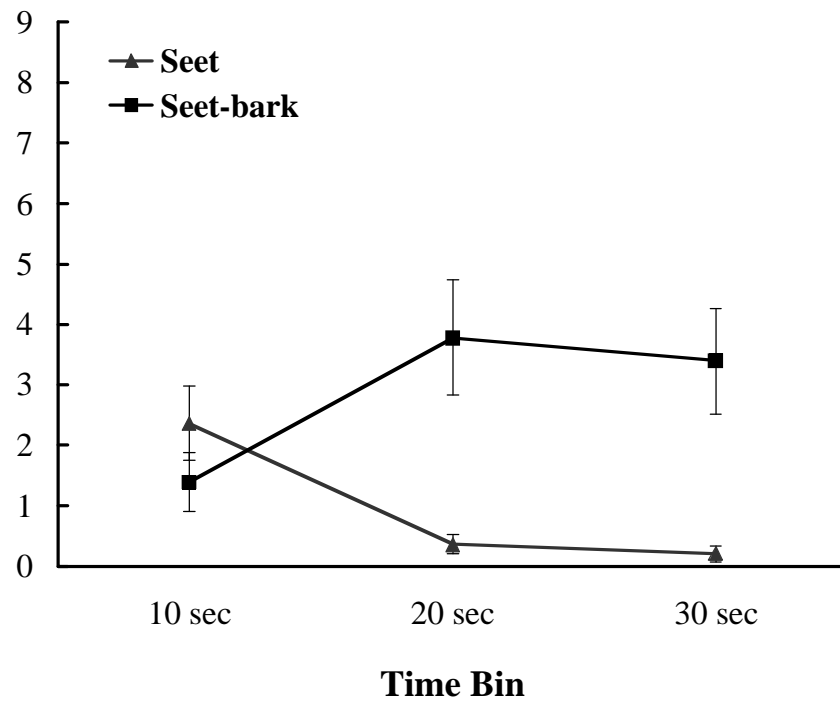
(b)
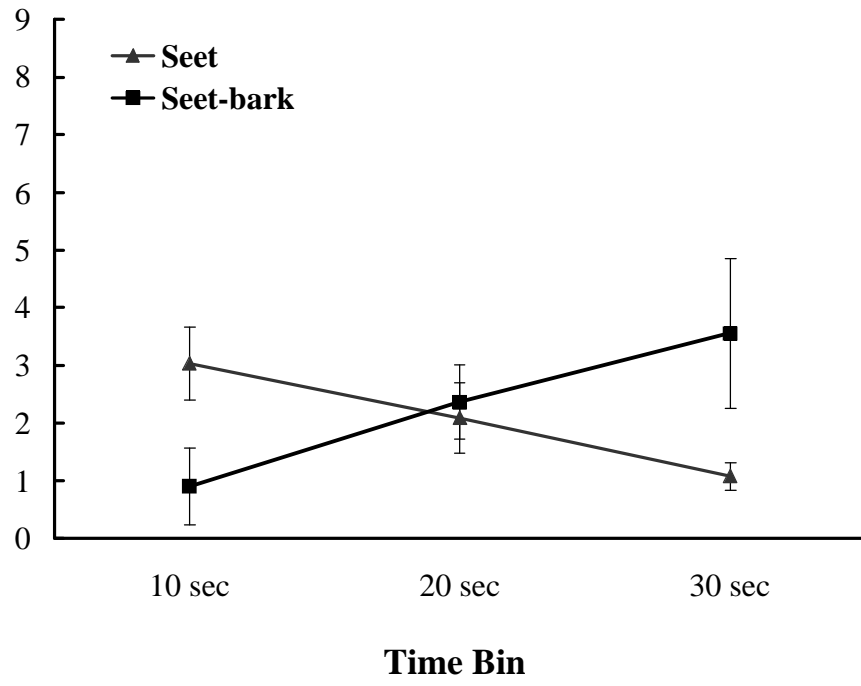
(c)
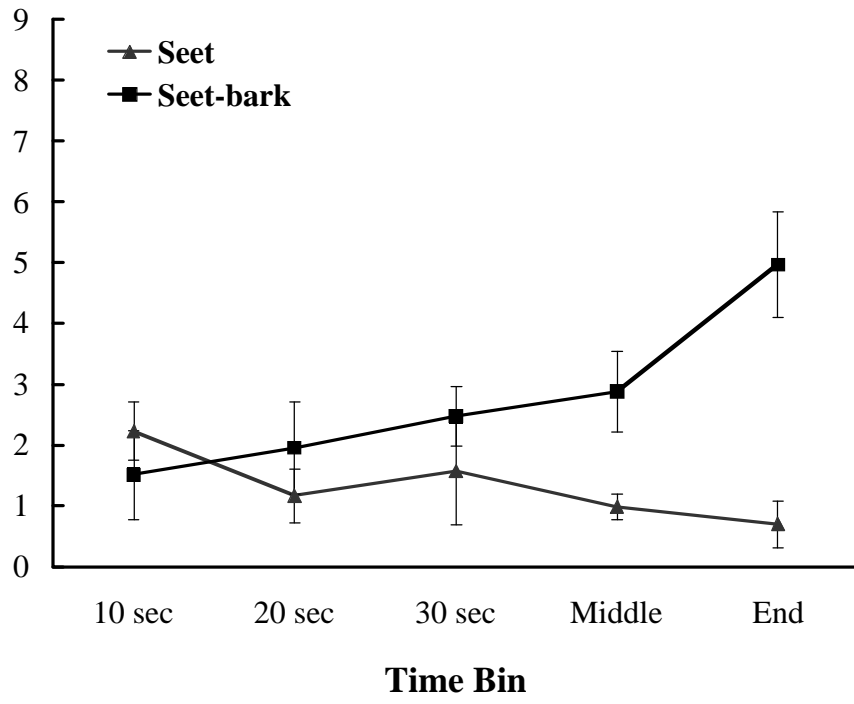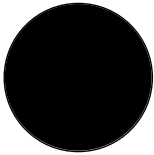
(d)
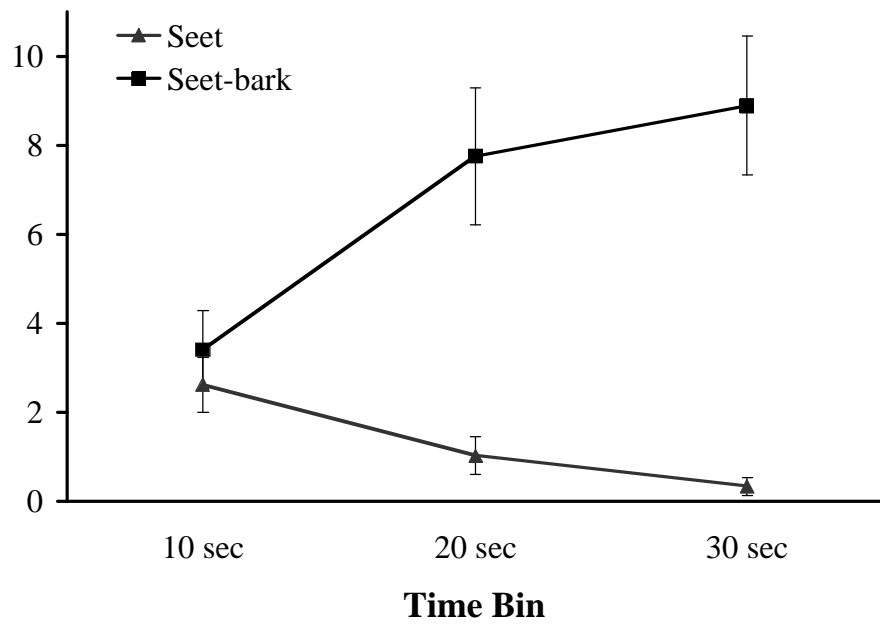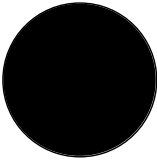
(e)

Figure 2.3. The time-course of seets and seet-barks within mixed bouts of calls produced during natural predator encounters (a) and in experimental trials involving taxidermied predator models (b), a moving terrestrial predator (c) and a moving aerial predator (d) and in encounters with intruder squirrels (e).

Second Call   (a)
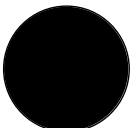
Seet   Seet-bark

First Call

Seet

Seet-bark

Second Call   (b)

Seet   Seet-bark

First Call
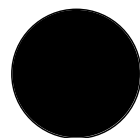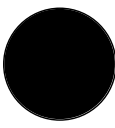
Seet

Seet-bark

Second Call (c.)

Second Call (d)

65

Second Call (e)

Figure 2.4. Graphic representation of transition probabilities for seet and seet-bark vocalisations within individual calling bouts produced during natural predator encounters (a) and in experimental trials involving taxidermied predator models (b), a moving aerial predator (c), a moving terrestrial predator (d) and in encounters with intruder squirrels (e). The size of the circle represents the relative frequency of a particular transition either within or between the two call types. In each case, the patterns show that seets tend to precede and follow other seets, while seet-barks precede and follow other seet-barks, and transitions that cross the two call types are relatively rare.

(a)

(b)

(c)

(d)

Figure 2.5. Photographs of the models used in simulated predator experiments:
taxidermied coyote (a), marten (b) and owl (c) and the live dog, Billy (d).

68

CHAPTER THREE

PREDATOR-ASSOCIATED VOCALISATIONS IN NORTH AMERICAN RED SQUIRRELS: TO WHOM ARE CALLS ADDRESSED AND HOW DO THEY FUNCTION?

3.1 INTRODUCTION

North American red squirrels (*Tamiasciurus hudsonicus*) are a solitary, territorial species that is well known for confronting predators and territorial intruders with a barrage of conspicuous vocalisations (Smith 1968; 1978; Embry 1970; Nodler 1977; Lair 1990). One recent study suggested that red squirrels might, in fact, produce a small set of structurally-discrete, predator-specific alarm calls (Greene & Meagher 1998). Thus, red squirrels were reported to produce a relatively soft, and high-frequency tonal 'seet' call in response to aerial predators, and a much louder, and broadband noisy 'bark' call in response to terrestrial predators. The relatively specific usage of these two predator-associated vocalisations in red squirrels suggested that the calls might function as referentially-specific warnings about predators similar to functionally referential alarm calls that have been reported for some nonhuman primates and other bird and mammal species (Seyfarth et al. 1981; Evans et al. 1993; Zuberbühler 2000; Manser 2001; Templeton et al. 2005; Kirchlof & Hammerschmidt 2006).

However, the results of additional recent research raise some doubts about the predator-specificity of red squirrel alarm calls. In a multi-year study conducted in the foothills of the Rocky Mountains in southern Alberta, Canada, Digweed & Rendall

(2009) confirmed that red squirrels produced two structurally distinct call types during encounters with natural predators and in systematic experiments involving simulated predators. The call types produced closely matched those reported previously and involved the comparatively soft, and high-frequency tonal seet call type and a much louder, and harsher 'seet-bark' call type composed of a short, tonal seet with an appended broadband 'bark'. However, the two call types were not used in a predator-specific fashion. Instead, the two call types were produced in mixed bouts to both aerial and terrestrial predators. Digweed & Rendall (2009) further found that, within protracted bouts of calling, there was a consistent temporal pattern of production of the two call types: the tonal seet call predominated in the early stages of all calling bouts and gave way to the harsher seet-bark call in the later stages of calling bouts as both real and simulated predators persisted in the area. The mixed use of the two call types in encounters with both aerial and terrestrial predators in combination with their consistent temporal patterning within calling bouts suggested that these predator-associated vocalisations in red squirrels might reflect not the identity of specific types of predator *per se* but rather the persistence of the threat they represented. A correlation between the production of one or other call type and a difference in the class of predator encountered might sometimes arise then but only because of a natural coincidence in the persistence of the threat that different classes of predator (aerial versus terrestrial) normally represent.

At this stage, it is not possible to discriminate definitively between these two accounts and so the hypothesised referential quality of predator-associated vocalisations

in red squirrels remains ambiguous. At the same time, it is not entirely clear to whom

these predator-associated vocalisations are addressed and ultimately how they function.

The latter questions are obviously critical ones to resolve and the answers might also help

to disambiguate the alternative mechanistic accounts just reviewed.

3.1.1 Alternative Functions of Predator Alarm Calls

Established theory observes that conspicuous alarm calls necessarily draw predators'

attention and thereby expose callers to greater predation risk themselves than if they were

to remain silent. Hence, there is assumed to be some benefit to callers that more than

compensates for these costs. A variety of possibilities have been considered, most

focusing on the benefits that can accrue to callers by warning different categories of

conspecifics about predators they might otherwise be unaware of. For example, for

individuals that live in groups composed primarily of closely related kin, the costs

associated with producing conspicuous alarm calls might be more than compensated by

inclusive fitness benefits that follow from the improved survival and reproduction of

relatives that are warned about predators (Hamilton 1964; Sherman 1980). In general, we

would expect that individuals with kin nearby should call more than those without, and,

indeed, research on several mammalian species supports this prediction (Sherman 1977,

1980; Schwagmeyer 1980; Cheney & Seyfarth 1981; Da Silva et al. 2002; Blumstein

2007; Wheeler 2008). For example, in Columbian ground squirrels (*Spermophilus

columbianus*), and black-tailed prairie dogs (*Cynomys ludovicianus*), parous and breeding

females that are surrounded by close kin produce alarm calls at higher rates than do males

and non-parous females (Hoogland 1983; MacWhirter 1992).

Warning kin might not be the only selected benefit to producing alarm calls, however. If survival of a mate is important to an individual's fitness, then individuals might also call to warn mates (Morton & Shalter 1977; Witkin & Fricken 1979; Krams et al. 2006), as has been proposed, for example, in great tits (*Parus major*). In this species, males produce alarm calls both within and outside their breeding ranges, but only if a female is nearby and not when males are alone, suggesting that alarm calls are targeted towards, and function to warn, potential mates (Krams et al. 2006).

It is also possible that alarm calling might serve additional social functions. For example, among solitary, territorial species, alarm calling might represent a form of "cooperation" among neighbours (Smith 1986). After establishing territorial relationships, often through protracted aggressive interactions, individuals in many species subsequently respond less aggressively to intrusions by neighbours than to intrusions by strangers, a phenomenon termed the 'dear-enemy' effect (Fisher 1954; Ydenberg et al. 1988). The dear enemy effect has been explored predominantly in territorial bird species (reviewed in Stoddard 1996; Hyman 2005). However, some mammals are also more tolerant of neighbours than strangers (Healy 1967; Randall 1989; Price et al. 1990). For example, in banner-tailed kangaroo rats (*Dipodomys spectabilis*), neighbour pairs were observed to interact more peaceably than were stranger pairs (Randall 1989). Alternatively, some social mammals experience increased competition from neighbours and thus are more aggressive toward, and less tolerant of, neighbours compared to strangers. For example, in the banded mongoose (*Mungos mungo*),

individuals showed more vigorous inspection and vocalising in response to olfactory cues

associated with neighbours compared to those of strangers (Muller & Manser 2007).

3.1.2 Potential Conspecific Warning Functions for Alarm Calls in Red Squirrels

Red squirrels are a solitary species, in the sense that they do not live in cohesive social

groups containing multiple other conspecifics the way some bird and mammal species do.

This solitary lifestyle means that, by comparison to more gregarious species, the value of

conspicuous alarm signalling to warn conspecifics might seem to be requisitely reduced

in red squirrels. However, red squirrels are not entirely asocial. Females and males

interact during the mating season; females invest in and interact with their offspring prior

to, and sometimes after, offspring dispersal from the natal territory (Gurnell 1987); and

males and females both interact with territorial neighbours (Price et al. 1990). As a result,

it is certainly possible that the conspicuous alarm calls produced by red squirrels function

as they do in other species to warn kin, potential mates, or territorial neighbours about

potential predators (Sherman 1977; Smith 1986; Krams et al. 2006).

There are a variety of testable predictions that flow logically from these different

functional hypotheses as they apply specifically to red squirrels (see Table 3.1).

3.1.3 Warning Kin

Mating is seasonal and polygynous in red squirrels. In the short mating season, males

compete vigorously for mating opportunities and are constantly on the move, often

traveling relatively long distances (up to 1km) to seek-out available mates (Gurnell

1987). Although the resulting spatial distribution of paternity is not fully known, this wandering pattern of males means that they will not necessarily sire offspring close to their home territory as opposed to farther away. In contrast, adult females are predictably associated with, and invest directly in, dependent offspring each year following birthing and pup emergence. Dispersal and settlement patterns of offspring are not well documented, but red squirrels appear to follow the usual mammalian pattern in which offspring settle in areas close to their mothers. Recent research indicates that females also sometimes bequeath a portion of their territory to offspring (Price & Boutin 1993). Taken together, these life-history characteristics suggest that females will have close kin nearby seasonally, in the form of young-of-the-year, whereas males may not; and that some females might also have adult offspring nearby as neighbours. Given this, if predator-associated calling in red squirrels functions to warn kin, then we would predict that: females should produce alarm calls more often than males; lactating females should call more often than non-lactating females; females should call more after pup emergence when offspring are more vulnerable to predators than before emergence when the offspring are less vulnerable in the nest (and relatively immobile in any case); and females whose neighbours include adult offspring should call more than those whose neighbours are not adult offspring.

3.1.4 Warning Mates

It is also possible that predator-associated vocalisations in red squirrels function as warnings to mates. In red squirrels, males invest nothing in offspring care post-copulation, while females lactate and provide additional investment in offspring during

74

early growth and development. As a result, the reproductive success of males post-copulation hinges on the short-term survival of former female mates, while the reproductive success of females post-copulation does not hinge on the continuing survival of former male mates. As a consequence, if alarm calls serve as warnings to mates, then we would predict that males should produce alarm calls more often than females; and males who are long-term residents should call more often than recently immigrated males because resident males will have had requisitely more opportunities to mate with local females.

3.1.5 Warning Neighbours

Finally, it is possible that predator-associated vocalisations in red squirrels function as warnings to neighbours. There is some precedent for neighbour tolerance in red squirrels. Results of a playback experiment found that territory holders produce more intense responses to playback of the species-specific territory advertisement call (termed a 'rattle') produced by strangers compared to the same type of calls produced by familiar neighbours (Price et al. 1990). Such tolerance might in part reflect the considerable costs associated with establishing clear territorial relationships with neighbours to begin with (Healy 1967; Randall 1989). If true, then there might also be a benefit to producing alarm calls to warn established neighbours about predatory threats, thereby preserving an established neighbour relationship and so avoiding the time, energy, and aggression that would be required to re-establish such a relationship with the squirrel that would inevitably enter to replace a former neighbour.

Both males and females maintain such territorial relationships in red squirrels, and there is no reason to think that the costs of establishing such relationships are greater for one sex than the other. Therefore, males and females would be predicted to produce alarm calls equally often. In addition, individuals with long-standing neighbours that reflect established territorial relationships worth preserving would be predicted to produce alarm calls more often than those with only short-term neighbours.

In this chapter, I examine patterns of alarm call production in a population of wild red squirrels for evidence of variation in call production aligned with these predictions.

3.2 METHODS

3.2.1 Study Site and Subjects

I conducted this study at the R.B. Miller Field Station in the Sheep River Valley of Kananaskis Provincial Park, Alberta (50°39′ N, 114°39′ W) over the course of three consecutive years (2005-2007). Research was conducted primarily between May and November representing the late spring, summer, and fall seasons at this latitude. Research focused on a population of 47 individually marked squirrels in a single, contiguous forest patch approximately 60 ha in size. In order to provide positive individual identifications, each squirrel was uniquely marked with a dye mark and a small numbered tag to be placed in one or both ears (National Band and Tag Company, Kentucky). Dye marks were visible at distances up to 20 meters facilitating positive identification under most conditions, while the numbered ear tags also facilitated recognition of marked animals at a distance and the unique numbers on the tags allowed certain identification across

seasons and across years when animals were re-trapped (for detailed methods see Digweed & Rendall 2009).

3.2.2 General Behavioural and Vocal Responses to Predators

Digweed & Rendall (2009) studied red squirrels' general behavioural responses and production of alarm calls in natural encounters with predators. In addition, three experiments using simulated predators were conducted. The first experiment involved presentation of taxidermied models of three species common in the study area and known to prey on squirrels, namely coyotes (*Canis latrans*), great horned owls (*Bubo virginianus*) and pine martens (*Martes americana*). The second and third experiments simulating a moving terrestrial and aerial predator, respectively. The terrestrial predator model was a dog of mixed breed that resembled a coyote in size and pelage. The dog was presented to squirrels in two conditions selected to represent a predatory threat under conditions of either low- or high-vulnerability. The aerial predator model was a small, green frisbee that was presented to squirrels by projecting it overhead in two conditions simulating either a fleeting or a persistent aerial threat (for additional details of experimental design see Digweed & Rendall 2009).

Squirrels' responses to real and simulated predators were similar. Briefly, squirrels who spotted a predator, or a predator model, immediately stopped their current activity and either froze momentarily if they were in a tree, or, if they were on the ground, bolted to the nearest tree where they climbed to a safe height (5m). They then typically remained at this safe height staring at the predator and, after a short interval,

began to stomp their hind feet and flick their tail back-and-forth over their back while

producing a steady stream of vocalisations that could last for several minutes, involve

hundreds of calls, and sometimes continue after the predator had left the area.

3.2.3 Data Collection and Analysis

Data for use in the present study derive from the record of vocalisations produced by

focal squirrels in response to the real and simulated predators described in Digweed &

Rendall (2009). Only two call-types were produced in these encounters, a tonal 'seet' call

and a more broadband 'seet-bark' call. The vocalisations were recorded using a digital

Marantz PMD660 recorder and a Sennheiser ME66 shotgun microphone with Sennheiser

MZH60-1 windscreen. In previous analyses, this record of vocalisations was tested for

evidence that seets and seet-barks were produced in predator-specific fashion. In this

chapter, I examine patterns of call production for variation among individuals belonging

to different demographic categories for which calling behaviour is predicted to vary

under alternative hypotheses of call function.

Although squirrels' behaviour in encounters with real and simulated predators

was similar, I nevertheless conducted separate analyses of calling behaviour in natural

predator encounters and in experimental predator simulations. However, there was no

reason to further subdivide the sample to test hypotheses of call function. For example,

there was no reason to assume *a priori* that the function of producing alarm calls should

be different for the different kinds of predator encountered, and, indeed my earlier work

revealed similarities in vocal production to all types of predator. There was also no

reason to assume *a priori* that the function of calling should be different for seets and

seet-barks. As a result, my analyses combine the production of seets and seet-barks and

consider all predator types together. I limited my tallies of call production to the first 30-

seconds of calling bouts to be consistent with my earlier analyses (Digweed & Rendall

2009). Statistical tests thus involved tallies of the combined number of seets and seet-

barks produced in the first 30-seconds of calling bouts during either natural encounters or

predator experiments. These tallies were tested for variation according to a series of

demographic distinctions described below.

3.2.4 Demographic Categories

To test the various research predictions, it was necessary to categorise individual

squirrels into a variety of demographic categories (Table 3.1). The distinction between

males and females was basic. Within males, an additional distinction was made to

distinguish resident males from recent immigrants. Residents were defined as males who

had held their territory for more than one season, while immigrants were males who had

arrived on a territory in the current season. Several distinctions were made among

females. First, females with pups were distinguished from those without pups. This

distinction was operationalised in the field by monitoring female lactational status during

routine trapping and inspection protocols. Second, for females with pups, I further

distinguished between those whose pups had emerged above ground and so were likely to

be more vulnerable to predators, and those whose pups had not yet emerged and thus

were more safely positioned in the nest. Third, females who were likely to have kin as

neighbours were distinguished from those whose neighbours were less likely to be kin.

Because I could not assess collateral kinship, or potential kinship extending back several years, this distinction was limited to offspring from the previous year. Hence, females whose neighbours were known to include an offspring from a previous year were distinguished from females for whom this was not known to be the case. Finally, for both sexes, I further distinguished between individuals who had long-term neighbours with whom they were likely to have an established relationship and individuals with only short-term neighbours. Individuals were defined as having long-term neighbours if they themselves had resided on their territory for more than one season and one or more of their neighbours had similarly resided on its territory for more than one season. Otherwise, individuals were defined as having only short-term neighbours, either because they themselves were recent immigrants or all of their neighbours were.

3.3 RESULTS

3.3.1 Vocal Responses to Natural Predators

Squirrels were involved in 34 natural disturbances. Of these, 22 involved known predators, either coyotes (*Canis latrans*), great grey owls (*Strix nebulosa*), northern goshawks (*Accipiter gentilis*), or pine martens (*Martes americana*). An additional 12 disturbances involved species that, on the surface, would seem to pose no predatory threat (e.g., ravens, weasels, deer). However, squirrels responded to the latter species with the same pattern of behaviours and vocalisations observed in encounters with known predators (Digweed & Rendall 2009). Therefore, disturbances involving both groups were combined for analysis.

Females were involved in 20 of the 34 encounters and males in the remaining 14. Some individuals were involved in more than one encounter; however, where this occurred, the individuals were serving as representatives of different demographic categories in the different encounters and so call production totals could not be averaged within individuals across these encounters. As a result, the data used in statistical testing represent individual disturbances rather than individuals *per se*.

Results of statistical testing of call production patterns at this level are shown in Figure 3.1. There was no significant difference in the rate of calling between males (N=14) and females (N=20; Mann-Whitney, two-sample test: $Z=-1.02$, $P=0.309$). There was also no tendency for lactating females (N=12) to call more than non-lactating females (N=8; $Z=0.95$, $P=0.341$) or for females to call more after pup emergence (N=11) than before (N=9; $Z=0.58$, $P=0.562$). Females whose neighbours were known to be offspring from a previous year (N=6) did not call more than females whose neighbours were not known to be offspring (N=14; $Z=0.79$, $P=0.429$). Among males, there was no difference in call production between resident males (N=11) who were more likely to have mated with local females, and immigrant males (N=3) who were less likely to have done so ($Z=0.16$, $P=0.875$). However, considering both males and females together, squirrels with at least one long-term neighbour (N-18) called more than squirrels with only short-term neighbours (N=15; $Z=-2.17$, $P=0.029$).

3.3.2 Vocal Responses to Simulated Predators

In total, 165 trials were conducted across the three predator simulation experiments: 63

trials involved taxidermied predator models (Experiment One); 47 trials involved

simulating a moving terrestrial predator using a live dog (Experiment Two); and 55

involved simulating a moving aerial predator using a frisbee (Experiment Three). This

experimental sample involved 21 different female subjects and 18 different male subjects,

many of whom thus participated in multiple trials across the three experiments.

Individuals did not necessarily contribute an equal number of trials to the sample,

however. At the same time, individuals could also be representing different demographic

categories across the multiple experimental trials they participated in. Therefore, for

statistical testing, I needed to eliminate possible confounds arising from differential

individual contributions to the overall sample, while at the same preserving each

individual's contribution to the different functional demographic categories they

represented. To accomplish this, I took the following precautions: a. in cases where an

individual participated in multiple experimental trials as a representative of one

demographic category (e.g., lactating female), I averaged their calling rates across these

multiple trials to arrive at a single score for this individual as a representative of that

particular demographic category; b. if the same individual was also the subject of more

than one trial as a member of another demographic category (e.g., non-lactating female),

as could happen given the extended time frame of this research spanning multiple seasons

and years, I calculated a separate average of their calling rates across these multiple trials

to arrive at a single score for this individual as a representative of this additional

demographic category. In this way, each individual ultimately contributed only one data point to any particular demographic category.

Results of testing call production patterns calculated in this way are shown in Figure 3.2 and almost perfectly replicate the patterns observed in encounters with real predators. There was no significant difference in call rates between males (N=18) and females (N=21; Mann-Whitney, two-sample test: Z=-0.31, P=0.757). There was also no tendency for lactating females (N=17) to call more than non-lactating females (N=9; Z=0.94, P=0.345), or for females to call more after pup emergence (N=21) than before (N=15; Z=1.38, P=0.167). Females whose neighbours were known to be offspring from a previous year (N=6) did not call more than females whose neighbours were not known to be offspring (N=20; Z=20.27, P=0.784). Among males, there was no difference in call production between resident males (N=13) and immigrant males (N=8; Z=1.19, P=0.232). Considering both males and females together, there was again a significant difference in calling rates between squirrels with at least one long-term neighbour (N=27) and squirrels with only short-term neighbours (N=21; Z=-2.87, P=0.0039). However, in this case, the pattern was reversed from that observed in natural disturbances: squirrels with short-term neighbours called more than did squirrels with long-term neighbours.

Squirrels in experimental trials registered and responded to all of the predator models; however, they did not always produce vocalisations in response to them (35.8% of all trials). Such 'non-calling' trials occurred more often with the stationary,

taxidermied predator mounts and in the low-urgency condition of Experiment Two where

squirrels encountered the live dog when they were already located safely up a tree. To

control for the possibility that these 'non-calling trials' might have influenced the result

patterns, I re-ran the previous analyses after excluding these trials. Results did not change

and are shown in Figure 3.3.

Once again, there was no difference in call rates between males (N=18) and

females (N=20; Mann-Whitney, two-sample test: Z=-0.53, P=0.599); between lactating

females (N=16) and non-lactating females (N=9; Z=0.68, P=0.497); between females

before (N=11) and after pup emergence (N=20; Z=0.68, P=0.496); between females with

offspring as neighbours (N=5) versus not (N=19; Z=0.14, P=0.887); or between resident

(N=13) and immigrant males (N=8; Z=0.94, P=0.346). Again, there was a significant

difference in calling rates between squirrels with only short-term neighbours (N=20) and

those with at least one long-term neighbour (N=26; Z=-2.39, P=0.016), the effect

matching that observed in the previous analysis of experimental trials with simulated

predators: squirrels with short-term neighbours called more than squirrels with long-term

neighbours.

Finally, to further test whether non-calling trials were influencing outcomes, I

used contingency analyses to test for possible patterns in the tendency to call or not in

experimental trials (Table 3.2). This analysis revealed no significant effects: there was no

difference in the tendency to call or not between males (N=18) and females (N=21;

χ_2=0.010, P=0.917); between lactating (N=17) and non-lactating females (N=9; χ_2=0.13,

P=0.712); between females before (N=15) and after pup emergence (N=21; χ_2=1.17,

P=0.280); between females with offspring as neighbours (N=6) versus not (N=20;

χ_2=0.29, P=0.592); between resident (N=13) and immigrant males (N=8; χ_2=0.012,

P=0.734); and between squirrels with long-term (N=27) versus short-term neighbours

(N=21; χ_2=0.74, P=0.391).

3.3.3 Vocal Responses to Natural and Simulated Predators: Seet-barks Only

Because seet-barks are far louder and more conspicuous than seets, and thus are arguably

more likely to be heard by distant listeners, I re-ran all the previous analyses after

excluding seet calls from the sample. Result patterns did not change, with one exception:

in the 'callers only' condition of the experiments using simulated predators, the previous

effect that squirrels with short-term neighbours called more than those with long-term

neighbours disappeared (Z=1.04, P=0.299).

3.4 DISCUSSION

Overall, my analyses of call production in a wild population of red squirrels during

encounters with natural disturbances and in simulated predator experiments do not

provide clear or consistent support for any of the traditional functional hypotheses

concerning anti-predator vocalisations. First, I did not find evidence that predator-

associated vocalisations in red squirrels function as warnings to kin. Females did not call

more than males, as has been documented in some female-philopatric species (Sherman

1977, 1980; Schwagmeyer 1980). I also found that red squirrel females who were

lactating and caring for dependent offspring did not call more than females who were

currently without dependent offspring, nor did they call more after their pups emerged

from the nest and thus were more vulnerable to predators than they were before

emergence, both of which are patterns noted previously for black-tailed prairie dogs and

Columbian ground squirrels (Hoogland 1983; MacWhiter 1992). Finally, females who

were known to have adult offspring as neighbours did not call more than females who

were not known to have offspring as neighbours.

Second, I did not find evidence that predator-associated vocalisations in red

squirrels function to warn potential mates either. Here, the reproductive success of males

after copulation is expected to hinge on the survivorship of female mates more than the

reproductive success of females hinges on the survivorship of previous male mates, who

provide no additional offspring care (Krams e al. 2006). As a consequence, the benefits

of warning previous mates about potential predatory threats should be greater for males

than it is for females, and males then would be expected to call more than females,

particularly resident males who had much greater opportunity to mate with local females

than recently immigrated males who had comparatively few local mating opportunities.

However, I found no differences in rates of calling between males and females or

between resident and immigrant males.

Finally, I found only weak and inconsistent evidence that predator-associated

vocalisations in red squirrels might function to warn territorial neighbours with whom

one has an established relationship that might be worth preserving. I did find that males

and females, who are equally likely to have such neighbours, called at equivalent rates.

However, strictly speaking, the absence of an effect, even if predicted *a priori*, can be considered only relatively weak support in favour of a hypothesis. More interestingly, I found a difference in calling rates between squirrels with at least one long-term neighbour and those with only short-term neighbours. However, the direction of this effect was not consistent. In encounters with real predators, squirrels with long-term neighbours called more than those with only short-term neighbours. But, in encounters with simulated predators, the pattern was reversed, individuals with only short-term neighbours called more than those with long-term neighbours. It is possible that this shifting pattern reflects a real difference in squirrels' behaviour in the two kinds of predator encounters. However, it is not at all clear what sorts of factors might create such a shift that would not also create other differences in the squirrels' behaviour and calling in the two situations, which was otherwise shown to be very similar (Digweed & Rendall 2009). As a result, although the potential for neighbour-effects might be fruitfully pursued in future work, the weak and inconsistent effects observed here are, for now, more parsimoniously interpreted as unreliable support for a neighbour-warning function for predator-associated calling in red squirrels.

3.4.1 Vocal Deterrence of Predators and Other Intruders

If the predator-associated vocalisations of red squirrels do not function as vocal warnings to kin, mates, or neighbours, then to whom are these vocalisations actually addressed and how do they function? One possibility is that the calls function as announcements to the predators themselves that they have been detected and that additional pursuit is unlikely to be profitable (Hasson 1991;Caro 1995). Similar predator-directed signals have been

described in other species, where they seem to function in deterring predators (Tilson & Norton 1981; Hersek & Owings 1993; Zuberbühler et al. 1999; Blumstein 2007). For example, when they spot a terrestrial predator, such as a lion or a cheetah, Thomson's gazelles in eastern Africa sometimes engage an exaggerated bounding display, called stotting. Stotting involves a moderate retreat from the predator using repeated bounding leaps rather than running directly away from it (Caro 1986). Stotting also makes individuals additionally conspicuous and almost certainly draws the attention of the predator to them. However, that effect may be commensurate with the selected function of the behaviour if the stotting effectively announces to the predator that it has been detected and that it is unlikely to succeed in preying on a fit individual that can afford to squander time and energy stotting rather than running directly away. Caro (1986) has shown that, in fact, cheetahs preying on gazelle are more likely to chase and kill individuals that do not stot compared to those that do.

A similar predator announcement and deterrent function has been suggested for alarm calls produced by some nonhuman primates. For example, Zuberbühler et al. (1997, 1999) have shown for several different monkey species in the Tai forest, Ivory Coast, that individuals produce loud, conspicuous alarm calls and sometimes approach and inspect predators, such as the leopard, that rely on stealth (or ambush) to capture their prey. In contrast, the same individuals retreat silently when they detect a pursuit predator, such as group-hunting chimpanzees, that are comparatively undeterred by having been detected and instead rely simply on outpacing and outflanking their prey. Furthermore, through observations of a radio-collared leopard, Zuberbühler et al. (1999)

confirmed that the conspicuous alarm calls produced by the monkeys can effect the behaviour of predators whose hunting success hinges on remaining undetected: following either natural vocalisations produced by the monkeys, or experimental playback of their calls, the radio-collared leopard tended to give up its hunt and leave the area.

An even broader precedent for this pattern of predator-directed calling and behaviour exists in passerine birds. As first documented by Marler (1955), many passerines produce two, structurally distinct types of alarm calls. One type is a loud, harsh (i.e., broadband) call that is typically accompanied by close approach, inspection and mobbing of a predator. The structural features of this call make it conspicuous and easy to localise and thus well-suited to recruiting additional mobbers to harass and thereby deter predators. In contrast, a second call type, often referred to as a 'seet', is comparatively soft, high-frequency and tonal. These properties make the seet call relatively difficult for predators to localise and the seet call is also typically accompanied by immediate withdrawal of callers from the area.

Taken together, these examples point to the possibility that the predator-associated vocalisations of red squirrels might serve a similar predator-deterrent function. A number of aspects of the calling and associated behaviour of the red squirrels are certainly consistent with such an account. For example, like passerine birds, the predator-associated calls of red squirrels include both a relatively soft, high-frequency and tonal seet variant and a much louder, harsh, broadband variant, either a 'bark', or a

'seet-bark'. The soft, high-frequency qualities of the seet mean that this call type transmits poorly in forest habitats and is unlikely even to be heard by other squirrels on neighbouring territories. However, at the same time, these same properties make red squirrel seets structurally similar to the seet calls of passerines. As noted by Greene & Meagher (1998), this structural convergence involves minimising the temporal, phase and intensity cues that are used by bird and mammalian predators to localise sounds (Brown 1983; Heffner 1998; Grothe 2003). As a result, red squirrel seet calls, like the ventriloquial seet calls of passerines, are probably difficult for predators to localise. In this respect, it is also significant that red squirrels produce seet calls disproportionately at the start of calling bouts in response to predators (Digweed & Rendall 2009). This temporal bias in the production of ventriloquial seets might be functional if it is important for squirrels to remain inconspicuous in the initial stages of predator encounters until the more specific nature of the predatory threat can be established.

However, when predators persist in the area, squirrels have been shown to switch to producing the loud, harsh call variant, which they then produce repeatedly for up to 10-minutes (Digweed & Rendall 2009). The loud, broadband structure of this call type and its protracted repetition make it extremely conspicuous. These features of the bark-type call variant, in conjunction with the disproportionate production of this call type in the later stages of encounters with predators, is consistent with a function in announcing to predators that they have been detected but only after the squirrels have identified the nature of the threat and are positioned relatively safely in a tree.

There are additional aspects of squirrels' responses to predators that are consistent with a predator-directed, deterrent function of vocalising. For example, when predators persist in the area, in addition to producing protracted streams of repeated broadband, bark-type calls, red squirrels, like passerine birds, often approach toward and inspect the predator rather than retreating. At the same time, they produce additional conspicuous foot-stomping and tail-flagging behaviours (Smith 1968; Gurnell 1987; Digweed & Rendall 2009). In solitary red squirrels, such foot-stomping and tail-flagging is unlikely to be visible to other squirrels in neighbouring territories that are, on average, 100-m distant through dense coniferous forest. Hence, these additional behaviours are unlikely to serve as a signal to conspecifics. However, they could be visible to predators at close range, and, indeed additional conspicuous behaviours like these have been reported to accompany vocalising in other squirrel species (Tamura & Yong 1993; Owings et al. 2001) and could serve a complementary function in drawing the predators' attention, emphasising that it has been detected, and thereby discouraging it from persisting in the area (Caro 2005). In some cases, tail-flagging behaviour, by itself, seems to serve a deterrent function for at least some types of predator (Hersek & Owings 1993; Rundus et al. 2007).

An additional feature of the calling patterns of red squirrels that is consistent with a predator-directed function of their calls is the fact that, paradoxically, the calls are not actually restricted to encounters with predators. They are also produced in encounters with other intruders (Lair 1990; Price et al. 1990; Digweed & Rendall 2009). These include humans and other forest interlopers but importantly also conspecific squirrels

that frequently trespass to steal cones stored in central middens and distributed caches in neighbours' territories. Notably, squirrels produce the same seet and seet-bark calls when they encounter conspecific intruders as they do during encounters with predators, and with the same temporal patterns (Digweed & Rendall 2009). On the surface of it, this convergence in calling behaviour in the two contexts seems puzzling and has bedeviled previous attempts to understand the alarm calling behaviour of red squirrels (Embry 1970; Searing 1977; Smith 1978). Why would predator alarm calls be given in encounters with other squirrels intruding on one's territory? What could possibly unite these disparate contexts?

One possibility is that conspecific intruders, like predators, also represent a serious threat to individual survival. In fact, trespassing and cone pilfering is ubiquitous in red squirrels and represents a major threat to survival (Rusch & Reeder 1978; Price et al. 1990). Cone losses to theft by neighbours can account for up to 84% of the stored food supply that individuals rely on to get through long, harsh winters in the temperate zone (Gerhardt 2005). Given the energetic constraints facing a small-bodied, non-hibernating mammal overwintering in boreal forests, cone loss might therefore represent as serious a threat to red squirrel survival as do many forms of predation. Viewed in this light, the common use of seets and seet-barks in both predator encounters and territorial intrusions may not be so puzzling. Instead, it may reflect a common attempt to announce detection of intruders of various kinds in an effort to deter them. In the case of conspecifics, squirrels take even more actives steps to repel territorial intruders. In addition to vocalising as they do in encounters with predators, resident squirrels in these

92

circumstances also aggressively confront and chase conspecific intruders out of their territory (Nodler 1973; Lair 1990; Price et al. 1990).

An important corollary of this account is that the predator-associated vocalisations of red squirrels are not about predators *per se*. As a result, they are probably not best interpreted as predator-specific, referential warnings similar to the referentially-specific alarm calls of some other animal species. Rather, the 'alarm' calls of red squirrels seem more conservatively interpreted as reflecting a broader concern with disturbances of various kinds that threaten territory integrity and individual survival be they predators, conspecific intruders, or other territorial interlopers, such as other mammal or bird species that forage opportunistically on stored cone supplies.

Table 3.1. Research hypotheses and predictions generated.

Demographic Categories	Warning Kin	Warning Mates	Warning Neighbours
Males (M) and Females (F)	F > M	M > F	M = F
Lactating (F/L) and Non-lactating (F/NL) Females	F/L > F/NL		
Females Before (F/B) and After (F/A) Pup Emergence	F/A > F/B		
Females With (F/W) and Without (F/WO) Offspring as Neighbours	F/W > F/WO		
Resident (M/R) and Immigrant (M/I) Males		M/R > M/I	
Individuals with Long-term (LT/N) and Short-term Neighbours (ST/N)			LT/N > ST/N

Table 3.2 Percentages of callers and non-callers used for Chi-square analysis in the demographic categories of: (a) males versus females; (b) females with offspring as neighbours versus without offspring as neighbours; (c) lactating versus non-lactating females; (d) females after versus before pup emergence; (e) immigrant versus resident males; (f) individuals with long term neighbours versus short term neighbours.

(a)

	Callers	**Non-Callers**	**Total**
Female	32.3%	17.4%	49.7%
Male	32.3%	18.0%	50.3%
Total	64.7%	35.3%	100.0%

(b)

	Callers	**Non-Callers**	**Total**
With	50.6%	28.9%	79.5%
Without	14.5%	6.0%	20.5%
Total	65.1%	34.9%	100.0%

(c)

	Callers	**Non-Callers**	**Total**
Lactating	20.5%	9.6%	30.1%
Non-Lactating	44.6%	25.3%	69.9%
Total	65.1%	34.9%	100.0%

(d)

	Callers	**Non-Callers**	**Total**
After	45.8%	20.5%	66.3%
Before	19.3%	14.5%	33.7%
Total	65.1%	34.9%	100.0%

(e)

	Callers	**Non-Callers**	**Total**
Immigrant	23.8%	11.9%	35.7%
Resident	40.5%	23.8%	64.3%
Total	64.3%	35.7%	100.0%

(f)

	Callers	**Non-Callers**	**Total**
Long-term	39.5%	24.0%	63.5%
Short-term	25.1%	11.4%	36.5%
Total	64.7%	35.3%	100.0%
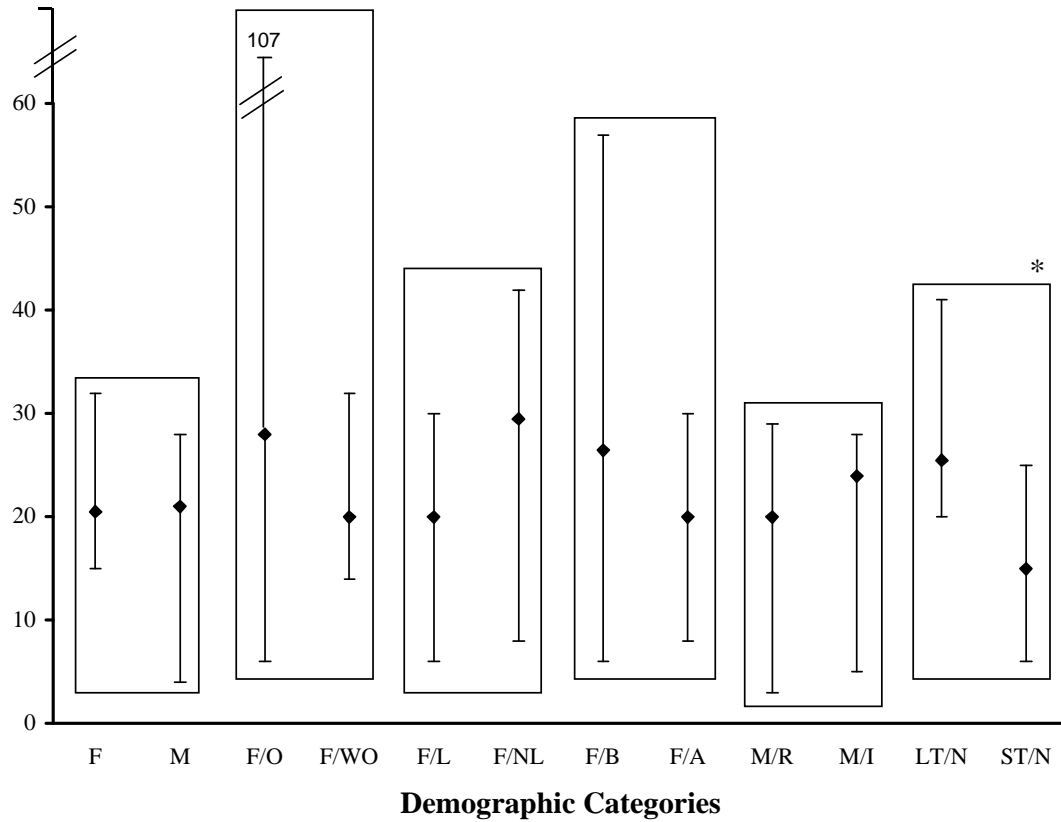
Figure 3.1. Mann-Whitney comparisons of the median number of calls produced during natural disturbances by individuals representing several different demographic categories. Categories listed are: M - male; F - female; F/O - females with offspring as neighbours; F/WO - females without offspring as neighbours; F/L - lactating females; F/NL - non-lactating females; F/B - females before pup emergence; F/A - females after pup emergence; M/R - resident males; M/I - immigrant males; LT/N - individuals with long term neighbours; ST/N - individuals with short term neighbours
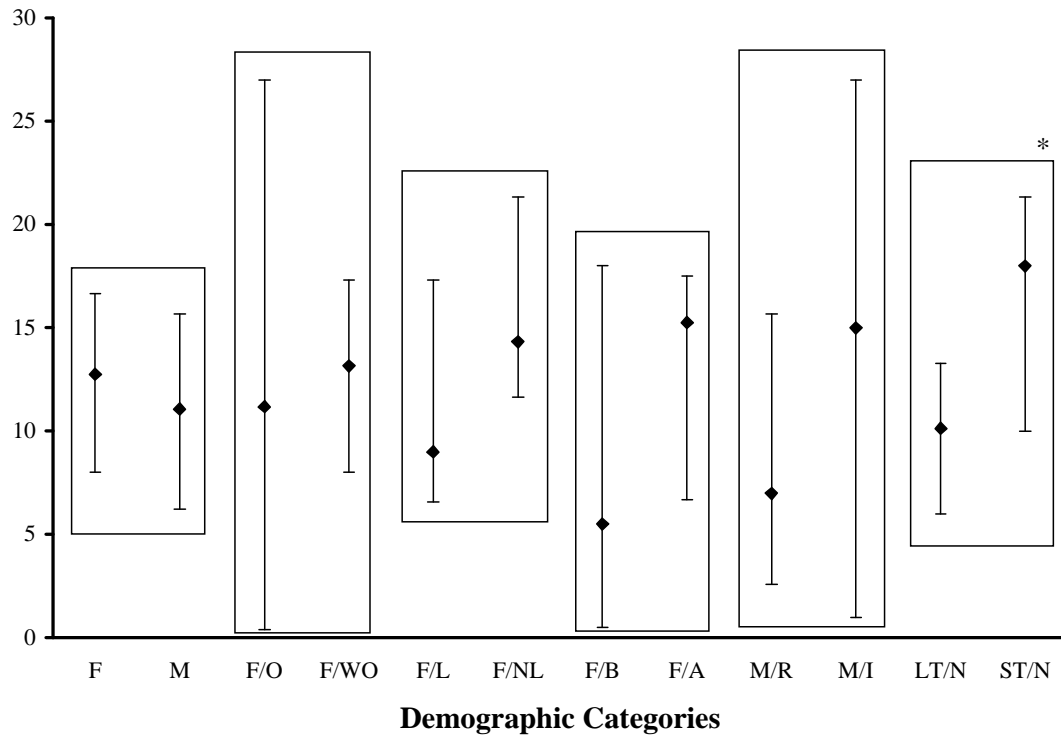
Figure 3.2. Mann-Whitney comparisons of the median number of calls produced in experimental trials using simulated predators by individuals representing several different demographic categories. Categories listed are: M - male; F - female; F/O - females with offspring as neighbours; F/WO - females without offspring as neighbours; F/L - lactating females; F/NL - non-lactating females; F/B - females before pup emergence; F/A - females after pup emergence; M/R - resident males; M/I - immigrant males; LT/N - individuals with long term neighbours; ST/N - individuals with short term neighbours.
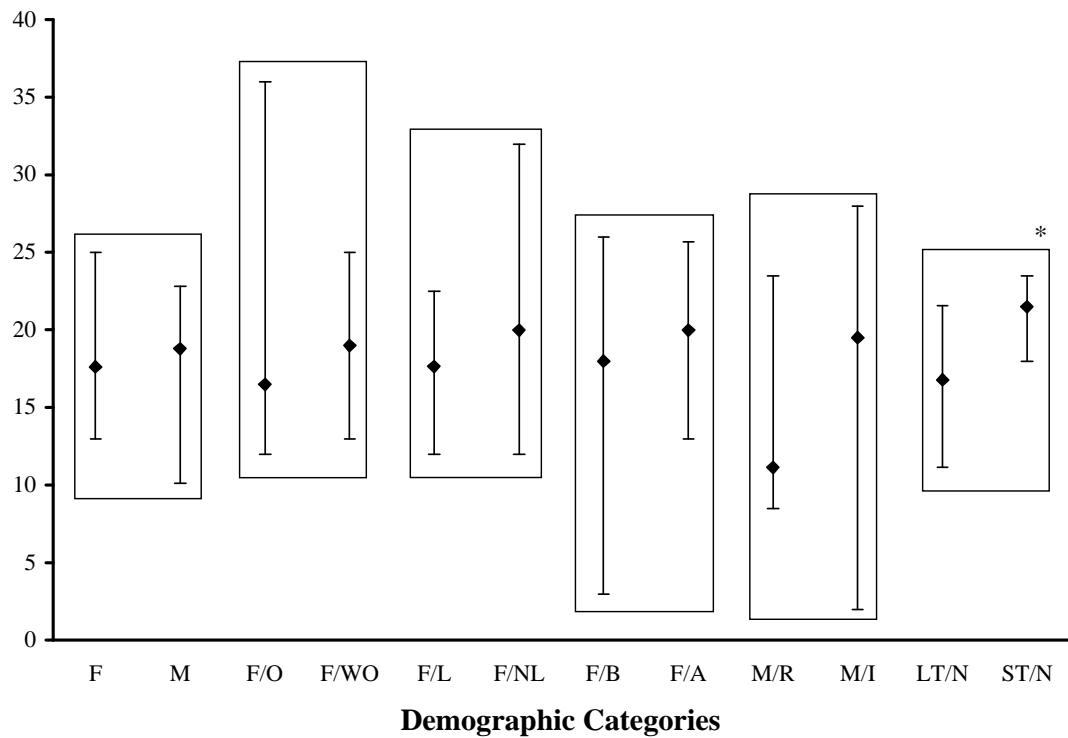
Figure 3.3. Mann-Whitney comparisons of the median number of calls produced in experimental trials using simulated predators by individuals representing several different demographic categories. Only individuals producing calls in experimental trials are included in this analysis. Categories listed are: M - male; F - female; F/O - females with offspring as neighbours; F/WO - females without offspring as neighbours; F/L - lactating females; F/NL - non-lactating females; F/B - females before pup emergence; F/A - females after pup emergence; M/R - resident males; M/I - immigrant males; LT/N - individuals with long term neighbours; ST/N - individuals with short term neighbours.

CHAPTER FOUR

PREDATOR-ASSOCIATED VOCALISATIONS IN NORTH AMERICAN RED SQUIRRELS: ADDITIONAL TESTS OF REFERENTIALITY

4.1 INTRODUCTION

There has been considerable theorising concerning the mechanisms that underlie and support systems of alarm calling in animals, much of it focused on the possibility that the calls themselves sometimes provide specific information about, or a reference to, particular aspects of predator encounters (Hauser 1996). For example, the structurally distinct alarm signals produced by some animal species appear to track the general category of predator encountered, such as aerial versus terrestrial predators, or more specific types of predators such as leopards, eagles, and snakes. Given their apparent predator-specificity, such calls have been labeled 'referential' because they appear to function as symbols for the predators themselves in much the same way that the words of language do (Cheney & Seyfarth 1990; Macedonia & Evans 1992; Evans 1997).

In order for a set of vocalisations to be considered referential, they must meet two specific criteria. First, the calls must exhibit some degree of 'stimulus-specificity', meaning that they are elicited only by a specific type, or subset, of predators and are not produced in other contexts, and the types of predators that elicit one type of call are different from those that elicit another type of call. Second, the calls must be sufficiently distinct from one another so that listeners can discriminate them and, importantly, that the responses the calls elicit from listeners must be 'context-independent'; that is, the calls

themselves must be sufficient to elicit appropriate escape response from listeners in the absence of supporting contextual information, including having already seen the predators themselves (Marler et al. 1992; Evans 1997).

A number of primate taxa have been reported to produce alarm calls that meet both criteria for referentiality (e.g. Zuberbühler 2000; Fichtel & Kappeler 2002; Kirchlof & Hammerschmidt 2006). The best documented example comes from the alarm call system of vervet monkeys (Seyfarth et al. 1980). Vervets are known to move both on the ground and in the trees and are thus exposed to a wide variety of predators, including large cats, raptorial birds, and snakes. Observational and experimental research has shown that vervet monkeys produce three structurally distinct alarm calls to these three different classes of predator, and that each predator induces a distinct behavioural response that is appropriate to it (Seyfarth et al. 1980). Furthermore, individuals that hear an alarm call engage in the appropriate response even if they themselves have not actually seen the predator. Hence, the calls demonstrate a degree of stimulus-specificity in their patterns of production and context independence in listeners' responses and thus seem to refer to or symbolise the predators in a way that supports adaptive behavioural responding (Seyfarth et al. 1980).

Subsequent research has documented similar systems of acoustically distinct, predator-specific alarm calls in a handful of other small mammals including, Columbian ground squirrels, chipmunks and meerkats (MacWhiter 1992; Da Silva et al. 1994; Manser 2001). North American red squirrels have also been reported to produce predator-

specific, referential alarm calls. Greene & Meagher (1998) reported that the squirrels produce two main alarm call types, a tonal seet call and a harsher, broadband bark call and the production of these two call types was closely associated with encountering either an aerial or a terrestrial predator, respectively. In other words, they reported a degree of stimulus-specificity in the production of the two call types. Based on this, they proposed that the calls functioned referentially to convey to listeners specific information about the type of predator encountered (Greene & Meagher 1998).

However, this study did not report on the behavioural responses of the squirrels to predators and whether they were differentiated for different classes of predators, nor were experiments conducted to test whether or not the different call types were sufficient to elicit potentially distinct escape responses. In other words, there is as yet no evidence that the predator-associated vocalisations of red squirrels meet the second criterion of referential signals, namely that they support context-independent responses in listeners. To address this important gap in our understanding of these calls, I report in Part I of this chapter a systematic set of playback experiments that test the specificity of red squirrels' responses to hearing seets and seet-barks produced by other squirrels during encounters with predators.

At the same time, my earlier work indicated that seet and seet-bark calls are typically produced in mixed bouts to all predator types and this finding alone seems to undermine the argument for predator-specificity in their usage. However, although seets and seet-barks do not themselves seem to map onto categorical distinctions in predator

class, it is still possible that functional, predator-specific variation exists within calls of one or both types. That is, there could be acoustically discrete subtypes within either the seet call or the seet-bark call that are associated with aerial versus terrestrial predators. The fact that the two broader call types are mixed within the bouts might then be tangential to their potential referentiality if cues to predator variation are not carried at this level but rather are conveyed by variation within these broader call types. To address this possibility, I report in Part II of this chapter detailed acoustic analyses that examine potential substructuring within both seets and seet-barks that might be aligned with the different classes of disturbance that elicit them.

PART I. RESPONSE SPECIFICITY

4.2 METHODS

4.2.1 Experimental Design

As an additional test of the potential referential quality of seets and seet-barks, I conducted a controlled playback experiment using calls recorded previously from squirrels in natural encounters with aerial and terrestrial predators. The experiment was designed to test the potential specificity of squirrels' behavioural and vocal responses to seets and seet-barks produced by other squirrels in the vicinity during encounters with predators. If seets and seet-barks have some referential value, as proposed by Greene & Meagher (1998), then squirrels that hear the calls of others should show some differentiation in their responses according to the type of predator that they represent.

The experiment was conducted using three sets of calls. The first two of these involved pure bouts of either seets or seet-barks, previously proposed to represent aerial and terrestrial threats, respectively. The third call set was a combination condition that involved a mix of both seets and seet-barks and was included specifically because my previous work showed that seets and seet-barks tend to be produced in mixed bouts to all classes of predator (Digweed & Rendall 2009).

Each set of calls was constructed in two forms, one in which the calls in a bout were concatenated together rapidly (a fast condition) and the other in which they were concatenated together more slowly (a slow condition). These fast and slow conditions were designed to allow testing of the additional functional possibility that the rate of call delivery conveys something to conspecifics about the imminency of the predatory threat involved or the urgency of the response that is required.

The experimental design thus entailed six experimental conditions comprised of three different call sets (pure bouts of seets, pure bouts of seet-barks, and mixed bouts that combined seets and seet-barks) presented at two different call-delivery rates (fast and slow).

4.2.2 Stimulus Construction and Presentation

I constructed stimuli for the six different experimental conditions using vocalisations previously recorded from eight different individuals in the study population. These vocalisations were recorded during natural encounters with aerial and terrestrial

predators. Seets and seet-barks for use in experiments were excised from within bouts of calls produced during such encounters.

The fast condition of both the seets and seet-barks involved a series of four different exemplars of each type produced by the same caller and concatenated into a continuous bout of calling lasting 60 seconds. I varied the intervals between adjacent calls within each 4-call series to avoid the artificiality that might result from the sound of four calls entirely evenly spaced and continuously repeated for 60 seconds. And I created three variants that preserved the interval variation between calls but shuffled its patterning to create a fully balanced set of inter-call variation. Thus, in the first variant of the 4-call series, the interval between the first and second call in each 4-call series was set at 600 ms, with the intervals between the second and third and between the third and fourth calls set at 300 ms. In the second variant of the 4-call series, the interval between the first and second call was set at 300 ms, the interval between the second and third call was 600 ms, and the interval between the third and fourth calls was 300 ms. In the third variant, the interval between the first and second call, and between the second and third call was 300 ms, while the interval between the third and fourth call was 600 ms. These three variants of the 4-call series were concatenated together, with a constant interval of 800 ms between each 4-call series. And this sequence of 4-call series variants was repeated to produce a single continuous stimulus bout lasting 60 seconds (Fig. 4.1).

The slow condition of both the seets and seet-barks was constructed in exactly the same fashion, except that the intervals between calls within a 4-call series and between

concatenated series was doubled (i.e., 1200 ms, 600ms and 1600 ms, respectively). The

entire duration of these stimuli was also therefore doubled (to 120 seconds) in order to

keep constant the number of individual calls that subjects heard across the fast and slow

experimental conditions. The only difference between the two conditions was the rate of

call delivery and, ultimately, the time period over which the calls were delivered (120

versus 60 seconds).

I constructed 3 different sets of fast and slow versions of the seet and seet-bark

stimuli, each set constructed of calls produced by a different squirrel but matched for

caller within a particular set (i.e., a seet:fast and seet:slow stimulus from each of three

different callers; and a seet-bark:fast and seet-bark:slow stimulus from each of three

different callers; Total = 12 different experimental stimuli).

The combination-call stimuli were constructed from naturally mixed bouts of

seets and seet-barks produced during predator encounters. They involved no internal sub-

structuring of call series by me but rather simply preserved mixed call sequences whose

rate of call delivery varied and approximately matched that used in my fast and slow

conditions of pure bouts of seets and seet-barks. These naturally mixed-bouts were then

truncated either at 60 ms or 120 ms to match the length of the other stimuli. Six such

bouts were used as experimental stimuli, three each in the fast and slow experimental

conditions. Each bout came from a different caller.

All experimental stimuli were assembled into individual stimulus files using PRAAT © 4.6.3 (Boersma 2001). Playback stimuli were then stored on and played from a Panasonic CF-P1 handheld computer. They were played back through a Mineroff SME-AFS portable speaker (frequency response 110Hz-12kHz). Standardised amplitudes were used for each stimulus and were established during a pre-testing period in a similar area of forest 1km away from the study site and out of the hearing range of squirrels who would subsequently serve as experimental subjects. Stimuli were played from a distance of 50m at a variety of different amplitudes to establish realistic levels for the different call types at this distance and in this type of forest. Thereafter, amplitude settings were held constant for each stimulus type in experimental trials.

4.2.3 Playback Trials

Experimental trials were conducted during the course of daily observations of focal squirrels only as appropriate conditions arose. These included the following requirements: that the focal squirrel had not encountered a predator that day; that it had been in its territory for the previous 15 minutes; that it had not had a territorial interaction with another squirrel in this period; that it had not itself vocalised in this period; and that no vocalisations (including seets or seet-barks) had been heard from neighbouring squirrels during this period.

When these conditions were met, a playback trial involved playing back calls from one of the six experimental conditions from a speaker hung approximately 2-meters up a tree located next to the central midden of an adjacent territory (approximately 50m

away). Playing calls from an adjacent territory allowed me to simulate a predator nearby without introducing the additional possible confound that the calls would be interpreted as representing a territorial intrusion were they heard emanating from a location within the subject's territory.

The focal squirrel was observed for 10-minutes post-playback to record its immediate reactions to the playback stimulus. During this time, all vocalisations and behaviours were recorded in an all-occurrences fashion. An extended 15-minute follow was then conducted on the focal squirrel to catalog any more delayed or protracted vigilance response squirrels might have had to the experimental stimulus. To facilitate analysis of any more protracted responses, I compared a subject's vigilance (head-up scanning behaviour) during this 15-minute focal follow period post-playback to the same individual's vigilance behaviour during a matched, 15-minute focal follow conducted the previous day. The latter period thus served as a baseline against which to test any possible differences observed in the period following experimental trials.

4.3 RESULTS

4.3.1 Immediate Reactions to Experimental Stimuli

A fully balanced set of 54 playback trials was conducted on nine squirrels, each individual serving as a subject in all six experimental conditions (Table 4.1). Squirrels appeared to register the calls presented to them. On presentation of the playback stimulus subjects typically stopped their current activity and either looked briefly (96.3%) or oriented their entire body (66.7%) in the direction of the speaker. However, they did not

ever produce vocalisations themselves in response to hearing the calls of others. Nor were

their initial reactions and orientations to the calls differentiated by experimental condition

(Initial reaction, Pearson's Chi-Square: χ_2=1.13, P=0.56, N=54; Orientation to speaker:

χ_2=0.70, P=0.70, N=54).

Beyond their initial reactions, subjects rarely showed obvious additional

responses. For example, they seldom moved any appreciable distance either up or down a

tree or across the ground immediately after playback (16.8% of trials) and there was no

variation in these movements as a function of experimental condition (Pearson's Chi-

Square: χ_6=5.87, P=0.44, N=54).

4.3.2 Protracted Vigilance Responses

Squirrel's vigilance behaviour was increased in the 15-minute period following playbacks

compared to baseline conditions (ANOVA: $F_{1,79}$=15.03, P=0.00021, N=81; Fig. 4.2).

However, there was no difference in vigilance as a function of experimental condition:

there was no main effect either of the type of call heard ($F_{2,48}$=1.8, P=0.18, N=54) or of

the rate of call delivery ($F_{1,48}$=0.11, P=0.73), and there was no significant interaction

between the two ($F_{2,48}$=0.10, P=0.9; Fig. 4.3).

4.4 DISCUSSION

In order for an alarm call type to be labeled referential or context specific there are two

main criteria that must be fulfilled (Evans 1997). First, calls should be structurally

discrete and produced only in response to a particular context. Second, calls should be

context independent, which means that in the absence of contextual information calls should elicit appropriate behavioural responses in listeners (Evans 1997).

My previous results on call production patterns in red squirrels tend to undermine the first of these two criteria for referential signals because I found that seets and seet-barks were produced together in mixed bouts in response to predators of all major types, as well as to conspecific intruders (Digweed & Rendall 2009). They did not therefore show the requisite stimulus-specificity required of referential signals.

Of course, the ultimate arbiter of the referential value of different signals is how listeners respond to them in the absence of supporting contextual information. So, although production of the two call types does not appear to be predator-specific, it is perhaps possible that listening squirrels can nevertheless effectively differentiate the eliciting contexts only from hearing the calls and engage in appropriately distinct responses for the different types of predator implicated.

However, results of a playback experiment specifically designed to test this possibility do not provide any support for it. The experiment used stimuli constructed from seets and seet-barks recorded during confirmed encounters with predators, but the squirrels' responses to the two, putatively referential call types were not differentiated in any observable way. Squirrels did register and respond to both types of calls played back to them. Indeed, they looked or oriented in the direction of the playback speaker in almost all experimental trials. They also showed a more protracted vigilance in the period

109

following call playback. However, neither their immediate reactions nor their more protracted vigilance responses were differentiated as a function of the type of call they heard. Nor were they differentiated by the rate of call delivery. As a result, although the calls were salient to other squirrels and induced some short- and longer-term responses from them, there was no evidence that the calls conveyed to listeners any more specific information about the type of predator encountered or the type of response that might be most appropriate.

Taken together, the apparent lack of specificity in the production of seets and seet-barks and the lack of any obviously differentiated responses in listeners hearing these calls strongly suggest that these predator-associated vocalisations of red squirrels do not meet the criteria established for referential signals.

At the same time, it is important to consider that there may yet be some potential for referentiality in these calls. For instance, it is possible that predator-specific information is conveyed to listeners not in the distinctions between seets and seet-barks *per se* but in subtle variations in the structure of calls within these two broader classes. This possibility is specifically addressed in the next section.

PART II. ACOUSTIC DIFFERENCES WITHIN SEETS AND SEET-BARKS

Although seet and seet-bark calls do not themselves appear to map onto categorical distinctions among predator types or elicit predator-specific behavioural responses from listeners, it is nevertheless possible that within each of these broad call types there exists acoustic variation that is associated with predator class distinctions. To test this possibility, I undertook a comprehensive set of acoustic analyses to explore the possibility that there are structural differences within seets and within seet-barks that reflect the three main categories associated with production of these calls, namely aerial predators, terrestrial predators and conspecific intruders.

4.5 METHODS

4.5.1 Study Site and Subjects

Research was conducted at the R.B. Miller Field Station in the Sheep River Valley of Kananaskis Provincial Park, Alberta (50°39′ N, 114°39′ W), which is situated in the foothills of the Canadian Rockies. The habitat in the Sheep River Valley is a mix of aspen (*Populus tremuloides)* parkland and montane (sub-alpine) coniferous forest composed primarily of lodgepole pine (*Pinus contorta*) and white-spruce (*Picea glauca*). Research focused on a population of 47 individually marked squirrels in a single, contiguous forest patch approximately 60 ha in size. In order to provide positive individual identifications, each squirrel was uniquely marked with a dye mark and a small numbered tag to be placed in one or both ears (National Band and Tag Company, Kentucky). Dye marks were visible at distances up to 20 meters facilitating positive

identification under most conditions, while the numbered ear tags also facilitated

recognition of marked animals at a distance and the unique numbers on the tags allowed

certain identification across seasons and across years when animals were re-trapped (for

detailed methods see Digweed & Rendall 2009).

Research was conducted in three consecutive years (2005-2007) between May

and November, representing the late spring, summer, and fall seasons. Data collection

focused on all natural encounters with known predators as well as encounters with non-

predatory species and with conspecific intruders. For each encounter, I noted the

disturbance or predator species involved and details of the squirrel's response to it, while

a continuous recording was made of all types of vocalisations produced. Vocalisations

were recorded using a digital Marantz PMD660 recorder and a Sennheiser ME66 shotgun

microphone with a K6 powering module and a Sennheiser MZH60-1 windscreen. All

vocalisations were digitally recorded at 44.1 kHz with 16-bit accuracy.

4.5.2 Vocal Sample and Measurement

My previous work indicated that this population of red squirrels produced primarily only

two call types in encounters with predators, non-predatory species and conspecific

intruders. These call types were the tonal seet and a more broadband, combination call, a

seet-bark. That work also indicated that these two call types were not produced in

predator-specific fashion but rather were produced together in mixed bouts where their

patterning reflected the persistence of any type of disturbance rather than specific classes

of predator *per se* (Digweed & Rendall 2009). Although seets and seet-barks were mixed

within calling bouts to all different classes of predator, it is nevertheless possible that there are salient differences in the acoustic features within each of these two call types in encounters with different predators that in some way then reflect the type of disturbance involved.

To test this possibility, I first limited the complete dataset of vocal recordings only to calls produced in encounters with confirmed predators or conspecific intruders. Encounters with conspecifics were included in this analysis for consistency with earlier work because my previous analyses showed that the squirrels produce the same call types in the same patterns in these encounters as they do in encounters with predators (Digweed & Rendall 2009). At the same time, because conspecific intruders represent a substantial threat to survival but in a very different way than do predators, it is possible that this variation might select for salient differentiation in seets and seet-barks produced in encounters with other squirrels compared to encounters with known predators.

To standardise the dataset and reduce potential confounding influences on call differentiation related to vocal differences between individuals, I attempted to construct a fully balanced sample including only individuals who had encountered all three of the disturbance categories to be tested: aerial predator, terrestrial predator and conspecific intruder. However, this was not entirely possible given inevitable natural variation in individual's exposure to these disturbances. In the end, the sample for analysis included 10 different individuals, two of whom encountered all three disturbance types, four of whom encountered both an aerial and terrestrial predator, and four of whom encountered

only a conspecific intruder (Table 4.1). These 10 individuals contributed a total of 18 different disturbance encounters balanced for disturbance type (i.e., six encounters for each of the three disturbance categories: aerial predator, terrestrial predator and conspecific intruder).

Each calling bout was then divided into five different time bins following methods used previously (Digweed & Rendall 2009). The initial 30 seconds of each bout was divided into three successive 10-second time bins (i.e.; 0-10 seconds, 10-20 seconds, 20-30 seconds). The latter portion of each bout varied in length (30 – 300 seconds) and so was divided equally into a middle and an end time bin. To balance calls produced across these time bins, I selected two seets and two seet-barks from each of the five time bins, resulting in a total of 10 seets and 10 seet-barks for each of the 18 different disturbance encounters. From this sample, I then selected only the best quality sounds for acoustic analyses. This resulting sample consisted of six seets and nine seet-barks from each of the 18 encounters, yielding a total call sample of 108 seets and 162 seet-barks, in each case divided equally between the three disturbance categories (i.e., 36 seets and 54 seet-barks produced to aerial predators, to terrestrial predators and to conspecific intruders).

4.5.3 Acoustic Analysis

To examine potential structural differences within seets and seet-barks, I measured a large number of specific acoustic features designed to comprehensively characterise the temporal, intensity and spectral characteristics of each call type. All measurements were conducted using PRAAT© 4.6.3 (Boersma 2001). For both seets and seet-barks, I

measured overall call duration. For seet-barks, I also specifically measured the duration

of the seet component, the bark component, and the silent 'gap' or interval separating

them. For both call types, I also characterised the abruptness of call onset, which

translates perceptually as its 'plosiveness'. I used two measures of call plosiveness,

specifically the time from call onset to maximum signal intensity expressed both as an

absolute time (ms) and as a proportion of total call duration (%).

I also measured several different spectral features of both call types. Because

seets were primarily tonal in nature, spectral measurements of these calls focused on the

fundamental (F_0), which translates perceptually as pitch. I used PRAAT to extract the F_0

(or pitch) contour for each seet and from this I retained as variables the frequency (in Hz)

and time (in milliseconds) of the F_0 at the start, middle and end of the call as well as at its

peak point in the call. I also measured the amplitude of the F_0 and higher harmonics from

a spectral slice centered on the midpoint of each call using a 250-point Fast Fourier

Transform (FFT). However, in most cases ($> 75\%$) seets were lacking harmonics of the

F_0 and so additional harmonic amplitudes were ultimately dropped from the analysis.

Although seets were generally tonal, there was some variation in their degree of

tonality. To capture this variation, I also developed a qualitative measure of relative

tonality by assigning to each call a number proportional to its relative tonality or

noisiness (1=noisy, 2=noise with minor tonal components, 3=noticeable tonal

components, 4=more tonality than noise, 5=tonal, no noise).

I measured the same spectral features of the seet portion of each seet-bark call. However, because seet-barks also contained an additional, broadband bark component I undertook several additional measurements of the bark component. The bark component of seet-barks typically had a noisy, broadband structure that lacked any stable F_0. Therefore, I used several different measurements to capture the distribution of spectral energy within the bark component. Here, I used PRAAT routines for quantifying the first four spectral moments of barks, which translate, respectively, as the center of gravity (mean), standard deviation, skewness and kurtosis of the energy in the call spectrum (Boersma 2001). Together, these variables help to characterise the area of average frequency emphasis as well as details of its complex distribution around this mean, either relatively evenly or asymmetrically and either skewed to higher or lower frequencies.

Finally, I also included a qualitative measure of bark tonality using the same scale developed for seets (1=noisy, 2=noise with minor tonal components, 3=noticeable tonal components, 4=more tonality than noise, 5=tonal, no noise).

4.5.4 Statistical Analysis

I used multivariate discriminant function analysis (DFA) to evaluate potential acoustic differences within both seets and seet-barks as produced in encounters with either aerial predators, terrestrial predators, or conspecific intruders. Separate discriminant analyses were conducted for each call type. DFA highlights the variables contributing to discrimination among groups and quantifies how accurately they allow groups to be discriminated (and classified). However, discrimination performance is dramatically

influenced by the number of predictor variables used in the analysis. Therefore, to avoid 'overfitting' the data by using a large number of acoustic features to discriminate a small number of groups I first conducted a Principle Components Analysis (PCA) on the multiple acoustic features measured for each call type.

PCA is a multivariate statistical technique that identifies and collapses overlapping variation between variables into a smaller set of orthogonal dimensions (or components). The resulting components are multivariate combinations of the original variables that retain all of the original variation in the dataset but package it into a much smaller number of independent factors (Tabachnick & Fidell 2007). I used PCA to reveal potential covariation in the acoustic features of both seets and seet-barks and to reduce the number of predictor variables that were then used in discriminant analyses of the calls. From the PCA analysis on each call type, I retained for subsequent DFA only those factors with eigenvalues greater than one because these factors account for the majority of the original variation in the dataset. PCA factors were then used as the variables in discriminant analysis classifications of seets and seet-barks.

The degree of discrimination among groups in discriminant analysis is typically assessed in two ways. First, the statistical significance of a given degree of discrimination is assessed using an overall test statistic, Wilks' Lambda. This test statistic varies from 0 – 1, where zero indicates perfect discrimination among groups and 1 indicates no discrimination among groups. The significance of the test statistic can be evaluated using an *F*-ratio or *Chi-square* transformation of the Wilks' Lambda value (Klecka 1980).

An additional and more practical metric for assessing the degree of discrimination among groups is provided by the relative success of the discriminant functions in classifying cases into their appropriate groups. Here, the degree of successful classification is typically evaluated according to how much it exceeds chance classification and this can provide a more practical sense of the degree of differentiation among groups. In this case, it will provide a more ecologically relevant sense of how well either seets or seet-barks can be reliably distinguished according to the context that elicited them. Typically, chance classification levels in DFA are determined by the simple ratio of the number of groups to be discriminated (i.e., 1/#of groups). However, all samples from a population will include chance variation that is only incidentally aligned with the groups of interest. Hence, because discriminant analysis is designed to partition variance to maximise differences between groups, it capitalises on this incidental variation in the call classifications it produces. Therefore, to control for this potential 'overfitting' of the data in call classifications, I undertook to establish chance for the particular sample of vocalisations collected using an iterated procedure of random call assignments followed by discriminant analysis classification of the randomised call sets. This procedure was run 50 times, for each of the two call types, with each run generating a Wilks' Lambda test statistic and a level of successful call classification. I then calculated an average Wilks' Lambda statistic for each call type to compare against that obtained in the two analyses where the real identity of the calls was preserved. And I calculated average classification successes for each call type that more accurately represented what chance was for these particular samples of seets and seet-barks.

4.6 RESULTS

4.6.1 Seets

Principle Components Analysis (PCA) on 11 acoustic features of the 108 seets yielded a

set of three orthogonal factors with eigenvalues greater than one. These three factors

accounted for a total of 71.5% of the variation in the original set of 11 acoustic features.

For all three of the PCA factors one or more or the original variables were significantly

associated with it (i.e., had factor loadings of 0.5 or higher; Table 4.2a).

Discriminant function analysis (DFA) using these three PCA factors yielded an

overall Wilks' Lambda of 0.87. This value was associated with a statistically significant

degree of variation in seets across the three disturbance categories ($F_{6,206}$=2.40, P=0.028).

At the same time, this Wilks' Lambda statistic was close to the theorised upper limit of

one for this test statistic, which would indicate no discrimination among groups. In fact,

based on the set of 50 classifications of randomised calls, the average Wilks' Lambda for

randomly assigned seets was 0.92, which is lower than the idealised theoretical limit of

one and only marginally different than the 0.87 value based on properly assigned calls.

Hence, the degree of differentiation in seets according to disturbance category appeared

to be quite weak.

Weak differentiation among groups was also evidenced in more practical terms by

the results of discriminant analysis classification of calls. Discriminant functions

successfully classified 52% of the calls to the correct disturbance category (Table 4.3a).

Chance for this sample, as determined through iterated classifications of randomised call

datasets, was 41%. Hence, discriminant analysis allowed successful classification of calls at levels only marginally above chance. This can be seen graphically in Figure 4.4 where calls produced in response to the different categories of disturbance overlap to a considerable extent.

Only the second PCA factor contributed significantly to this level of differentiation among seets according to disturbance type. This factor was associated with F_0 variables and accounted for only 28.8% of the measured variation in the overall sample. Hence, the majority of measured variation in this sample of seet calls was not related to differences in the type of disturbance that elicited them.

4.6.2 Seet-barks

PCA on the 21 acoustic features of 162 seet-barks yielded a set of five orthogonal factors with eigenvalues greater than one. These five factors accounted for a total of 69.5% of the variation that occurred in the original set of 21 acoustic features. For all five of the PCA factors one or more or the original variables had factor loadings of 0.5 or higher and was thus significantly associated with it (Table 4.2b).

DFA using these five PCA factors yielded an overall Wilks' Lambda of 0.78. Once again, this value associated with a statistically significant degree of variation in seet-barks across the three disturbance categories ($F_{10,310}=4.00$, $P<0.001$), but the test statistic was close to its theorised upper limit of one indicating relatively weak discrimination by disturbance type. Based on the set of 50 classifications of randomised

120

seet-barks, the average Wilks' Lambda for randomly assigned calls in this sample was

0.92. Hence, the degree of differentiation in seet-barks according to disturbance category

also appeared to be relatively weak.

Such weak differentiation is again evidenced in more practical terms by relatively

poor classification of calls to the correct disturbance category (Table 4.3b). For seet-

barks, DFA correctly classified 51% of calls where chance established for this sample of

calls was 40% (see Fig. 4.4).

The first, fourth and fifth PCA factors contributed significantly to this level of

differentiation among seet-barks. These factors were associated with a variety of

temporal and spectral features of seet-barks (see Table 4.2b) and respectively accounted

for 27.8%, 9.7% and 7.8% of the measured variation in these calls (total variation

accounted for 43.3%). Hence, once again, more than 50% of the measured variation in

seet-barks was not related to differences in the type of disturbance that elicited them.

4.7 DISCUSSION

Previous research suggested that red squirrels produce acoustically distinct alarm calls for

the different predator types of aerial and terrestrial threats they face (Smith 1978; Greene

& Meagher 1998). However, my more recent work has indicated that these two call types

are not about different predator types *per se*. Instead, seets and seet-barks are mixed

within calling bouts elicited in encounters with all predator types and including territorial

confrontations with conspecific intruders. In all cases, the two call types are produced in

a consistent pattern that involves shifting from producing seets early in a bout to producing seet-barks later in a bout. This calling pattern seems to reflect the persistence of the disturbance irrespective of its type (Digweed & Rendall 2009).

At the same time, it is possible that within mixed bouts of seets and seet-barks there are distinct subtypes of the two broader categories of call that reflect the different disturbances encountered (i.e. aerial predator, terrestrial predator or conspecific intruder). Detailed acoustic analyses were undertaken to address this possibility explicitly. However, results do not provide convincing evidence for such substructuring of the two call types. Discriminant analyses did point to a statistically significant degree of discrimination within both call types according to the disturbance that elicited them. However, for both call types, the overall test statistics (Wilks' Lambda) were very close to their upper limit of one, which indicates only very weak discrimination among groups.

More convincingly, discriminant analyses on both call types allowed successful classification according to the disturbance that elicited them at only modest levels (53% successful classification for seets and 51% for seet-barks). At first, these levels of successful classification appear to represent an appreciable improvement on chance, which would be 33% for these samples, which involved three disturbance categories. However, this level of chance is a theoretical ideal and does not take account of additional variation in this sample that is only incidentally associated with the three disturbance categories. When chance for these samples was established quantitatively using an iterated series of randomised call classifications, it was established to be 40%

and 41% for seets and seet-barks, respectively. Compared against these levels, classification success from discriminant analyses using the actual predator-encounter associations represents only a very slight improvement on chance.

Furthermore, there were also no patterns in the misclassification of seet and seet-barks across the disturbance categories, which might be expected if there were some substantive subtypes of the two calls. For example, one might expect that errors in classification of seets or seet-barks produced in encounters with conspecifics would be biased to the terrestrial predator category as opposed to the aerial predator category, given that both conspecific intruders and terrestrial predators are encountered in similar locations, namely on the ground. If the errors in classification showed some pattern like this, there might be some additional reason to infer some substructuring of the calls that was either too subtle for discriminant analysis to reveal fully, or that is actually realised along slightly different dimensions than those used here. However, there was no such patterning of errors. Errors in classification were almost equally divided among the other disturbance categories (Table 4.3a,b).

At the same time, it is important to recognise the possibility that there could be other factors that were not controlled in these discriminant analyses that were nevertheless incidentally correlated with the different disturbance categories. These might help to account for the modest level of classification success without there actually being any real and consistent substructuring of the calls according to disturbance type. For example, although I attempted to construct a fully balanced sample of calls for use in

123

discriminant analysis that would eliminate the possibility that individual squirrels contributed differentially to the outcomes, this was not entirely possible. In the end, individuals were not equally represented in all disturbance categories and there was also a sex bias: eight of the individuals in the sample were female and only two were male. As a result, some of the variation in the call sample that was parsed in discriminant analyses as variation according to the different disturbance categories might, in fact, reflect variation attributable to individual identity or any of the inevitable variety of other factors associated with it. For example, individual differences in the structure of vocalisations are well-documented in many species and so too are differences among individuals in other factors such as age, sex, and body size known to introduce variation in call structure (e.g., Snowden & Cleveland 1980; Hare 1998; Rendall et al. 1996, 1998; Maurello et al. 2000).

Overall, the very weak evidence for substructuring of seets and seet-barks according to disturbance category revealed in discriminant analysis does not provide a convincing basis for inferring communicatively significant referential subtypes of these two broader call categories. Taken together, both the results of playback experiments that showed no obvious functional differentiation in listener's responses to the broader call categories of seets and seet-barks and the call production results reported in earlier chapters that showed consistent mixing of seets and seet-barks in bouts of calling produced in encounters with all types of predators as well as conspecific intruders, there is little reason to infer that these vocalisations of red squirrels support a system of predator-specific, referential communication.

Table 4.1. Individual squirrels whose seet and seet-bark calls were used in acoustic analysis and the squirrels that were used in playback presentation trials.

Individual (M/F)	Aerial Predator	Terrestrial Predator	Conspecific Intruder	Playback Experiment
Ajax (F)			X	
Aphrodite (F)	X	X		X
Artemis (M)				X
Athena (F)	X	X		
Eros (M)	X	X	X	X
Holmes (M)				X
Hudson (M)			X	
Isadora (F)				X
Negra (F)	X	X		X
Rip (F)			X	
Scratch (F)			X	X
Snap (F)	X	X		
Triton (M)				X
Watson (F)	X	X	X	
Zip (F)				X

Table 4.2. Descriptive statistics for the acoustic features of seets (a) and seet-barks (b) used in Principle Components Analysis (PCA) analyses, and association of those features with the PCA factors used in discriminant function analysis (DFA).

(a)

Variables Measured	Mean	PCA Factors
SEET:		
Duration (ms)	45.66	1
Plosiveness (ms)	27.57	1
Plosiveness (as % of duration)	60.72	3
Start Pitch (Hz)	4600.05	2*
Peak Pitch (Hz)	5215.64	2*
Peak Pitch (ms)	21.81	1
Mid-point Pitch (Hz)	5441.33	
Mid-point Pitch (ms)	22.23	1
End Pitch (Hz)	4717.16	2*
Tonality (score of 1-5)	4.01	
Fundamental Frequency Amplitude (dB)	32.76	2*

* significant influence in discriminant analysis for predator category classification.

(b)

Variables Measured	Mean	PCA Factors
SEET:		
Duration (ms)	25.58	1*, 2
Duration Gap (ms)	10.76	3
Duration Total (ms)	69.93	1*
Plosiveness (ms)	19.84	1*
Plosiveness (as % of duration)	77.96	5*
Start Pitch (Hz)	4723.06	1*, 2
Peak Pitch (Hz)	5354.32	1*, 2
Peak Pitch (ms)	13.20	
Mid-point Pitch (Hz)	5279.52	1*, 2
Mid-point Pitch (ms)	12.82	1*, 2
End Pitch (Hz)	4823.05	1*, 2
Tonality (score of 1-5)	3.95	
Fundamental Frequency Amplitude (dB)	40.74	
SEET-BARK:		
Duration (ms)	32.74	3
Plosiveness (ms)	45.94	1*
Plosiveness (as % of duration)	31.37	3
Center Of Gravity (Hz)	6985.18	
Center of Gravity Standard Error (Hz)	420.86	4*
Center of Gravity Skewness	0.69	4*
Center of Gravity Kurtosis	0.07	4*
Tonality (score of 1-5)	1.65	

*significant influence in discriminant analysis for predator category classification.

Table 4.3. Classification results from discriminant function analysis (DFA) of seets (a) and seet-barks (b).

(a)

	ACTUAL	PREDICTED Aerial	PREDICTED Terrestrial	PREDICTED Conspecific	Total
Count	Aerial	**23**	9	4	36
	Terrestrial	13	**9**	14	36
	Conspecific	9	3	**24**	36
	Total				**52% (56/108)**

(b)

	ACTUAL	PREDICTED Aerial	PREDICTED Terrestrial	PREDICTED Conspecific	Total
Count	Aerial	**34**	12	8	54
	Terrestrial	13	**27**	14	54
	Conspecific	22	10	**22**	54
	Total				**51% (83/162)**
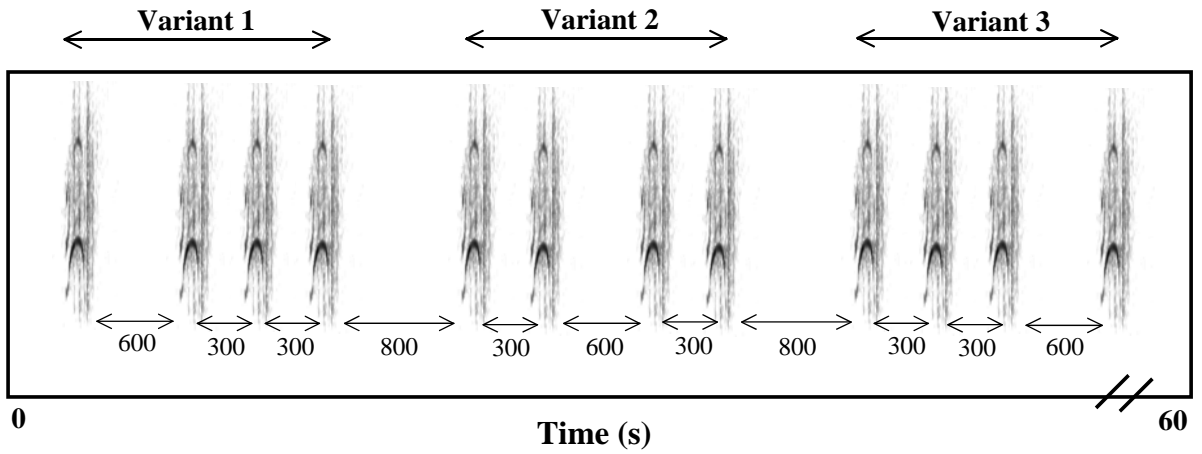
Figure 4.1. Graphic to illustrate how playback stimuli were constructred for use in experiments. Three different variants of a 4-call series, each one varying the internal spacing of calls within a series, were concatenated together and this concatenation was then repeated to produce a total stimulus lasting 60 seconds. This graph illustrates the case for seets used in the fast condition. The same procedure was used for seet-barks.
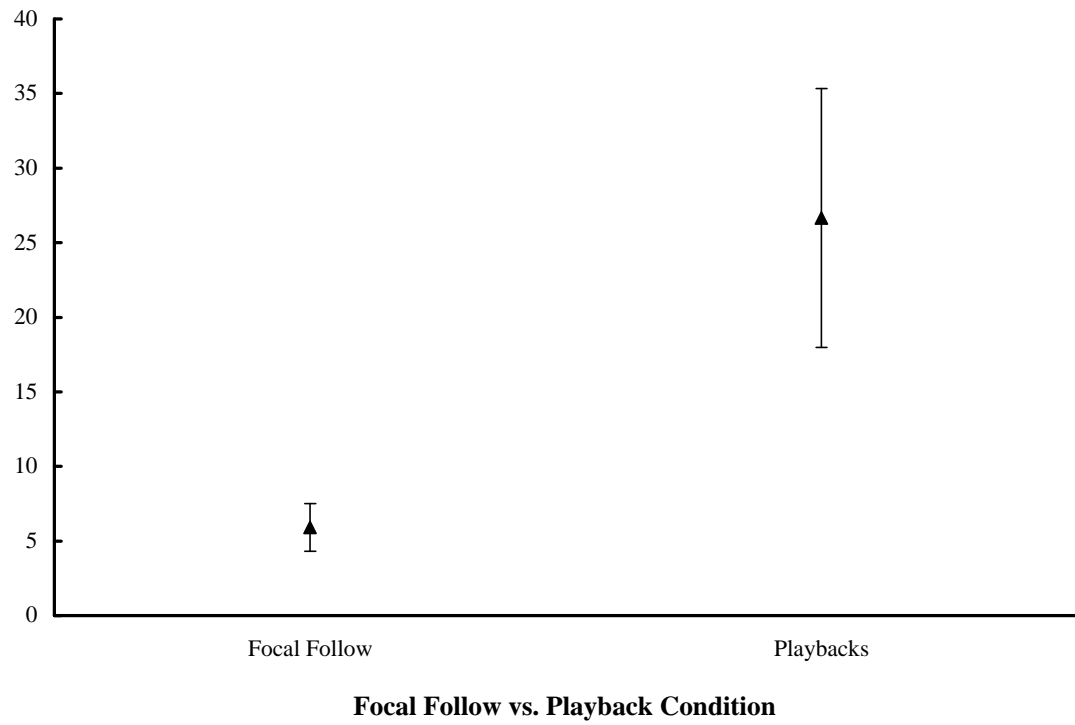
Figure 4.2. Graphic depicting the percent of time spent vigilant in the 15-minutes following presentation of the playback stimulus in experimental trials by comparison to baseline conditions.
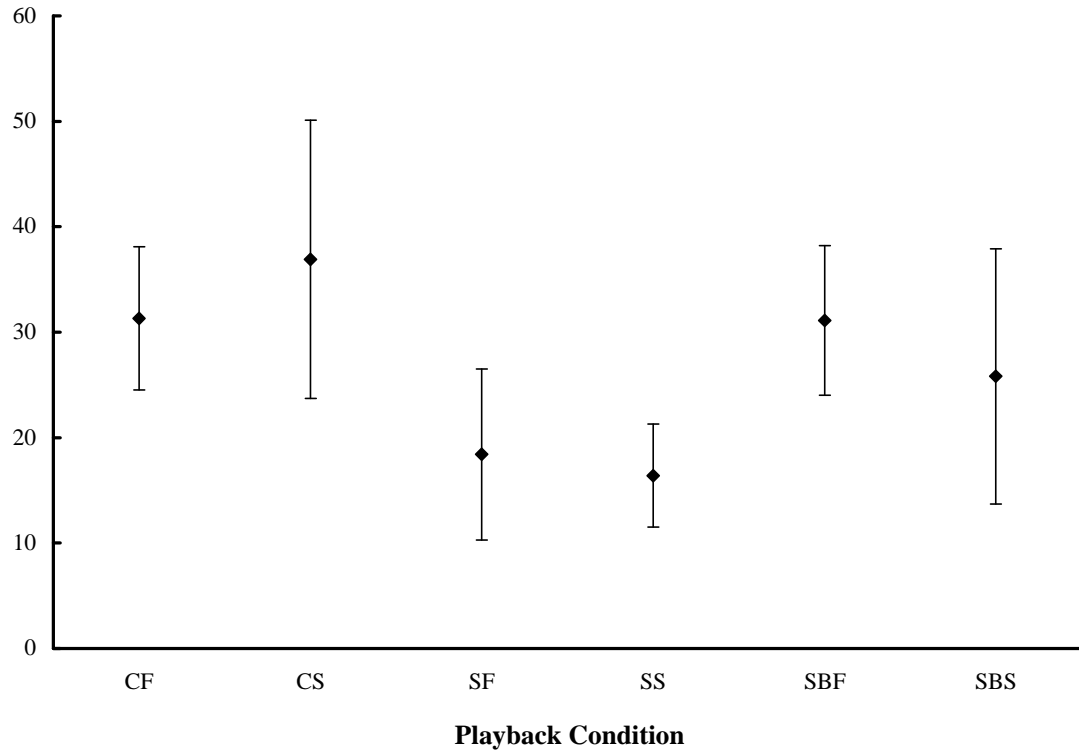
Figure 4.3. Graphic depicting the percent of time spent vigilant following presentation of the playback stimulus in each of the six experimental conditions: CF=combination fast; CS=combination slow; SF=seet fast; SS=seet slow; SBF=seet-bark fast; SBS=seet-bark slow.

(a)

(b)
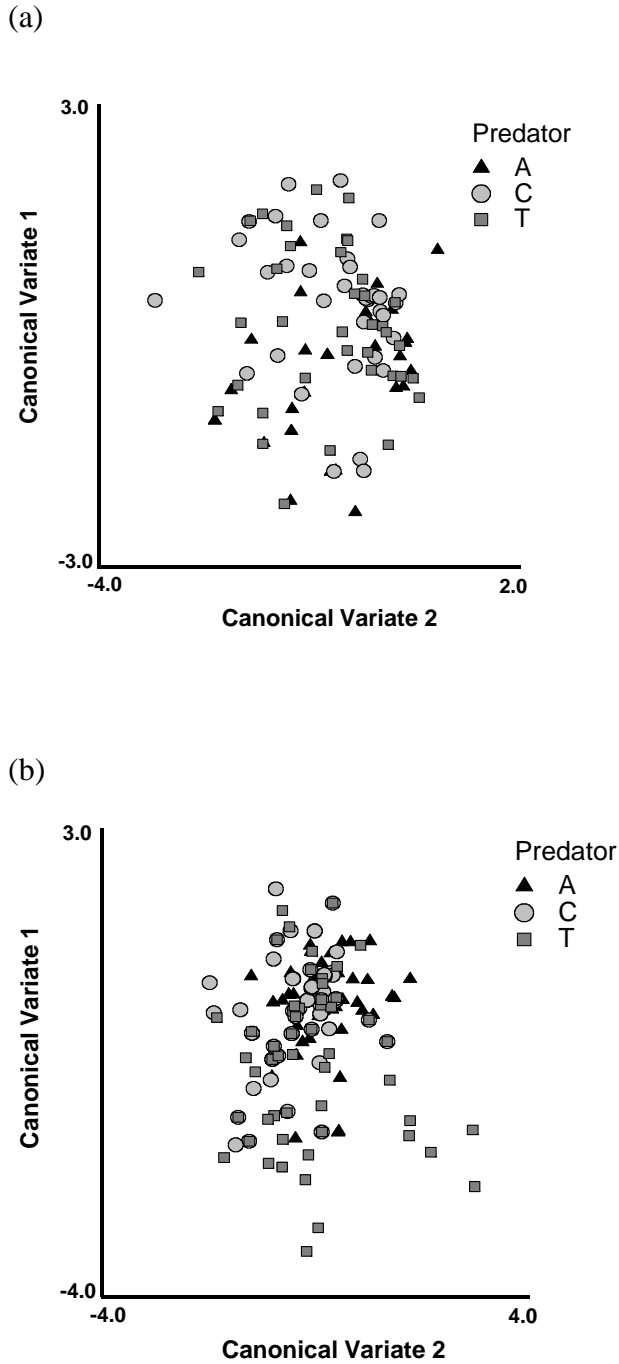
Figure 4.4. Scatterplots plotting (a) seets and (b) seet-barks produced during natural disturbances involving either aerial predators (A), terrestrial predators (T) or conspecific intruders (C). Calls are plotted along the first two canonical variates from discriminant analyses. For both call types, calls produced to different classes of disturbance overlap to a considerable degree.

131

CHAPTER FIVE

SUMMARY

The North American red squirrel is a small territorial mammal that produces loud, conspicuous alarm calls when encountering a variety of disturbances. Previous work on red squirrels suggested that these alarm calls were predator-specific (Greene & Meagher 1998). Specifically, squirrels were reported to produce a high-frequency, tonal seet call when encountering an aerial predator, and a harsher, broadband bark-type call when encountering a terrestrial predator. However, like many brief studies on anti-predator vocalisations there were several outstanding ambiguities that were not properly addressed. Perhaps the most detrimental of these ambiguities involved neglecting listener responses and thus the ultimate function of calls. Although Greene & Meagher (1998) addressed the production of calls from the signaller's perspective, they neglected to consider listeners and therefore to whom the calls are actually targeted. In fact, because red squirrels are solitary and highly territorial for the majority of the year, with the exception of a short mating season, it is not clear that the calls are even directed at conspecifics (Gurnell 1987). Moreover, outside of the mating season, squirrels are well known for being equally aggressive to kin and non-kin, further suggesting that warning others might not be the primary selected function of alarm calls (Nodler 1973; Lair 1990; Price et al. 1990). If calls are not directed at conspecifics, then there is little reason to expect the calls to 'refer' to particular predator types. My studies have shown that by divorcing questions of mechanism and function and not embedding research within the natural life-history context of the organism, previous research has yielded a potentially

132

distorted view of alarm vocalisations in red squirrels.

5.1 RED SQUIRREL ALARM CALLS AS REFERENTIAL SIGNALS

My research did confirm a core finding of previous work, namely that red squirrels produce two types of alarm vocalisations in encounters with predators: a high-frequency tonal call variant (the seet), and a harsher, broadband call variant (the seet-bark). However, both in natural encounters with predators and in simulated predator presentation experiments that I conducted, these calls were not produced in pure bouts of one or other call type as would be expected if they served a referential function, but rather in mixed bouts that included both call types. In addition, such mixed bouts of calls were produced to all major classes of predators and also to non-threatening species and conspecific intruders.

There were necessarily some limitations in this work. For example, the sample of natural predator encounters witnessed was small and the range of simulated predator experiments conducted to complement them was necessarily restricted in scope. Hence, future work might profit from an expanded set of naturalistic observations and from additional experimental manipulations. For example, one noteworthy feature of the experiments I conducted using predator models was the importance of "predator" movement. Movement may be important simply because it makes the predator model more salient to the squirrels and so they are more likely to detect and register it, or perhaps because it also makes the model more natural and thus better simulates a real predatory threat. In either case, future experiments might profit from additional efforts to

133

reproduce the natural movement patterns of the various aerial and terrestrial predators that threaten red squirrels. Improvements like this have been developed recently for similar kinds of research by using robotic models (Rundus et al. 2007; Partan et al. 2009) and, as applied to red squirrels, they might provide further clarification of the calling patterns and possible functions of the calls.

However, it is unlikely that such improvements will fundamentally change the general pattern of results reported in this study which provided little evidence for stimulus-specificity in the production of alarm calls in red squirrels, and thus contradicted a key criterion for referential signalling (Macedonia & Evans 1993).

At the same time, my research attempted to test the second key criterion of referential signals, specifically the requirement that the responses they elicit in listeners be context-independent. Here, the results of playback experiments using seets and seet-barks and stimuli that mixed the two call types in more naturalistic bouts revealed no evidence for differentiated listener responses. Although squirrels registered and responded to calls played back to them, their immediate reactions and orienting responses were not differentiated according to the type of call heard as would be expected if the calls had some referential value. Squirrels' showed more protracted vigilance in the period following call playback suggesting that the calls were salient to them and put them on guard. However, there was nothing further in their vigilance responses that suggested they learned anything more specific about the type of threat that might be conveyed by the calls themselves.

Once again, these results are not entirely without qualification. For example, although the calls used as stimuli in the playback experiments were originally recorded in natural encounters with predators, they did not control for the type of predator involved. One might then argue for conducting additional experiments that use as separate stimuli seet vocalisations recorded in encounters with aerial predators and seets recorded in encounters with terrestrial predators, as well as seet-barks recorded in encounters with aerial predators and seet-barks recorded in encounters with terrestrial predators. This might allow a more definitive conclusion about listeners' responses vis a vis the potential predator-specificity of the calls themselves.

However, at the same time, the potential for such predator-specific subtype distinctions within seets and seet-barks is weakened by additional acoustic analyses reported in this study which show little evidence for such variation within the two call types. These analyses identified some weak subtype distinctions associated with variation in the kinds of disturbance associated with them. But much of what little subtype variation there was could have reflected a variety of unrelated factors stemming from, for example, unequal individual representation in the call sample used. Of course, this does not entirely preclude the possibility for some subtype variation in seets and seet-barks associated with different predator categories, but results of my acoustic analysis make this possibility comparatively unlikely.

Therefore, notwithstanding some limitations in the design of the playback

experiments I conducted, the lack of any obvious behavioural differences in squirrels

responses to seets and seet-barks, and the lack of evidence for further subtype variation

within these two classes, together contradict the second major criterion of referential

signals, the requirement that they elicit functionally distinct responses that are

independent of supporting contextual information (Macedonia & Evans 1993; Evans

1997).

At the same time, because my research suggested that red squirrel alarm calls do

not have predator-specific, referential value, it introduced additional ambiguity into what

the ultimate function of the calls might be and to whom the calls are actually addressed?

In other words, do the calls function to warn kin, mates or conspecific neighbours as they

do in a variety of other species (Sherman 1980; Hyman 2005; Krams et al. 2006)?

Analyses of call production patterns during a variety of disturbances did not provide

support for any of these possibilities. There was no compelling evidence that red squirrel

females called to warn offspring or collateral kin, that males called to warn mates, or that

males and females called to warn neighbours with whom they had established

relationships. Although these negative findings cannot, by themselves, point to any

additional specific function for these calls, they do tend to further undermine the proposal

that the calls represent a form of referential communication for warning conspecifics

about predator-specific dangers.

5.2 AN ALTERNATIVE ACCOUNT OF RED SQUIRREL ALARM CALLS: DISTURBANCE PERSISTENCE AND INTRUDER DETERRENCE

Taken together, my results do not provide support for the proposed referential function of alarm calls in red squirrels (Greene & Meagher 1998). However, there were a variety of patterns in the calling behaviour of the squirrels that pointed to an alternative functional account. For example, mixed bouts of calls to predators and a variety of other disturbances showed consistent internal patterning with seets preceding seet-barks, and this internal patterning of the two call types tracked the persistence of the disturbance. In other words, irrespective of the type of disturbance encountered, bouts of calls began with seets and then shifted into seet-barks as the disturbance continued. This pattern suggested that the most salient dimensions of encounters with predators and other disturbances, such as conspecific intruders, might in fact be the persistence of the disturbance they represented. The obvious corollary of this possibility is that the calls might be directed not at distant conspecifics but directly at the source of those disturbances, whether they are predators or conspecifics intruding on one's territory. And, the ultimate function of calling might then be to announce the detection of these intruders and possibly then to deter them in some way or repel them from the area.

This emphasis on the importance and salience of a variety of disturbances, including conspecifics, is consistent with the broader life-history of red squirrels. Red squirrels are a small-bodied species inhabiting north temperate habitats where limited food supplies and cold temperatures create a very difficult survival problem for a non-hibernating mammal. Red squirrels live solitarily and aggressively defend a small

territory year-round in which they harvest and store cones that are critical for overwinter survival (Boutin & Schweiger 1988). As a result, although predators represent a serious threat to survival, so too do conspecifics that intrude on one's territory to steal stored cones (Gerhardt 2005). In fact, cone pilferage is not limited to other squirrels but May also include other cone-eating species (e.g., chipmunks, jays). Hence, the list of potential predators, food competitors and cone pilferers is quite broad. Collectively, these species pose continuing serious threats to survival. It is possible that the kind of pugnacious behaviour for which red squirrels are popularly known – actively confronting and vigorously 'scolding' intruders – is a selected response to this variety of threats, one that is functional across a range of disturbances. Viewed in this light, the common use of seets and seet-barks during encounters with known predators, but also in encounters with non-predatory species and with conspecific intruders is no longer puzzling but makes functional sense. It reflects a common attempt to deter or repel intruders of various kinds by conspicuously announcing that they have been detected, and, in some cases (e.g., conspecific intruders), accompanying this by aggressively chasing the intruder.

5.3 UNRESOLVED ISSUES

This account helps to explain observed calling patterns in the population of squirrels studied and it is also consistent with important aspects of red squirrel life-history previously unconsidered in earlier papers (Greene & Meagher 1998). However, there are a number of unresolved issues. For example, some details of the structure of seets and seet-barks appear well-suited for this kind of predator- and conspecific-deterrent function. Seets are soft and tonal and therefore difficult to detect and localise which may

be functional in the early stages of predator encounters when the threat has not yet been fully evaluated. In contrast, seet-barks are harsher, broadband calls that are comparatively easily detected and localised and are produced in the later stages of encounters after time has allowed some better evaluation of the threat.

However, it is not clear that hearing these calls would equally deter all species that prey on squirrels. Coyotes are limited in their ability to pursue squirrels once they are safely located in a tree; hence, they might be deterred by announcements that they have been detected (see Zuberbühler et al. 1999). In contrast, pine martens are about equally adept in the trees and on the ground, and, in fact, are known to pursue squirrels in both areas. As a result, they might not be deterred in the least. In fact, in this case, announcing detection with conspicuous vocalisations might simply draw the marten's attention and thus be quite maladaptive. At the same time, though, it is not clear that this is truly a weakness of the disturbance persistence account because I do not have sufficient data on encounters with different predators to know whether there is some variation in squirrels' propensity to call when encountering coyotes versus martens. If they tend to remain silent when they encounter pine martens in particular, then the account is not weakened.

A further limitation of the proposed deterrent function of red squirrel alarm calls is the lack of data on how the predators (or conspecific intruders) themselves actually respond to the calls. For example, does the combination seet-bark call allow squirrels to announce their detection of predators without facilitating easy localisation by the predator as predicted by this account? Do the calls actually cause predators to give-up their

hunting in the area? Although there is certainly a precedent for such a function of alarm signals in other species (Zuberbühler et al. 1999), this would be an important issue to test for red squirrels in the future.

A final obvious question that emerges is that, if seets are soft, tonal, and difficult to detect over more than a few meters, why do squirrels produce this type of call at all? They appear not to be functional for communicating with distant conspecifics. They appear similarly limited in communicating even with predators close by. One possible answer is suggested by the wider production of seets not just during encounters with predators and conspecific intruders but in several other quite unrelated contexts. For example, when traveling through the trees and making large jumps between them, squirrels often produce seets. They also produce seets when simply moving down the trunk of a tree towards the ground, which is a more vulnerable location for a squirrel. What could these different contexts have in common that would explain the common production of seets in all of them?

One commonality is that all of these contexts are associated with some uncertainty on the part of the squirrel in combination with some possibly increased levels of arousal. These contexts also all tend to be associated with, or precede, periods of increased and sustained motor activity. One possibility then is that seets might be a byproduct of a more general set of processes associated with preparing the squirrel for physically taxing activity including effortful movement through the trees, a rapid escape from a predator, a prolonged chase of a conspecific intruder, or a long protracted bout of

vocalisations announcing the detection of predators or conspecifics.

There is certainly a precedent for vocalisations like this that are byproducts of other physiological functions. An excellent example involves the phenomenon of laryngeal braking in rat pups. This phenomenon is associated with a functional attempt by pups to warm themselves in situations where they have become isolated from their mother or from other pups in the nest. The pup's attempt to warm itself involves metabolising reserves of brown fat tissue via a process of nonshivering thermogenesis (Blumberg & Alberts 1990). This involves increasing blood oxygenation by increasing subglottal air pressure through muscular constriction of the larynx. As a byproduct, an audible sound is often produced as air escapes incidentally through the constricted vocal folds of the larynx. Rat mothers are sensitive to these sounds and will return to the nest to retrieve pups. However, it is not clear that the sounds are designed for this maternal retrieval function or whether they are an unselected byproduct of a pup's attempt to regulate its own temperature to which mothers have some sensitivity (Blumberg & Alberts 1990; Blumberg & Sokoloff 2001).

It is possible that the seets of red squirrels reflect much the same kind of physiological process. Perhaps seets are also produced largely incidentally in conjunction with a related functional process that is designed to increase peripheral blood flow and oxygenation in preparation for taxing, vigorous activity. This possibility remains speculative but warrants future study. For example, experiments could be conducted in the laboratory that manipulate blood oxygenation, for example, by depriving a squirrel of

oxygen in a hypoxic chamber, or by physically taxing them on a running wheel (or similar apparatus) and measuring their production of seet vocalisations relative to baseline conditions. The prediction is that, under these experimental conditions, squirrels should produce seet calls similar to those recorded under natural conditions, but, in this case, in the absence of any external eliciting stimulus (i.e., a predator or conspecific intruder).

5.4 RESEARCH SIGNIFICANCE

To the extent that my research has contributed some additional understanding of the alarm calls of red squirrels, it has profited from considering mechanistic and functional issues simultaneously, and in doing so paying particular attention to important life-history characteristics of the species. Thus, while systems of referential signaling might seem possible for any species, in fact, certain combinations of social and ecological factors might predispose them. Put differently, certain combinations of social and ecological factors might limit the value of referential modes of communication.

North American red squirrels inhabit a complex three-dimensional environment that offers a variety of escape options from predators. They also face a variety of different predators that might select for differential use of the various escape options that are available to them. However, ultimately, squirrels tend to respond to most threats in a similar way, by retreating to a relatively safe location in a tree and monitoring the threat from that location. At the same time, red squirrels are comparatively non-social. Hence, the functional value of communicating vocal warnings to kin, mates, or other group

members might be reduced in red squirrels compared to some other species that are far more gregarious.

Furthermore, red squirrels face additional important challenges to survival related to food pilferage from a variety of competitors. Together, the adaptive problems posed by predators and food competition in red squirrels appear to have selected for a common response that involves confronting the source of disturbances directly and deterring them either through protracted bouts of vocalisations that communicate their detection, or, in the case of conspecific intruders, through such calling accompanied by active chasing and aggression.

In this species, then, there appears to be little social or ecological pressure favouring a system of multiple, predator-specific vocal messages such as have evolved in some other species (Evans et al. 1993; Da Silva et al. 1994; Templeton et al. 2005; Kirchlof & Hammerschmidt 2006). However, this insight emerges only by considering and testing multiple alternative functional and mechanistic hypotheses and doing so cognizant of these basic components of the species' life-history.

My research might also contribute to the small but growing database of studies that have explored a specifically deterrent function of predator-associated signals (Woodland et al. 1980; Caro 1986; Hasson 1991; Zuberbühler et al. 1999; Blumstein 2007). As a result of this growing literature, alarm signals that were originally thought to function specifically as warnings to kin or mates are increasingly re-interpreted as being

directed at the predators themselves and functioning in some way to deter them (Hasson 1991). However, the number of examples of such signals remains small and, hopefully, my work will add something to this literature, at the least further emphasising the need to consider predator-deterrence as an alternative when exploring the function of predator-associated signals.

Finally, my research has been guided by an alternative general approach to studying communication. For many years, studies of alarm communication have adopted a linguistic approach, which, by analogy to human language, suggests that animals will parse their worlds into specific (referable) categories that represent important objects or events in their world. However, this linguistically-inspired approach tends to forget that different species inhabit very different worlds than humans, and they also have very different life histories, all of which might yield very different parsing of their worlds and communication systems that are not quite the same as language (Owings & Morton 1998; Owren & Rendall 2001).

Von Uexküll (1957) famously recognised this point and specifically argued that different animals will often experience their environments in very different ways, yielding very different '*Umwelts*', or worldviews. Although the concept of the *Umwelt* was not designed specifically to explain communication, it can be embraced to provide a more inclusive approach to its study, as communication represents the interaction of the organism with the variety of social, physical and ecological factors that collectively describe the environment a species inhabits.

In recent work on the alarm call system of red squirrels, the linguistic perspective was adopted and the species was proposed to produce different alarm calls to different predator types (e.g. Greene & Meagher 1998). However, as noted above, this language-centered approach tended to overlook the very different social, ecological and physical environments that characterise red squirrels. My research has tried to move away from this linguistic perspective and adopt a more 'ecologically sensitive', or *Umwelt,* approach to vocal communication in red squirrels by specifically considering and incorporating key life-history traits of the species into the account that is ultimately offered for their predator-associated vocalisations.

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