The effects of energetic and physiological state on the foraging decisions of birds

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ABSTRACT

Animals make many decisions throughout their lives and there is good reason to suggest that these decisions are related to their energetic state. However, there is a need for more experimental data linking animals' behavioural decisions directly with their energetic state. Using behavioural assays and measurements of energetic state, I conducted a number of experiments that investigated the relationship between an animal's foraging decision-making and their energy levels. In Chapter 2, I examined the effects of energetic state on the rationality of foraging decisions of European starlings (*Sturnus vulgaris*). I found that there were no breaches of rationality and that there were no relationships with energy reserves. In Chapter 3, I investigated risk-sensitive foraging behaviour and its relationship with temperature and behaviour in rufous hummingbirds (*Selasphorus rufus*). However, the birds were indifferent to risk and there was no relationship with any of the variables that were included in the analysis. The remaining four experimental chapters dealt with problems related to the state-based consumption of chemically defended prey in European starlings. The results from these experiments confirmed many long-held assumptions regarding the state-based consumption of chemically defended prey. They also confirmed some recent predictions of stochastic dynamic programming models, which were less obvious. Generalist predators, such as European starlings, can also adapt to new food sources quickly and develop sophisticated behaviours that may mediate their intakes of dangerous compounds. The results of this thesis show that a bird's state can play an important role in mediating the foraging decisions that they make, especially regarding chemically defended prey. More generally, these results have important implications for how the dynamic systems of foraging behaviour and energetic state are viewed, and demonstrate a path as to how these different areas can be integrated.

Chapter 1

INTRODUCTION

You can't always get what you want, but if you try sometimes you might find you get what you need

M. Jagger, 1969

You can't think rationally on an empty stomach, and a whole lot of people can't do it on a full one either

Attributed to Lord Reith

The belly rules the mind

Spanish proverb

I chose two lines from the song "You can't always get what you want" from the Rolling Stones record *Let it Bleed* of 1969, to illustrate the concept of the biological trade-off. Biological trade-offs occur when an organism has a conflicting set of demands to meet, but that they cannot satisfy all of them fully. However, they can satisfy each adequately in order to survive. Defined more technically, trade-offs are linkages between factors that constrain the evolution and expression of two or more traits (Stearns 1992). One of the most famous and well-studied trade-offs is that between somatic growth and reproduction (Williams 1957, 1966a,b). For example, in red deer (*Cervus elephus*), the yearly survival has been shown to be age dependent with increasing mortality with increasing age (Clutton-Brock 1982; Clutton-Brock *et al.* 1988). However, the survival of calves was also dependent on their birth mass, with heavier calves having a greater rate of survival. Younger mothers tended to be in better condition and so, gave birth to heavier calves. As the hinds aged, they were less able to produce high quality offspring because their general condition declined through senescence.

The second quote is attributed to the prominent public servant Sir John Reith and neatly encapsulates the other subjects of this thesis: energetic state, decision-making, and rationality. The final proverb was included because it also illustrates the fact behaviour can and energy are inextricably linked. The relationship between bodily energy reserves and the variety and quality of behaviour has been widely demonstrated (e.g., Barnett & Briskie 2007). This study found that when birds were supplemented with extra food, the amount and quality of their dawn singing displays increased.

What animals do with their time has been one of the primary concerns of ethologists and behavioural ecologists for the last half-century. One fact to come out of this research is that animals are constantly faced with choices: what to eat, with whom to mate, where to live, and whether to rest or search for food. Over the course of the day, a foraging animal is expected to make many foraging choices. The functional study of foraging has focussed on how an animal's foraging behaviour relates to fitness or some proxy that correlates highly with fitness. The currency of the fitness proxy is still a matter of some debate. Energetic state is a short-term proxy of fitness that is gaining favour because of its ecological realism and because of recent advances in modelling animal behaviour using dynamic programming models (see Houston & McNamara 1999; Clark & Mangel 2000 for details of biological applications of SDP models).

1.1 The importance of energetic state in animal behaviour

McNamara and Houston (1999) have suggested that all behaviour should be dependent upon an animal's state. This may be a slight exaggeration, but we would know a lot more about behaviour if we could link changes in energetic state with changes in behaviour. Therefore, studies that integrate behavioural data with physiological data on energetic state are more powerful than studies that consider behaviour and physiology separately. This is because the behaviour of animals can be matched to the subject's energy levels simultaneously. This controls for a lot of the variation in behaviour that could be accounted for due to state and behaviour being measured at different times. It is difficult to study the behaviour of most large vertebrate species for the duration of their lives. It is also impractical to follow animals for their lifetimes in order to determine the effects that short-term changes in condition have on fitness. This is where the concept of energetic state becomes important as it can be used as a short-term surrogate for lifetime reproductive success (LRS).

Energetic state is a set of variables that describes the condition of an animal at any given time and is an absolute measure of its energy reserves (Houston & McNamara 1999). It is important to match the time horizon of interest with suitable state variables. For example, it would be impractical to measure daily changes in body size of a large ungulate, as it is unlikely to change significantly over the course of a day. A more biologically meaningful measure of state throughout the day might be gut contents or body mass. Therefore, a number of different physical components–such as body fat, gut contents, blood sugar levels, and glycogen reserves in the liver–could be used as state variables in my experiments. All of these variables can change over short time-spans meaning that they can be correlated with short-term changes in behaviour. In most cases, one or two state variables are sufficient in order to represent the levels of energy reserves–and energetic state–for behavioural experiments (Blem 1990). Fat reserves are known to correlate directly with body mass in birds and are one of the most common variables used to represent energetic state (Blem 1976; Witter *et al.* 1994).

The integration of energetic state with animal behaviour is an important development in the study of animal behaviour. However, there are a number of challenges in both measuring energetic state and relating it to the fitness of subjects. First, as the ungulate example in the previous chapter demonstrates, finding the correct measure of energetic state is important. I chose to measure energetic state in this thesis, by utilising measures that reflect changes in a birds' levels of energy over a over a matter of hours to days. Therefore, I used bird mass and a visual scale of the furcular fat levels as measures of energetic state. These measures were used because they can reflect changes in individual's levels of energy reserves and they can change over the course of a day to response to food availability. Mass has been found to correlate with a fat reserves in previous studies (Gosler 1994; Witter *et al.* 1995; Dall & Witter; Barnett & Briskie 2007). Therefore, if increases in mass are associated with increases in fat levels, then there should be highly significant relationships between mass and furcular fat scores in the experiments. The method of fat scoring and weighing are a good compromise between invasiveness and reliability in their relation to the energetic state of birds but as always, it is not a perfect correlation with energy reserves. Second, energetic state is used in stated based models of behaviour as a direct proxy for fitness. Energetic state can be used in this manner because improved energetic state leads to improved general condition of females and males which increases their quality to produce a larger number of offspring or offspring of high quality. Moreoever, many of the models of the strategic regulation of body mass in small birds in response to predation formulate the problem in a manner where animals must survive an arbitrary period before they can breed (McNamara & Houston 1990; Houston & McNamara 1993; McNamara *et al.* 1994). Therefore, the mass regulation strategy can directly influence a birds' reproductive success through influencing the probability of survival until breeding.

1.2 The link between foraging, energetic state, and fitness

The link between foraging efficiency and fitness is a major cornerstone of behavioural ecology. Most models of optimal foraging are based on the fact that animals are striving to maximise fitness (or a currency that can be used as a proxy for fitness). There is evidence to suggest that there is an association between foraging efficiency and fitness. Schoener (1971) was one of the first authors to explicitly state that fitness increases with net rate of energy gain from foraging although, confirmation of this assumption took longer. Lemon (1991), who was working on zebra finches (*Teaniopygia guttata*), found there was a negative relationship between the amount of time spent foraging and female reproductive output. This was despite females from all treatments obtaining similar amounts of food. The difference was thought to be due to the females having to expend more time obtaining their food in treatments where they were forced to work harder than less hard working females. This finding has subsequently been replicated in other studies (Lemon & Barth 1992; Lemon 1993; Williams 1996; Wiersma & Verhulst 2005). Therefore, the net rate of energetic gain per unit of time is a valid currency for foraging experiments because it has been found to correlate with reproductive success.

There is much literature that has shown in birds that there is a positive relationship between the condition of parents and their reproductive output (see Newton 1998, pp. 145-189 for a review). Condition is related to energetic state insofar as it is a general term that is used to describe the physical state of an organism. Energetic state is a factor that contributes to the condition of an animal. However, energetic state and condition cannot be viewed as synonyms as an animal's condition can encompass a greater number of factors than energetic state. For example, measures of condition could also include measures of immune system challenge from infection or parasites. Therefore, measures of condition can incorporate many extrinsic factors (e.g., air temperature, population density, and food availability) and intrinsic factors (e.g., the state of the endocrine system, level of bodily energetic reserves, the level of immune challenge, and parasite or disease load). Energetic state is thus, only one factor that can affect an animal's condition. However, it is also the case that these factors all overlap and to some extent and may make simple interpretation of condition difficult. Although the concept of condition is often loosely defined, it is the most commonly used concept of relative animal well being that is used in field based studies of ecology, evolution, and conservation.

Generally, it has been found that increases in condition lead to increases in reproductive success. For example, it has been shown that bird populations increase in size because of food supplementation (see Newton 1998, pp. 145-189 for a review). These increases in reproductive success can be due to increasing the number of mates, increasing the amount of reproductive effort within a clutch (both directly through energy investment in eggs and indirectly through increased behavioural investment). Generally, the relationship between reproductive success and condition is well supported in most taxonomic groups although a full review of this subject is outside the scope of this thesis (see Clutton-Brock 1988; Newton 1989, 1998 for reviews).

1.3 Thesis aims

The diet preferences of animals have been examined generally and it has been found that animals become less selective when food density declines (Stephens & Krebs 1986). This is likely to be due to declines in the foraging animal's energetic state. As favoured food become less abundant, the amount of energy per unit of time will decline because the number of prey items encountered per unit time declines. Therefore, the amount of energy that can be extracted from the environment falls. In order to compensate for this, animals will consume food that they would not consider at other times (e.g., Beukema 1968; Kislaliogu & Gibson 1976; Williamson 1980; Ernsting & Van der Werf 1988). However, there is little direct evidence linking these changes in foraging behaviour with changes in animals' fat reserves. Therefore, it is the aim of my thesis to examine the effects of energetic and physiological state on the foraging decisions that animals make.

I chose to examine three disparate areas of foraging behaviour and how they were affected by the birds' energetic and physiological state. The three areas where I focussed my attentions were: (1) rationality in relation to foraging context; (2) the energy budget rule of risk-sensitive foraging; and (3) the energy-toxin trade off and consumption of chemically defended prey. I examined the effects of energy on foraging decisions through negatively manipulating the birds' energetic state and correlating these changes in state with changes in decisions of the birds. Using these methods allowed me to examine the three topics in which I was interested:

1) Do birds display breaches of rationality, and are these breaches in rationality related to energetic state?
2) Do birds display risk sensitive foraging behaviour that is consistent with the energy budget rule in natural situations?
3) Do birds consume chemically defended prey when they are more energetically stressed?

Through careful behavioural observation and measurement of environmental and state variables, I addressed these questions systematically in this thesis. All behaviour is dependent on energetic state to an extent, meaning that birds ought to pay attention to their energy levels when making foraging choices. Therefore, it is likely that the decisions that birds make when they are energetically stressed are likely to be different compared with when they have higher levels of energy reserves.

1.4 Thesis outline

This thesis can be broken into three sections dealing with three different areas of research. There are conceptual links between these sections insofar as they all examine some aspect of foraging behaviour and incorporate some aspect of physiological state. The first section consists of Chapter 2, which deals with rationality and how it interacts with energetic state of subjects. Recently, Schuck-Paim *et al.* (2004) proposed a model of apparent breaches in rationality based on the differences in marginal values in fitness that a reward has whilst the subject is in different energetic states. Their argument was that apparent breaches of rationality were based on differences in the marginal fitness payoffs of the rewards in different energetic states. Therefore, they argued that the maximal fitness payoffs switched between options at different energetic states. Hence, what are seen as breaches of rationality might be seen as rational decisions after all. They tested this prediction in European starlings (*Sturnus vulgaris*) and concluded the model was a good fit to the birds' behaviour. However, there are a number of problems with this study that can be broken into two areas: (1) flaws in the experimental design and (2) flaws in the analysis and interpretation of the results. Therefore, I aimed to replicate their results using the same species, but using a different experimental apparatus and method of state manipulation.

The second section (Chapter 3) is devoted to a test of the energy budget rule (EBR), which is one of the hypotheses proposed to explain risk sensitive foraging behaviour (Stephens 1981). At this point, I wish to make a distinction between what I have termed energy reserves above and the energy budget rule of risk-sensitive foraging (see Chapter 3). Energy reserves, as stated above, are a set of physical characters, which describe an individual's current energy levels at a specific point in time. The energy budget rule is a strategy for minimising the risk of starvation in a variable environment. It states that an animal should base its current foraging decisions on both its current energy levels and environmental factors that affect the its energy expenditure (e.g., ambient temperature) and the amount of energy available from a food item. An animal (such as a small diurnally active bird) living in a variable environment must accumulate a certain amount of food in order to guarantee survival during its night fast (when it cannot forage). Therefore, it will need to gain all of its energy to survive an entire 24-hour period during the daylight hours. The EBR states that animals might be selected to pay attention to the variability of a food source as well as the mean size of the food's payoff. The bird should pick reward options with low variance when its levels of energy are sufficient to meet the overnight energy demands. If its reserves are below what are normal for the time of day, then it might face the possibility of overnight starvation if it does not find food quickly. Therefore, it might be faced with finding larger than average rewards meaning it has to adopt a risk-prone strategy and hope for a run of good luck in order to have a chance of survival.

The EBR has limited empirical support, as there are studies that indicate that risk sensitive foraging behaviour is consistent with the EBR. Others find little support for EBR in determining the risk sensitive foraging behaviour of animals (see Kacelnik & Bateson 1996; Bateson & Kacelnik 1998 for reviews). The best demonstration of the EBR is the study of Caraco *et al.* (1990) who used differences in temperature to cause changes in the energy budget in dark eyed juncos (*Junco phaenotus*). Therefore, I attempted to replicate this result in rufous hummingbirds (*Selasphorus rufus*) using natural variation in air temperatures. Accordingly, I investigated the risk-sensitive foraging behaviour of the birds at three times of day–early morning, mid-day, and the evening–where birds might have had different energy budgets due to differences in temperature. I also did this early and later in the breeding season as temperatures increased significantly throughout the breeding season.

The final section (Chapters 4-8) examines the energy/toxin trade-off that has recently been formalised using stochastic dynamic programming (SDP) models (Kokko *et al.* 2003; Sherratt 2003, Sherratt *et al.* 2004). These models make many interesting predictions regarding the situations when animals should consume chemically defended prey. They are based on the premise that predators may consider defended food sources and utilise them at times of energetic need (e.g., Brower *et al.* 1968; Speed 1993b). There is evidence to suggest that educated predators will attack chemically defended prey when they are energetically stressed (e.g., Sexton *et al.* 1966; Gelparin 1968; Chai 1986; Hileman *et al.* 1995; Gillette *et al.* 2000). However, none of these studies directly measured energetic state of the predators. Therefore, in Chapter 5, I examined the underlying assumption of the SDP models. This is that predators increase their consumption of chemically defended prey when their energy reserves decline. I also conducted a simultaneous binary presentation of both defended and undefended prey to determine: (1) if birds could differentiate between the prey based on the on the conditioned stimulus (i.e., background colour) and (2) if energetically stressed birds were able to make educated decisions regarding the prey.

Chapter 5 is a demonstration of the energy-toxin trade-offs involved in the consumption of chemically defended prey. However, it could be criticised for being ecologically unrealistic. Therefore, in Chapter 6, I decided to replicate my results of Chapter 5, but with two differences: (1) the energy/toxin trade-off was examined in more detail as birds gradually lost and gained mass and (2) three prey types that differed in their levels of defence were used instead of two. Few studies have examined if birds can detect modest differences in the levels of toxin content of their prey. Therefore, I predicted that if birds were able to learn the differences among prey then they should prefer prey based on their toxin content. I also predicted that the order of these preferences should remain constant as birds lost and gained mass.

Chapter 7 was similar to Chapter 6 in design, but instead of having three prey types containing different levels of quinine, the experiment provided the birds with two defended prey types that had the same mean levels of quinine. The difference came from the amount of variation around the mean that each prey type had (thus creating an experimental system similar to a risk-sensitive experiment). This created a case where the visual signal did not allow the bird to predict the quinine content of any one prey that had the variable quinine. If the birds were strategically managing their body burdens of quinine as predicted by the SDP models (Sherratt *et al.* 2004), then it would be predicted that the birds would prefer the prey with a constant level of defences. I also manipulated the mass of birds in manner similar to Chapter 6. Given that the previous two chapters have confirmed state-dependent consumption of chemically defended prey, I predict that the birds should increase consumption of both chemically defended prey. I also predict that the birds should maintain their preferences throughout the experiment.

The final experiment (Chapter 8) concentrated more on the strategic regulation of toxins, which is another focus of the model of Sherratt *et al.* (2004). This model predicted that under some circumstances that the addition of mimics to a mimicry system could decrease the rate of attack on the all prey types. This was as long as these mimics also increased the abundance of food available to the predator. I conducted an experiment to test this prediction by feeding the birds with prey of three types (alternative and visually indistinguishable model-mimics). In the low mimic frequency treatments, the birds encountered equal numbers of models and mimics. In the high mimic frequency treatment, models were encountered at the same rate but their number of encounters with mimics increased. The rate of encounter with the mimics increased as did the predators encounters with undefended prey. I predicted as with the SDP model that the overall rate of attack would decrease in the high mimic frequency treatment compared with the low mimic frequency treatment.

1.5 Conclusions

There has been much theoretical research into energetic state and how it affects mass regulation strategies and breeding behaviour in animals (Houston & McNamara 1999). However, there has been much less empirical research as to how energetic state affects the foraging decisions of animals. This might be because we are now only becoming aware of how to fully test state-based models of animal foraging decision-making in an integrated manner (Houston & McNamara 1999; Hutchinson & McNamara 2000). I aim to investigate how energetic state interacts with foraging decisions that animals make. Given that the level of energy reserves affect most animal decisions, it is probable that it also has an effect on the foraging decisions of birds.

Chapter 2

THE ENERGETIC STATE AND RATIONALITY IN EUROPEAN STARLINGS

2.1 Introduction

The application of normative economic models to evolutionary biology has been an incredibly fruitful path of research within this field. One of the areas where economic models have been most applied is in the classical models of foraging behaviour (see Schoener 1971; Pyke *et al.* 1977; Krebs & McCleery 1984; Pyke 1984; Kamil & Roitblat 1985; Stephens & Krebs 1986; Morrison *et al.* 1990; and Ydenberg 1998 for reviews). Classical models of foraging assume that foraging animals also make rational decisions. From an economic perspective, rationality generally centres on the consistency of the decisions made and the maximisation of something (e.g., utility), although there are many interpretations of rationality. For example, Kacelnik (2006) outlined three ways that the concept of rationality had been used in in three distinct academic disciplines: economics, philosophy, and biology.

It is becoming increasingly clear that rationality from a biological perspective may be different from an economic perspective (Gigerenzer & Todd 1999; Kacelnik 2006). This is because from a biological perspective, animals may be selected to make fast and accurate decisions in the face of often great environmental complexity. Normative models of foraging are based on the economic conception of rationality. This means that animals are thought to base their foraging decisions on the utility of a foraging option. In the case of animals, utility is normally equated with fitness, or a short-term currency that equates directly with fitness, meaning that animals' preferences for foraging options should be consistent irrespective of the other options that are concurrently available.

One of the reasons that normative models have been criticised is because if an animal's foraging preferences can ultimately be related to fitness, then their preferences ought to be consistently related to the value of the payoff. This means that animals ought to form preferences that conform to the principles of rational decision-making.

Recently, it has been found that animals choose among foraging options in a manner that is inconsistent with these assumptions (Shafir 1994; Hurly & Oseen 1999; Waite 2001a,b; Shafir *et al.* 2002; Bateson 2002; Bateson *et al.* 2002, 2003; Schuck-Paim *et al.* 2004). These findings suggest that animals may use comparative evaluative mechanism rather than absolute evaluative mechanisms when choosing among foraging options. Comparative evaluative mechanisms have been suggested in animals whereby choice options are evaluated relative to the other options available (starting with Shafir 1994). Comparative evaluative mechanisms are a subset of heuristics which states that the option with the most favourable combination of dimensions is chosen, meaning that choice is dependent on the other options available at the time (i.e., it is context dependent). In a natural environment, animals are often faced with more than one option available at any one time, it is probable that they utilise some form of comparative method to evaluate foraging options. However, it is important to note that comparative evaluative mechanisms in themselves are not irrational, but might result in preferences that seem irrational according to the economic conception of rationality (see section 2.1.1 for a discussion of ecological rationality).

Absolute evaluative mechanisms in contrast, posit that animals should assign a fixed value to each option and select the option that yields the maximum payoff. Therfore, an absolute evaluative method is unlikely because a bird's memory of the absolute values will change over time. The accuracy of memory declines with increasing time since the memory was reinforced or used, and an animal's foraging environment is generally highly heterogeneous. Therefore, the number of absolute values for all dietary options might have to number into hundreds, if not thousands, for all items that might be incorporated into a diet over a year. Violations of rationality have been demonstrated in humans, lower vertebrates, and invertebrates which have led some authors to suppose that there exists some universal cognitive mechanism (Shafir *et al.* 2002; Bateson *et al.* 2002, 2003; Bateson & Healy 2005). However, questions remain over the generality of their findings and whether these findings are significant within a biological context.

There have been many demonstrations of irrationality in humans in context related problems (e.g., Tversky 1969; Huber *et al.* 1982; Huber & Puto 1983; Wedell 1991). More recently, biologists have found analogous examples of violations of rationality in two taxa of animals: Insecta: Hymenotpera (*Apis mellifera,* Shafir 1994, Shafir *et al.* 1999, 2002) and various bird species (Hurly & Oseen 1999; Waite 2001a, b; Bateson 2002; Shafir *et al.* 2002; Bateson *et al.* 2002, 2003; Schuck-Paim *et al.* 2004). These violations were discovered after psychologists used methods derived from economics and decision theory to investigate if animals are rational decision-makers. These methods rely upon a form of economic rationality (Kacelnik 2006), which states that for an agent's decisions to be considered rational, they must conform to a number of principles. One of these principles is transitivity which can be defined as when an animal's preference-or-indifference relations (denoted as $\geq$) for x, y, and z are such that if $x \geq y$ and $y \geq z$, then $x \geq z$ (Luce & Riaffa 1957, p. 28; Tversky 1969). However, it is important to note that although cases of intransitivity may not fit within the economist's definition of rational decision-making, intransitive animals may still be following adaptive rules of decision-making (Houston 1997).

There are also many other principles that need to be adhered to for the decisions to be considered rational (e.g., Luce & Riaffa 1957, pp. 25-30, Kacelnik 2006). The principle of the independence of irrelevant alternatives (IIA) is another cornerstone of rational decision-making. The IIA states that the expectation of relative preference between options will be unaffected by the presence or absence of other options (Luce & Riaffa 1957; Luce 1977; Tversky & Simonson 1993). Two aspects of the IIA that I will consider in this chapter are the concepts of regularity and the constant ratio rule. Regularity is the principle that states that the addition of a third choice option to a pair of options should not increase the preference for either of the preexisting options. The constant ratio rule states that the relative proportion of choices made for each of two options should be unaffected by the addition of another less favoured option.

The asymmetrically dominated decoy (ADD) method was developed to test for violations in IIA (Huber *et al.* 1982; Huber & Puto 1983). Normally, the ADD method consists of testing the relative preference for two options that differ along two dimensions (e.g., food amount and food handling time). Subjects compare different options along these dimensions and pick the option that offers the most profitable mix along these two dimensions. The option that has its relative rate of preference increased when a decoy added to the set is considered the target. The ADD method tests the constant ratio rule through adding a 'decoy', which is equal or dominated by the target in one dimension, but inferior in the other dimension. The competitor also dominates the decoy in the dimension that the target dominates, but is inferior in the other (see Figure 2.1). This method has been used successfully to demonstrate that the addition of a decoy does significantly alter the rate of capture for one of the two pre-existing choice options (Huber *et al.* 1982; Huber & Puto 1983). It has also been shown that animals violate the conditions of IIA (e.g., Hurly & Oseen 1999; Shafir *et al.* 2002; Bateson 2002; Bateson *et al.* 2002, 2003; Schuck-Paim *et al.* 2004). These results indicate that although animals' decision-making, at times, may approximate optimal decision-making, there are times when these conditions are violated. Therefore, this brings the supposed relation between foraging payoff and fitness into question, which we assume animals are acting to optimise (see Stephens & Krebs 1986 for a review).

Table 2.1 summarises the results of studies that have investigated regularity and the constant-ratio rule, which are both predictions derived from IIA. Eight experiments have investigated regularity and three of them have shown a significant group effect. Four of these experiments also provided evidence of individual differences in behaviour; two of them found that some individuals violated the principle of regularity. However, in neither of these studies, were these individual violations consistent between individuals. This means that increase in preference for a focal option was different between individuals (Table 2.1). The other aspect of the IIA which has been considered by previous studies is the constant-ratio rule. There have been seven experiments from six studies that have tested the constant-ratio rule in animals (Shafir *et al.* 2002; Bateson 2002; Bateson *et al.* 2002, 2003; Hutton 2003; Schuck-Paim *et al.* 2004; see Table 2.1 for a summary). Of these seven studies, six have found breaches of the constant-ratio rule for group results. Three of these studies provided individual results of which one (Bateson 2002) showed individual breaches of the constant-ratio rule. However, in the studies that did not provide individual data, the proportion of

Table 2.1. A summary of animal studies that have investigated breaches in the various predictions of IIA. The symbols + and – were used to indicate results showing a breach of rationality and no breach respectively. Numbers represent the number of individuals showing breaches of principle/total sample size (— indicates that individual data was not presented). Na = not applicable.

	IIA		
Species	Regularity	Constant Ratio Rule	Source
Honeybees	–, —	+, —	Shafir *et al.* (2002)
Grey jays	+, —	+, —	Shafir *et al.* (2002)
European starlings	–, 2/8	+, 6/7	Bateson (2002)
	–, 0/7	–, 0/7	Hutton (2003)
	+, —	+, —	Schuck-Paim *et al.* (2004)
Rufous hummingbirds	+, —	na	Hurly & Oseen (1999)
	–, 7/16*	+, —	Bateson *et al.* (2002)
	–, 8/11*	+, —	Bateson *et al.* (2003)

* Indicates that although some of the birds displayed violations of regularity in their individual choices, there were differences in the directions of the effect meaning that the effect was not consistent among individuals.

individuals showing individual breaches of rationality must have been very high for the group results to be significant. The only negative results (i.e., Hutton 2003) failed to show a single individual breach of the constant-ratio rule. This result seems at odds with the other studies and the reasons for this result could indicate that birds do display absolute evaluative mechanisms in some circumstances.

There are many explanations possible for contextually induced preference reversals of which, Wedell (1991) outlined three: (1) the dimensional weight model, (2) the value shift model, and (3) the dominance-valuing model. He presented results from three experiments which tested each of these hypotheses and found support for the third hypothesis, the dominance-valuing model. This model is based on the subject's perception and the heuristic strategies that they utilise to solve a problem and predicts the main reason that animals behave irrationally is to avoid the possibility of making a poor decision. The dominance-valuing model differs qualitatively from the dimension weight and the value shift models in that, although processing proceeds dimensionwise to detect dominance, the weighting of and values along the dimensions are assumed to remain fixed. Instead of a dimension-based approach, choice preference for the targeted alternative is assumed to be altered by the perception of the dominance relationship (Wedell 1991, p.770). Therefore, this model is compatible with the heuristic based approaches of the heuristics and biases school (Tversky & Kahneman 1974; Tversky & Simonson 1993; Kahneman & Tversky 1996) and the later ecologically based heuristics approach typified by the Adaptive Behaviour and Cognition (ABC) group (Gigerenzer & Goldstein 1996; Gigerenzer & Selton 1999; Gigerenzer *et al.* 1999; Hutchinson & Gigerenzer 2005). However, there are many possible explanations for these observations and there is still much to be learned as to the cognitive mechanisms and the meanings of these observations (Huber *et al.* 1982; Wedell 1991). We know very little about the direct neurological phenomena which accompany such behaviour. However, there is growing acceptance that the mechanism for evaluation must be comparative and that some heuristic or rule-of-thumb process is responsible for such violations of rationality.

2.1.1 Normative methods versus heuristics, biases, and rules-of-thumb

A critical review of the normative approach of foraging will yield many flaws in this way of thinking about animal foraging behaviour. Whilst it is true that the predictions of simple models can approximate the behaviour of animals (Schoener 1971, Pyke *et al.*

1977, Pyke 1984, Kamil & Roitblat 1985; Stephens & Krebs 1986), generally the classical approach will fail with increased behavioural, physiological, or ecological complexity. One of the reasons for this, are the assumptions that biologists make regarding the behaviour of foraging animals in these models. For example, foragers were thought to have complete knowledge of a system. This assumption simplifies foraging models, because continued forager learning could be excluded as a factor, making the models simpler to calculate. More recently, forager learning processes have been included in theoretical models of foraging behaviour which have improved their biological realism (see Valone 2006 for a review). Therefore, to make foraging models more realistic, we need to first utilise a more integrative approach that includes all aspects of the constraints and benefits that affect individuals. Second, we need to realise that animals might not be acting to optimise within a normative framework, but may be satisficing.

Herbert Simon (1956) introduced the concept of satisfication into the economics literature. This idea was that individuals would choose the first choice option that satisfies a certain need or most of their needs, yet do not necessarily provide the optimal solution. Therefore, satisfication yields results that are good enough, but may not be the best solution. Indeed, there may be many other solutions to the problem, which yield equal or better results. However, in the appendix of Simon (1956), he stated that once this strategy had been picked, the subject could then act to optimise within the context of the satisficing rule. This effectively moved optimisation down a level, and introduced the possibility of optimisation with constraints. Tversky (1969) demonstrated intransitivities in humans and suggested that the assumption that subjects apply fixed utilities to options that they then compare in order to select the option that maximises utility may be flawed. He suggested that animals might actually use a comparative evaluative mechanism because it was a more efficient method of calculating differences between options. Amos Tversky and Daniel Kahnemann went on to publish many more studies in this field and their approach came to be known as the heuristics and biases school (e.g., Tversky & Kahnemann 1974; Kahnemann *et al.* 1982). This approach stated in the face of complex problems, animals might simplify problems by adopting strategies that they termed heuristics. These heuristics, which offered fast and frugal solutions, were often hampered by producing biases in the decision-making process. However, it is also possible that priming effects could have an influence on these biases

(Fantino & Fantino 2005). The effects of priming have not been adequately controlled for in animal experiments and remain a major problem in these studies.

Later researchers criticised the heuristics school for their lack of ecological relevance and the maintenance of a normative core to human and animal decision-making processes (Gigerenzer & Goldstein 1996; Gigerenzer & Todd 1999; Todd & Gigerenzer 2000; Hutchinson & Gigerenzer 2005). Gerd Gigerenzer and colleagues at the Adaptive Behaviour and Cognition (ABC) Group at the Max Planck Institute for Human Development also maintain that decision-making should utilise simple algorithms based on simple psychological models. They argue that animals should utilise fast and frugal heuristics and bounded rationality to solve seemingly complex problems. They developed the idea of ecological rationality where animals are considered differently from how they were treated by the heuristics and biases school. The rationality that animals use–ecological rationality–where animals spend most of their time trying to make decisions in ever changing environments is different from the heuristics and biases school which used traditional definitions of rationality. The traditional definitions of rationality maintain that subjects are trying to maintain an internal order and consistency. Therefore, fast and frugal heuristics deliver solutions to problems that are accurate, quick, and low-cost in the face of ecological complexity (Gigerenzer & Todd 1999; Gigerenzer & Hutchinson 2005). Hence, the observation that animals' preferences fail to match predictions of rationality may be wide of the mark. This is because animals are using ecological rationality that may be different from the traditional views of rationality. Therefore, results may not be the results of biases as supposed by the heuristics and biases school. Rather, the biases may result from the trade-off involved in the need for free living animal to make accurate decisions quickly in an ecologically complex environment. The study of ecological rationality is still in its infancy, but it offers a new way to think about cognitive evolution and decision-making that is far more realistic in how animals deal with their environments.

2.1.2 Alternative explanations for context-dependent results

Within the animal and human literatures, there have been those who defended the normative approach in the face of these hostile criticisms (e.g., Maynard Smith 1977; Cohen 1981; Stephens & Krebs 1986, pp. 207-215). Their rebuttals have tended to focus on four arguments: (1) performance errors, (2) computational limitations, (3) the application of the wrong normative model, and (4) a different construal of the task by the subject than was intended by the researcher (Stanovich & West 2000). I will consider each of these criticisms briefly below.

The argument that animals are making performance errors is an argument when animals' behaviour has not conformed to model predictions. Because of its liberal use, it has become amorphous and almost meaningless although inherent error is a valid concern in any empirical enterprise. Performance errors have often been the first argument that biologists resort to in the face of results that did not match their predictions. For example, Rechten *et al.* (1983) stated that birds were unable to discriminate between large and small prey thus causing partial preferences in great tits (*Parus major*) diet for small prey. However, other factors such as energetic state were ignored in the experiment. Moreover, this issue could have been resolved with simple simultaneous presentations of both large and small prey.

Computational limitation is another argument that has been used to explain the many results that are thought to breach the formal principles of rational decision-making. This argument is based on the fact that the problems posed by researchers may be too complex for animals to solve, or they do not have the cognitive machinery to reach the expected normative response. Little is known about the neurological bases of the cognition involved in evaluating between different foraging problems. Tversky (1969) supposed that evaluative comparisons might involve fewer calculations when comparing between two or more options. However, the current level of understanding of the cognitive and neurobiological bases of evaluation means that his hypothesis is still untested. However, this idea was one of the first examples, of what would later become known as the heuristics and biases school. Heuristics and later bounded rationality have become common explanations for breaches in rationality that has been found in humans and animals.

If data were used to falsify an incorrect normative model, this would lead to an erroneous result. This argument has been used a lot more in the human literature than in the animal literature (Stanovich & West 2000). As mentioned above, few animal studies have attempted to validate few of their experimental parameters. Although it is possible that the wrong model is being applied a data set, without adequate validation this can neither be proved nor disproved. This brings me to the final criticism, difference in perception between the animal subject and the researcher. Such biases could lead to the subjects construing the problem in a different way than was intended by the experimenter. Therefore, failure to support the model's predictions, is not a true negative, but a false negative or more correctly, it results in the hypothesis not being tested. Stanovich and West (2000) also pointed out that individual differences in responses could lead to results that lead to violations of rationality and could help explain the inconsistencies that exist in some of the animal data (e.g., Bateson *et al.* 2002, 2003).

One problem with applying descriptive economic models to animal behaviour is that they fail to include the biological factors that may affect decision-making both within individuals over time and between individuals. One such factor is energetic state, which may affect decision-making in many ways that are only now becoming apparent. I will examine one recent hypothesis for state based violations of rationality in the next section.

2.1.3 The role of energetic state in ADD effects

Schuck-Paim *et al.* (2004) conducted an experiment in order to examine if the energetic state of birds' had any effect on the violations of rationality that had been observed in other studies. The authors manipulated the energetic state of starlings to assess their preferences for different foraging options that varied in two dimensions in accordance with the asymmetrically dominated decoy experimental design. One half of the birds had their energy intake controlled so that they received an amount of food that was sufficient to meet their energetic demands. The remaining six birds received insufficient food from the trials that should have caused the birds to have lower energetic states than the fed group. Each of these two groups was given two treatments of different choice options. Two of the choice options were the same in both treatments and these were the focal pair of options. They had the same 'short-term' rate of intake of 0.5 units of food per second. Short-term rate of energetic gain has been suggested as a possible currency that birds utilise in decision-making processes (Bateson & Kacelnik 1996). One option delivered two pieces of food after a four second wait (focal option for delay [F_D]) while the second option delivered five pieces of food after a ten second wait (focal option for amount [F_A]). A third option (the decoy) differed between treatments in the payoffs and waiting times they offered birds, which were asymmetrically dominated by the focal options. In the high intake treatment, the decoy for amount (D_A) had an equal payoff to the F_A but had a longer delay of 20 seconds. The low intake treatment, the decoy for delay (D_D) delivered one piece of food after a four second wait. Using this these options presented in binary and trinary arrays, Schuck-Paim *et al.* (2004) were able to test the rationality of decision-making of birds depending on energetic state and the nature of the asymmetry between decoys and focal options.

Schuck-Paim *et al.* (2004) argued that their data indicated that in situations where birds were energetically stressed (the group where intake was not controlled during the low intake treatment [D_D]), they tended to display breaches in rationality. However, the birds' decisions were rational when they had higher levels of food intake (D_A treatment). These apparent breaches in rationality at different energetic states were due to differences of the marginal fitness values of the rewards in different energetic states. This meant that the option that delivered the higheset fitness was dependent on the birds' energetic state, but the decisions were rational given that they were choosing the option that maximised fitness. Initially, this was a very interesting finding because it indicates that the birds' 'irrationality' in other studies might have been caused by differences in energetic state and the birds may have been behaving in a rational manner. However, the study has problems that can be classified into two types: (1) experimental design and (2) analysis and interpretation of data.

The design of the experiment has two main flaws. First, the authors stated that the short-term rate of return for a focal option was 0.5 food units per second and 0.25 food units per second for a decoy option. This was calculated as the number of food items received for choosing the option until the time the food was delivered. However, the effect of the inter trial interval of 60 seconds was not considered which given their model is state-based is surprising. Energetic state is a function of the food derived over the entire experimental period. Given that there was capacity for the times it took birds to complete the experimental session to differ between individuals and between bouts, the state changes of birds could also have been different. If the short-term rate is recalculated to take account of these factors then the birds received: 0.0712 food items per second for F_A, 0.0312 food items per second for F_D, 0.0625 items per second for D_A, and 0.0156 food items per second for D_D (calculated as experienced delays plus inter trial intervals). Second, there was no direct measurement of the energetic state of birds before, during, or after each experimental session. Therefore, there is no direct evidence to suggest that birds during low intake treatments had lost more reserves than birds in the high intake treatments.

There were also major flaws in the analysis and interpretation of the results. First, the breach in regularity and in the constant ratio rule was found when comparing the binary (low intake) with trinary (high intake) treatments. Their statistical treatment was problematical because this comparison was not valid. They conducted independent statistical tests between treatment effects for the binary versus trinary presentations in low and high food intake treatments. However, these effects are interactions meaning that the data should have been analysed in a single model and an interaction term calculated. Given the choice preferences of birds that are made between trinary and binary options are known to be context dependent, context effects are important and so comparisons ought to maintain the contextual relationships that are inherent in the method. Therefore, the results that the authors claimed are invalid given the method used to obtain them.

Second, the authors Schuck-Paim *et al.* (2004) also asserted that some of the context-dependent violations of rationality found in other studies could have been due to inadvertent effects of pre-experimental deprivation periods on the subject's energetic state. These periods are imposed upon bird in order to increase their hunger and so make them more likely to participate in the experiment. Although this is a valid concern in some experiments, it cannot explain the results that have been obtained from free-living subjects that were able to find their own food and so were not reliant upon the food provided in the experiment (Bateson *et al.* 2002, 2003; Shafir *et al.* 2002). These experiments provided birds with supplements on top of their normal diets. Therefore, their fat reserves were probably greater compared with non-supplemented conspecifics of the same population.

The Schuck-Paim *et al.* (2004) model is interesting in that it proposed a state-based explanation of the violations of rationality that have been recorded in previous studies. However, their experiments to test their hypothesis were poor tests of their model. There were many confounding effects that were uncontrolled for and their statistical analysis was incorrect. Therefore, in order to test their hypothesis a state manipulation that alters the levels of fat reserves of subjects may be a better way of testing this idea.

2.1.4 Experiment introduction

In the section above, I outlined some of the problems of the Schuck-Paim *et al.* (2004) experiment. I wanted to try to replicate the results of this experiment using the same experimental species, but in a different experimental design. Hutton's (2003) findings that European starlings did not violate the constant-ratio rule are also interesting because they support the use of absolute evaluative processes. I used the method used in

Hutton (2003), which was developed by Melissa Bateson. Therefore, my experiment had two aims which were: (1) to investigate if birds demonstrated violations of the constant-ratio rule and (2) if preferences between binary and trinary food presentations remained constant or changed with measurable changes in energetic state. If the Schuck-Paim *et al.* (2003) model is correct, I expect that the birds would violate the constant-ratio rule when birds were in poorer energetic states.

2.2 Methods

2.2.1 Subjects

European starlings are large ground-foraging insectivorous passerines that are widespread throughout Europe. They are a semi-social species and are partially migratory with the proportion of birds migrating increasing with increasing latitude. Naturally, their masses can range between 75-90 g with males being heavier than females. When captured and taken into aviaries, birds can lose between 5-10 g in mass due to reduced body reserves of fat and atrophy of flight muscles. Starlings are considered generalist foragers due to their great geographic range and success as a naturalised species in other parts of the world (e.g., North America, Australia, Southern Africa, and New Zealand). Their diets consist of mainly invertebrates and insects, which are supplemented by fruit and nectar where available (Feare 1984).

The subjects were eight wild-caught (European starlings [5 males, 3 females]) caught under licence from English Nature (Licence Nos. 19991381 and 20001512). Prior to the commencement of the experiment, birds had been kept communally in a large indoor free-flight room and used occasionally in operant box experiments, but none had been used previously for context experiments. Five days before the start of training, birds were moved into individual in wire cages (445 mm high x 750 mm wide x 450 mm deep), which were housed in an air-conditioned room maintained at ca. 17 °C with a 14:10 hour light: dark cycle. Four birds were kept in each of two neighbouring rooms. This was because in order to observe birds during the experiment, I had to place the birds against the back wall of the room. The shelves were only able to accommodate four cages in any one room along the back wall. The birds were fed Purina wild game starter, mealworms (*Tenebrio molitor*), and fresh fruit pieces. They also had continuous access to water at all times of the day. Birds could see and hear other birds at all times.

2.2.2 Fat scores and weighing

Birds were caught daily between 0900 hr and 1000 hr Greenwich Mean Time (GMT) to have their fat levels assessed and to be weighed. I assessed the bird's fat level using a modified visual scale of subcutaneous fat around the trachea (modified from Gosler [1996, 2004], Table 2.2), which has been demonstrated as a good indicator of overall reserves (Blem 1990). To do this, I immobilised the bird by placing it on its back into the palm of one hand and straightening its neck so that its bill was pointing forward. With my other hand, I held the legs on the tarsi. The position of the birds is important because if the necks of different individuals are at different angles, it can influence the reading because the tissue bunches when the neck is at its normal angle. Once immobilised, I blew back the feathers around the base of the neck to ascertain its fat score. This method was also used in subsequent chapters when fat scores were taken. I then weighed the birds to the nearest 0.1 g using an electronic balance (Ohaus Scout SC6010). Birds were immobilised for weighing by placing them, head first, into a conical plastic bag until their body was tight against the walls of the bag. The bag had the apex of the cone removed to allow airflow into the bag. Birds were generally in the bag for between 10-20 seconds. Prior to the start of the experiment, I used the eight masses of birds that had been used during training to calculate the free-feeding masses of birds so that I could then calculate the birds 90 % free-feeding masses.

Table 2.2. System developed to visually assess subcutaneous adipose tissue around the tracheal region of starlings.

Fat Score	Visible Characteristics
0	No fat visible in tracheal cavity (T.C.)
1	< half T.C. covered in fat
2	> half T.C. covered in fat
3	Entire T.C. covered in fat
4	Fat in T.C. level with keel
5	Fat bulging above the level of keel
6	Fat extending beyond T.C. and bulging

2.2.3 Feeder design and construction

Feeders were made from a ceramic tile with a short piece of polyvinyl chloride (PVC) glued to it. The tubing had an internal diameter of 38 mm cut at one end at a 90° angle to the longitudinal plane of the tube and the other end was cut at 45° to the longitudinal plane (Fig. 2.1). The tube was affixed to the tile at an equal distance from the left and right sides of the tile with araldite on the 45° cut to create an angled tube running at 45° from the horizontal tile. The short side of the tube was nearest the front face of the tile and the angle of the tube took the opening away from the tile. The three feeders were all identical except for the length of the feeder from the bottom lip of the opening to where it met the ceramic tile (measured along the bottom side of the PVC tubing). The three lengths that were chosen were 20 mm, 50 mm, and 60 mm (Fig. 2.1). Birds had previously been shown to have preferences for shorter tubes when all other things were equal (Hutton 2003). I will henceforth, refer to the bottom length of the feeder as "length" throughout the rest of the chapter because this is the measure of length that was used to differentiate between feeders. The mealworms were placed on the tile at the base of the shortest part of the tube where it met with the tile. This method was inspired by Waite's (2001a) experiments on gray jays that were required to venture different distances into wire mesh tubes. The length of the tube was a negative dimension, because the longer tubes may have exposed the birds to higher risks of predation and may have had higher energetic costs of food retrieval associated with increasing length.

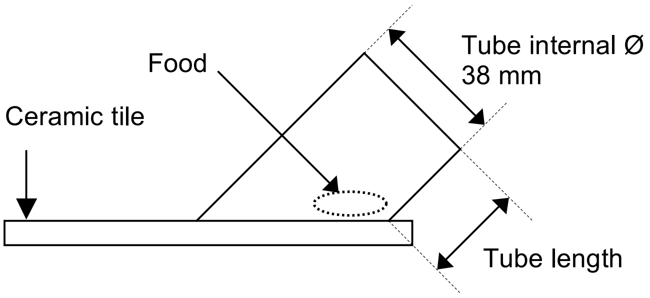


Figure 2.1. A side elevation of a feeder that was used in this experiment. The tube lengths used were 20 mm, 50 mm, and 60 mm (figure modified from Hutton 2003).

2.2.4 Training

During the training phase of the experiment, birds were maintained on *ad libitum* food for soft-billed birds, with additional fruit and mealworms provided each morning. Water was available to birds at all times. Food was removed from their cages two hours prior to the commencement of the training. Training took place by habituating birds to the three feeder types in order of tube length from shortest to longest. Training was carried out by first habituating birds to 20 mm feeders containing one *Tenebrio* sp. larvae (henceforth, mealworms). I presented all the birds with a feeder at once and left the room for three minutes. I returned after three minutes to see if birds had consumed the mealworms. Once all birds were consuming mealworms without me present (this took a maximum of 17 presentations), I began presenting the feeders and then moving away from birds until I stopped at a distance of about five metres. From there, I observed them while I was in their full view. This was so that I could observe the birds and time their latencies to feed. Birds were considered habituated when it took on average less than 20 seconds for them to consume the mealworms from the time I moved away from the cage. Once the birds were reliably feeding from the feeders, they were habituated in the same manner to 50 mm and 60 mm feeders which both contained five mealworms. Overall, the process of training lasted 9 days as it proceeded at the same pace for all birds as I only moved onto the next step once all birds were performing the behaviour without hesitation. This was so all birds experienced the same number of training feed presentations. I made one feeder presentation to all birds every 12 minutes. Each feeder type was marked with coloured insulation tape in one of three colours (blue, yellow, or red), which corresponded with a feeder length for that individual. The colours for each feeder were different in order to facilitate association between colour and feeder type and colours were balanced between individuals as much as possible (in order to counteract colour biases). After 9 days of training, all birds were consuming the mealworms from all three-feeder types without signs of fear. On the tenth and eleventh days, I conducted 30 forced trials (15 on each day) on each individual in which birds had one of the three feeders presented with its reward. Again, the feeders were presented once every 12 minutes in a random order for each individual. The presentations were staggered so that only one individual was worked at a time. The forced trials were completed to familiarise the birds with the three feeder types and their rewards. It also allowed birds to get used to having all feeders presented in one session. Once the forced trials were completed, I began the experimental trials.

2.2.5 Experimental protocol and daily routine

This experiment examined the effect of two factors: (1) the relative preferences of birds for targets and competitors in the absence of decoys (binary presentations) compared with when a third decoy option was present (trinary presentations) and (2) the effect that energetic state had on the relative preferences of birds in binary and trinary presentations. The experiment consisted of four phases where energetic state and context were manipulated in the manner of a 2 x 2 experiment design. Hence, the birds experienced four phases during the experiment (a binary and trinary phase for each state treatment). Each phase consisted of 30 simultaneous presentations of either two feeders (binary phases) or three feeders (trinary phases) while the birds were in different energetic states (90 % free-feeding mass and free-feeding mass) which were split between two days (15 on each day). Therefore, the entire experiment was made up of 120 presentations split between the two state treatments and the two context treatments. I conducted the experiment in a counterbalanced design for state and context treatments. This meant that half the birds were run first at 90 % of their free-feeding masses and the other half were run at their free-feeding masses. The order in which the context treatments were run was then split between the two mass groups.

The experiment was designed using the asymmetrically dominated decoy method (Huber *et al.* 1982; Huber & Puto 1983). The three feeder options varied along two dimensions (tube length and food reward, Fig. 2.2). The food reward (payoff) was the positive dimension because birds always preferred greater numbers of mealworms (Hutton 2003). Tube length was a negative dimension because birds preferred shorter lengths (Hutton 2003; Melissa Bateson, personal communication). This preference for shorter feeders may have been related to the birds perception that they were exposing themselves to a higher probability of being captured by a predator. Therefore, the 50 mm feeder containing the five mealworms was designated the “target”, whereas the

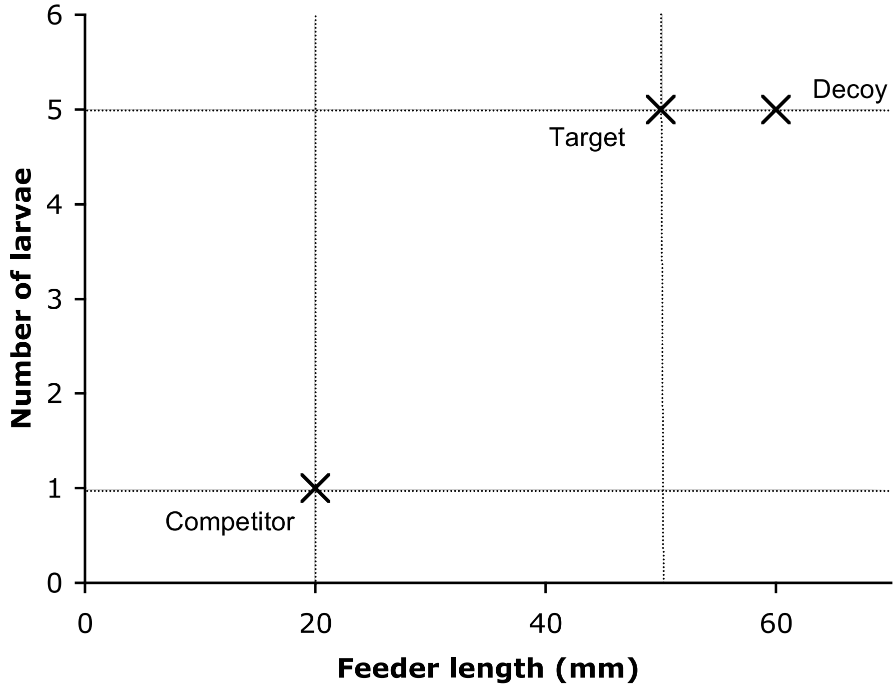


Figure 2.2. The treatments used in this experiment conformed to the asymmetrically dominated decoy design. The number of larvae was a positive dimension while the feeder length was a negative dimension.

"competitor" contained a single mealworm in the 20 mm feeder. The "decoy" contained five mealworms in the 60 mm feeder (Fig. 2.2). The mealworms measured approximately 8 mm long and were sourced from a pet food supplier.

The daily routine started with fat scoring and weighing of the birds between 0900-1000 hr GMT. The birds were then allowed at least two-hours access to food before the onset of food deprivation. This was done in order standardise the effects that the gut contents of the birds might have on the results. This was because in the 90 % free-feeding mass treatment, birds tended to consume their food before lights were switched off at the end of the day. This meant they might have had longer fasts compared with when they were free-fed. Thus, by allowing all birds at least two-hours access to food, it allowed them to have food in their stomachs prior to the commencement of the two-hour period of food deprivation. This standardised the differences in gut contents between treatments, but maintained the effects of other state variables. This meant that short-term state differences were standardised between state treatments, but the long-term differences in state were maintained. Following the completion of the two-hour food access period, the food was removed from the birds' cages. They were then food deprived for a further two-hours and the experiments started generally between 1300-1500 hrs. Daily sessions lasted up to three hours although they occationally exceeded this (up to 15 feeder presentations) with the birds' only access to food during the experiment sessions being the payoffs they received from the feeders. The birds had a choice trial every 12-minutes meaning that I was generally able to complete one presentation for the eight birds within the 12-minute cycle. The birds were presented with either a pair (in the binary phases) or three feeders (in the trinary phases). In binary presentations, the feeders were placed near the ends of the cage with the tubes facing towards the inside the cage. In trinary presentations, two feeders were placed near the end (~ 50 mm from end) of the cage and one placed in the centre next to the door. Once I had placed the feeders in to the cage, I moved briskly away and activated a hand-held stopwatch. I used this to calculate the birds' latency to feed and total handling time. From these times, I was able to calculate the latency to feed and the average handling time per larva. I calculated preference as the birds' first choice in the trial. I terminated the trial once the bird had taken its head out of the feeder and began to move away from it. I did this by moving towards the bird's cage in order to prevent it from feeding from another feeder. At the end of the daily experimental session, the birds' food was placed back in their cages and they were left until the next morning's state measurement. At the end of the experiment, all birds were kept in their cages to allow their masses to reach their pre-experiment level and then they were placed back into free flight for use in further experiments.

2.2.6 Statistical Analyses

Analyses were performed using SPSS (version 11.0) for Macintosh computers and version 13.0 for PCs. I performed statistical tests using a linear mixed model (LMM) which included repeated measures tests on the data when all assumptions of the models were met. All proportionate data were arcsine square root transformed and converted to degrees. I did not conduct tests for individual birds, as subject was included as a factor in the model meaning the statistics were conducted in an integrated manner. I calculated the relative preference of targets to competitors below.

$$\text{Relative preference} = \frac{(\text{Proportion of targets} - \text{proportion of competitors})}{(\text{Proportion of targets} + \text{proportion of competitors})} \qquad (2.1)$$

Means are presented ± SE, and all statistics are two-tailed with the significance level set at 0.05. I used the least significant difference (LSD) method to compare contrasts within fixed factors. I adjusted alpha levels were using a Bonferroni correction (α/n) when I made multiple comparisons.

I also collected data on the effectiveness of birds at getting prey from feeders and the amount of handling time that birds spent on consuming each prey item. In order to calculate the proportion of larvae obtained from each feeder, I averaged the number of larvae consumed by the number of presentations. The data were pooled by bird, feeder type, state treatment, and trial context. In order to calculate the handling time per prey, I calculated this as the amount of time from when the bird put its head into the feeder until the bird either moved away or stopped feeding. I then divided this by the number of mealworms eaten to obtain the handling times per prey consumed. I analysed both sets of data that these calculations generated using LMMs.

2.3 Results

2.3.1 Side and colour biases

Birds often have biases for a particular side of the cage on which they prefer to attack food. In order to exclude the possibility that birds were biasing their decisions for one particular side, I conducted chi-squared tests for each individual for both the binary and trinary treatments. The results for the binary presentations of the target and competitors show that there were no significant biases for any of the birds (Table 2.3). In the trinary trials, there did seem to be more of a tendency for birds to choose the centrally placed feeder although none of the results were significant (Table 2.3). In six out of eight birds, the highest number of choices was for the centre option. Six out of the eight birds also had *P*-values of less than 0.1 (marked with an asterisk). Although this suggests that there might have been a slight bias, it was not deemed bad enough for the data to be corrected.

Table 2.3. Tests for side biases using χ^2 tests for binary and trinary presentations of prey ($\chi^2_{(0.05,2)}$: binary=3.841, df=1; trinary=5.991, df=2; * denotes $P<0.1$).

	Binary			Trinary			
Bird	Left	Right	Test Statistic	Left	Centre	Right	Test Statistic
4	30	30	0.00	18	28	14	5.2*
15	27	33	0.60	12	21	27	5.7*
21	30	30	0.00	12	25	23	4.9*
30	29	31	0.07	22	26	12	5.2*
34	33	27	0.60	21	25	14	3.1
35	30	30	0.00	15	28	17	4.9*
40	26	34	1.07	22	25	13	3.9
44	33	27	0.60	21	12	27	5.7*

2.3.2 Effect of food restriction on energetic state

The aim of this experiment was to manipulate the energetic state of birds. In order to do this I compared the masses of birds when they were allowed *ad libitum* access to food with when theyds had limited food availability to maintain the masses at 90 % of their free feeding mass (FFM). It was not possible to maintain the birds' masses at exactly 90 % of their FFM. However, the birds masses were significantly lighter in the food-restricted treatments ($\bar{X}$ ± S.E.: 100 % FFM=77.05 ± 1.51 g; 90 % FFM=70.49; paired *t*-test: $t=-15.085$, df=7, $P<0.0001$) as were their levels of furcular fat reserves ($\bar{X}$ ± S.E.: 90 % FFM=1.0 ± 0.3; 100 % FFM=2.6 ± 0.3; paired *t*-test: $t=-8.844$, df=7, $P<0.0001$). Individual regression analyses also revealed that the relation between mass and fat scores were highly significant (4 f: $F_{1,44}=48.274$, $P<0.001$, $r^2=0.523$; 15 f: $F_{1,39}=77.542$, $P<0.001$, $r^2=0.671$; 21 m: $F_{1,40}=62.301$, $P<0.001$, $r^2=0.615$; 30 m: $F_{1,28}=5.966$, $P=0.021$, $r^2=0.181$; 34 m: $F_{1,40}=14.837$, $P<0.001$, $r^2=0.276$; 35 f: $F_{1,40}=43.596$, $P<0.001$, $r^2=0.528$; 40 m: $F_{1,27}=19.631$, $P<0.001$, $r^2=0.430$; 44 m: $F_{1,44}=29.508$, $P<0.001$, $r^2=0.407$). This indicates that mass losses were also associated with mass losses in birds.

2.3.3 Discrimination between options

The birds displayed differences in the number of times they chose each option (Fig. 2.3). I constructed LMMs in order to examine if energetic state was a significant factor affect birds' preferences. I included feeder type and context as fixed factors. Subjects were included as random subject variables and the dependent variable was the transformed proportion of times each feeder was chosen. There were no effects of the mass treatments for either the trinary or the binary presentations meaning that the data could be pooled for this part of the experiment (LMM: binary, $F_{1,21}$=0, P=1; trinary, $F_{2,42}$=0.005, P=0.947). During binary trials, birds chose to consume significantly greater numbers of targets compared with competitors (repeated measures ANOVA: $F_{1,7}$=144.296, P<0.001, Fig. 2.3). This pattern was also mirrored in the trinary trials with there being significant differences between the three options that the birds chose ($F_{2,14}$=120.574, P<0.001, η^2=0.945, Fig. 2.3). There are differences between the feeders in the number of times the birds chose to consume each option. Comparisons of the differences in the means indicated that birds preferred the targets to the competitors (LSD: target – competitors, P<0.001; targets – decoys, P=0.001; and decoys – competitors, P<0.001). Therefore, the target was consistently favoured over the decoy,

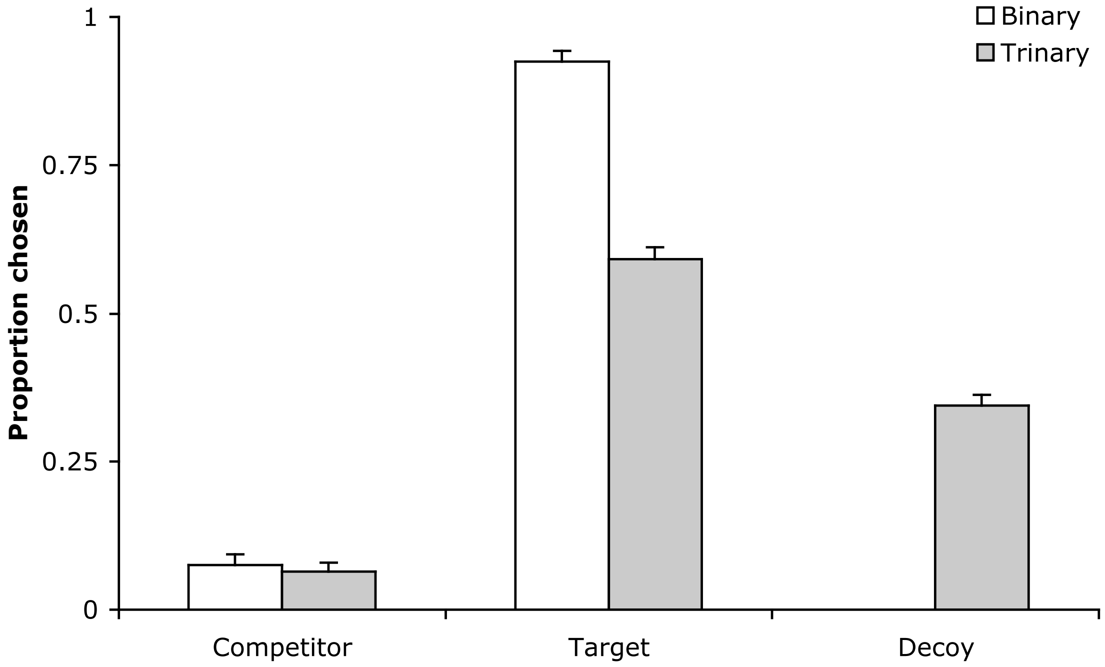


Figure 2.3. The mean (+SE) proportions of choices for each food option presented for both the binary and trinary phases of the experiment. The data was pooled for state treatment because there were no differences for state in both the binary and trinary presentations.

the decoy was favoured over the competitor, and the target was favoured over the competitor.

2.3.4 Independence of irrelevant alternatives (IIA)

The addition of the decoy in the trinary trials did not increase the relative proportion of choices for either the target or the competitor meaning that the birds' decisions were regular (Section 2.3.3 and Fig. 2.3). Therefore, the principle of regularity was not violated in the experiment, which is one of the cornerstones of rational decision-making. Figure 2.4 shows that the relative preference of the target which was calculated using formula 2.1 (see section 2.2.6). The constant-ratio rule states that the addition of a decoy of lesser value in trinary trials should not affect the relative proportions of the preference for the initial two options when compared with the results in the binary trials (although the decoy in this experiment was not of lesser value). I constructed a LMM, which included energetic state and context as fixed factors. Subjects were included as subject variables meaning that the analysis controlled for differences between individuals. The arcsine square root transformed relative preferences of birds were the dependent variable. When I compared the relative preferences for the target and competitor, I found that there was no significant difference between choices made in the two mass classes (LMM: $F_{1,21}=1.595$, $P=0.221$, Fig. 2.4). Comparisons between the binary and trinary presentations indicated that the decoy did not affect the birds' preferences ($F_{1,21}=0.918$, $P=0.349$, Fig. 2.4). Therefore, there is no evidence to suggest that the birds' choices in the trinary trials were affected by the addition of the decoy. I tested for differences in behaviour between individuals and found that variance between individuals accounted for only 19 % of the total variance of 0.021 which was not statistically significant (Wald Z test: $Z=0.867$, $P=0.386$). The other 79 % of variation was accounted for by within individual variation and error.

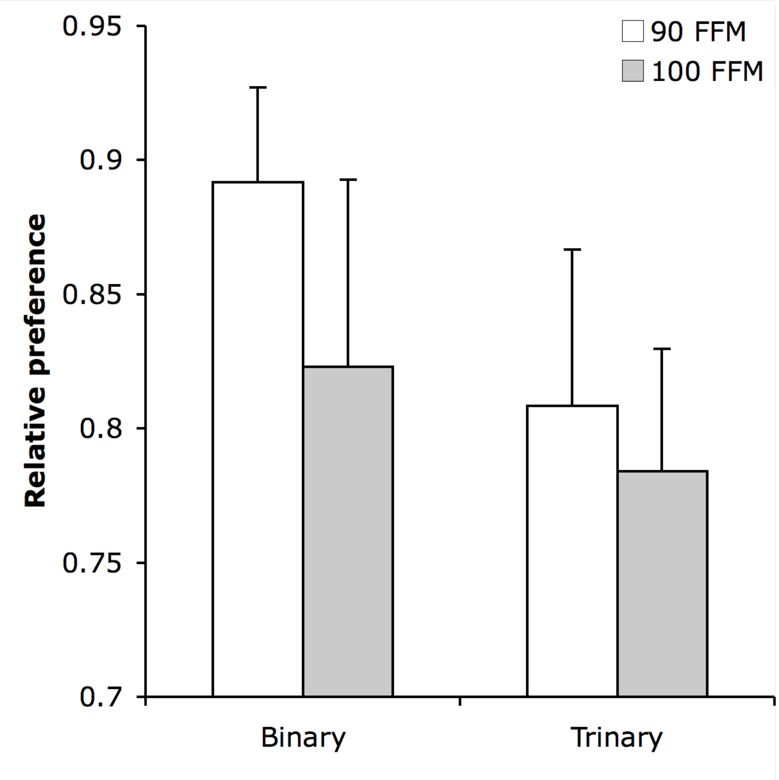


Figure 2.4. The mean relative preferences (+SE) of birds' in relation to context and food availability.

2.3.5 Payoff for each option

It was assumed that birds' ability to obtain mealworms from the three feeder types was similar among all three types. I conducted two analyses where I compared the proportion of mealworms consumed from each feeder type and the amount of handling time per mealworm extracted from each feeder type. For the first analysis, I constructed a LMM for the proportion of mealworms eaten from each of the feeder type. I included context, state, and feeder type as fixed effects and subjects were included as a random subject variable. The arcsine square root transformed proportions of the mealworms eaten was the dependent variable. I found that there was a significant difference in the proportions of mealworms eaten for the three feeder types ($\bar{X}$ ± SE: target=0.979 ± <0.001, competitor=1.000 ± 0.001, decoy=0.939 ± 0.001; LMM: $F_{2,57}=30.099$, $P<0.001$, Fig. 2.5). When I compared extraction rates between the three feeder types, I found that the extraction rates between all three feeder types were significantly different from one another (LSD: target – competitor, $P<0.001$; target – decoy, $P=0.003$; competitor – decoy, $P<0.001$). I also found that there was a significant mass effect becasue birds tended to extract a higher proportion of mealworms when they were in the food restricted phase of the experiment ($F_{1,57}=4.206$, $P=0.045$, Fig. 2.5).

However, there was no difference in extraction rates between binary and trinary trials ($F_{1,56}$=0.374, P=0.543).

I also conducted a similar analysis on the handling time per mealworm eaten as the dependent variable and all other factors identical to the above model. I found that there was no effect of feeder type ($\bar{X}$ ± SE: competitor=0.86 ± 0.118 sec, target=0.93 ± 0.105 sec, decoy=1.16 ± 0.138 sec, $F_{2,53}$=2.175, P=0.124), mass manipulation ($\bar{X}$ ± SE: restricted=0.87 ± 0.103 sec, free-fed=1.03 ± 0.105 sec; $F_{1,53}$=1.706, P=0.194), or treatment ($\bar{X}$ ± SE: binary=0.92 ± 0.112 sec, trinary=0.964 ± 0.099 sec; $F_{1,52}$=0.221, P=0.64) in the handling time per larva.

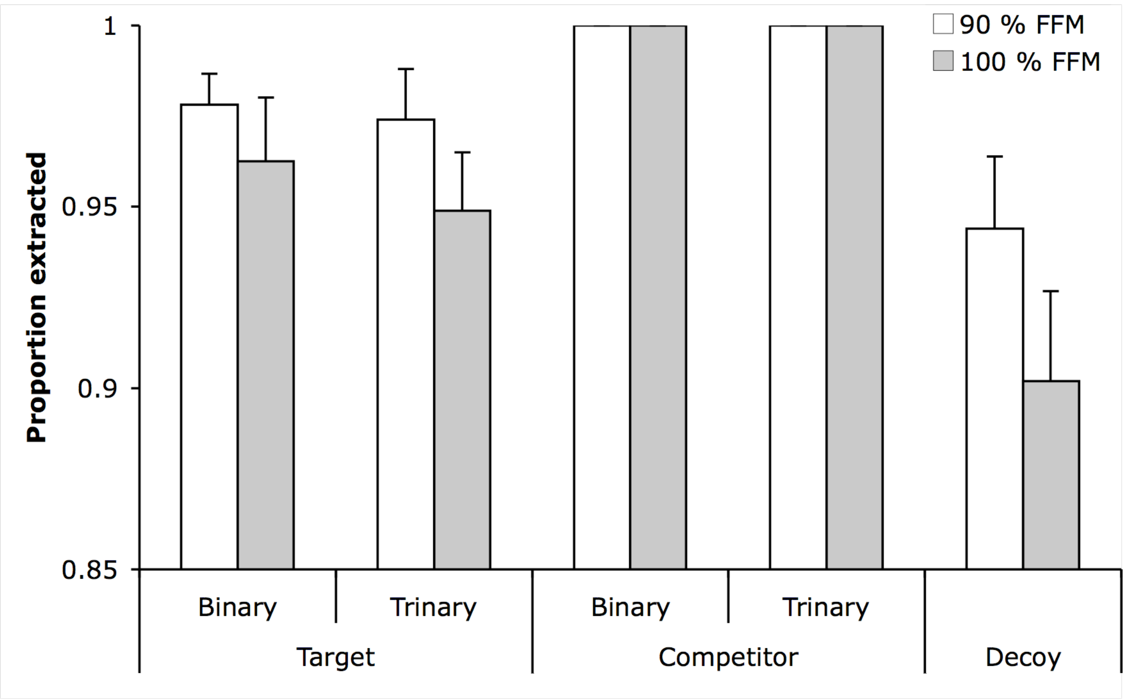


Figure 2.5. The mean proportion (+ SE) of larvae that birds were able to extract for each feeder option and for each mass treatment.

2.3.6 Feeding latencies in relation to feeder length

Latencies to feed can give an indication of subjects' motivation to feed from a feeder. Thereore, I constructed a LMM of the latency to feed data which included energetic state, context, and the feeder type as fixed variables. I included subject as a random subject variable and the dependent variable was latency to feed. These data show clear differences for the availability of food with the birds taking longer to make a choice when they had free access to food compared when they had restricted access to food ($\bar{X}$ ± SE: 90 % FFM=5.12 ± 1.971 sec, 100 % FFM=13.29 ± 1.902 sec; LMM: $F_{1,56}$=8.925, P=0.004, Fig. 2.6). There were no other significant factor effects in the analysis:

treatment ($\bar{X}$ ± SE: binary=9.39 ± 2.217 sec, trinary=8.921 ± 1.746 sec; $F_{1,56}$=0.002, P=0.965), feeder type ($\bar{X}$ ± SE: target=6.93 ± 2.013 sec, competitor=12.22 ± 2.316 sec, decoy=7.74 ± 2.77 sec, $F_{2,56}$=1.745, P=0.184).

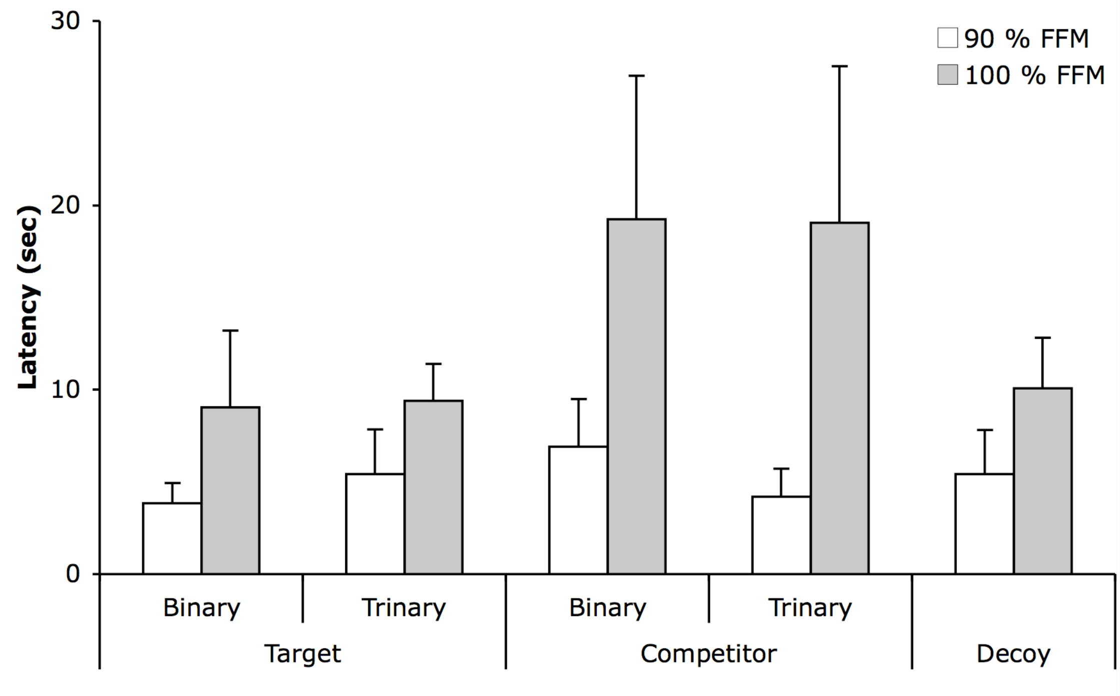


Figure 2.6. The mean latencies (+SE) of the birds to chose a feeder in relation to feeder length, food availability, and treatment.

2.4 Discussion

The results from this experiment indicate that the birds did not violate the principle of regularity or the constant-ratio rule and there was no effect of state on the birds' preferences. This result is consistent with the results of Hutton (2003) who also found that European starlings' preferences did not change between binary and trinary presentations. Therefore, these results suggest in starlings, at least, that they may utilise an absolute evaluative method. If this were the case, this would provide support for the normative approach to modelling foraging behaviour. It would also raise doubts over the previous studies that have found that animals consistently violate the constant-ratio rule (Bateson 2002; Bateson *et al.* 2002, 2003; Shafir *et al.* 2002). However, this interpretation of the results may be incorrect.

Through a detailed *post-hoc* analysis of my data (Section 2.3.5), I found that birds ranked feeders more in line with the number of prey extracted from the feeders. This is because although the birds have been shown to prefer shorter feeders when it was the only dimension presented (Hutton 2003), this dimension was not weighted equally with the number of mealworms that they were able to obtain from each feeder. Therefore, it is likely that the birds were construing the problem in a manner that was not intended. This means that the data cannot be interpreted as a test of the constant-ratio rule and so cannot be used to support for the normative approach. Hutton's (2003) study also utilised a similar method to this study, meaning that her data may also have suffered from similar biases. Hence, there cannot be said to be any studies that have conclusively supported the normative approach despite the results of Schuck-Paim *et al.* (2004).

The tube feeder length was adopted as a negative dimension in these experiments because birds such as starlings may find it unpleasant to stick their heads into such a confined space. When a bird sticks its head into a confined space, its field of view becomes greatly reduced. This means that they may be exposed to a greater risk of capture if they were to be attacked by a predator. However, it is known that animals are able to facultatively adjust their behaviour to match their perceived risk of predation (Caro 2005). Therefore, birds in a laboratory may perceive a much lower risk of predation than a free-living animal. Moreover, training is a factor likely to lead to a negation of the birds natural aversion of foraging from highly restricted places. Other studies have used negative dimensions that have a measurable cost (Waite 2001a; Shafir *et al.* 2002). For example, the experiments of Shafir *et al.* (2002) gray jays and bees were required to enter tunnels in order to obtain the rewards. The length of the tubes varied and so the costs of entering the tunnels may have been much more obvious and possibly much more risky. Moreover, the gray jays used in Shafir *et al.* (2002) were free-living animals meaning that their actual and perceived risks of predation were potentially much greater than the laboratory housed birds used in this study and Hutton (2003).

Given that the birds are likely to have valued the dimension of length as being much less important than payoff when making decisions about which feeder to choose, this study cannot be said to have adequately tested the Schuck-Paim *et al.* (2004) model. Their hypothesis is an interesting idea although it cannot be applied to all situations because the majority of studies that have utilised the ADD experimental design have been on free-living animals that have probably obtained greater reserves than conspecifics not participating in the experiment. It would be interesting to test this hypothesis in with a treatment that unequivocally manipulated state as I was able to do in this experiment. It is unlikely that free-living animals could be used in negative state manipulations. This is because it would be difficult to negatively manipulate a subject's energetic state without it supplementing its diet with food obtained from the environment.

In this experiment, there was no effect of energetic state on the preferences of birds although there was a significant effect of energetic reserves on the latencies to feed. Latencies can be used as a behavioural indication of the bird’s energetic state. When the birds had fewer reserves, they were quicker to approach the feeders compared with when they had greater reserves. Hence, there were behavioural correlates that indicate that birds had fewer energy reserves. Schuck-Paim *et al.* (2004) also found that when birds were subject to low food intake, their latencies to first peck were significantly shorter than in the high intake treatment. In this experiment, there was also a lower rate of extraction from the two longer feeders when they were at their free feeding masses. This indicates that when the birds had greater levels of energetic reserves, they were less eager to feed from the feeders and less willing to persevere with continuing to feed from the feeders. This may indicate that overall, the birds were more motivated to feed from feeders when they were more energetically stressed.

The aim of this experiment was to investigate whether breaches of regularity and the constant-ratio rule could be demonstrated and if the decisions of birds were different depending on their energetic states. The results demonstrate that the birds displayed no violations in rationality as have been demonstrated in invertebrates (Shafir 1994; Shafir *et al.* 2002), non-human vertebrates (Hurly & Oseen 1999; Shafir *et al.* 2002; Waite 2002 a, b; Bateson *et al.* 2002, 2003; Schuck-Paim *et al.* 2004), and humans (e.g., Simonson & Tversky 1992). This result is unlikely to be the result of a lack of power because birds were forced to make 120 decisions split between four treatments. Previous studies have found violations of rationality with similar numbers of replicates (e.g., Waite 2001a). Moreover, my results demonstrate the importance of validating the dimensions used in an experiment. This is something that has not been done in many of the previous experiments that have used similar methods in order to disprove normative models of foraging (Shafir 1994; Hurly & Oseen 1999; Bateson *et al.* 2002, 2003). My results emphasise that supposed constraints imposed upon animals in an experiment may not be perceived in the manner envisaged by researchers.

There have been a number of studies that have investigated rationality in animals by testing for intransitivity in the binary preference relationships of three choice options. These experiments have had mixed results because whilst they suggest that there may be comparative evaluative mechanisms, the data is less than convincing. For example, in Shafir's (1994) study, only three out of fifteen bees showed breaches of rationality, which while interesting, is not enough to argue for a specific hypothesis. Indeed, the remaining twelve subjects displayed behaviour consistent with both absolute and comparative evaluative mechanisms. Therefore, this lack of ability to falsify either hypothesis when no violation of rationality was recorded was a major problem for this experiment. Waite's (2001a) results were far more important as he demonstrated that an entire sample of 12 birds showed consistently similar results. This suggests that the effect was a widespread property of the decisions made by the entire group. Hurly & Oseen (1999) and Bateson (2002) conducted risk-sensitive foraging experiments in rufous hummingbirds (*Selasphorus rufous*) and European starlings respectively, and found that some of the individuals violated aspects of rational decision-making. However, none of these experiments utilised the ADDs to investigate breaches in rationality.

Although there is evidence animals have been shown to violate principles of rational decision-making, there are doubts as to if the observations are biologically meaningful. The biological relevance of the data is questionable, as not all studies have found consistent results between individuals (Bateson *et al.* 2002, 2003). Consistency of preference is another important factor in rational decision-making (Simon 1959). However, this factor has rarely been addressed in biological studies. This tends to indicate that a common cognitive cause was not the underlying process leading to violations of rationality. If the violations were due to a common feature of the cognitive architecture, then it might be expected that animals would display preference orders and changes in preference that were consistent between individuals and in the same individuals over time.

The differences in the cognitive architectures and central nervous systems between insects and higher vertebrates are unlikely to give rise to universal cognitive bases for these behaviours. This is despite the fact that insects might be capable of similar learning tasks as vertebrates (e.g., Giurfa *et al.* 2001) and the fact that superficially, similar data can be generated in these two taxa. It is more likely that these similarities are derived from convergence rather maintenance of some primordial feature of the cognitive architecture of all animals. Moreover, violations in rationality have only been investigated in five species, which makes the hypothesis of a universal cognitive mechanism extremely premature. Some of the analysis and data manipulation in previous studies has been questionable as some of the studies only used a small sample of the total amount of data collected (e.g., Bateson *et al.* 2003) or excluded individuals that they deemed were not behaving in an appropriate manner (Bateson 2002). Moreover, many of these authors have been willing to accept their alternative hypothesis without adequately excluding other possible explanations, which might confound interpretation.

2.4.1 Conclusions

Although it has been claimed that animals demonstrate the same violations of rationality as humans, there is sufficient doubt to remain cautious due to sufficient methodological and analytical problems. Moreover, as Kacelnik (2006) points out, it is questionable whether studying animal behaviour in the context of economic rationality is a good thing. The results of this study revealed that the birds were probably construing the problem in a manner that was not consistent with my original intention. Reconstructing the problem to consider only the impact of the payoff dimension revealed that birds were behaving in manner that would have been consistent with normative models. However, this does not mean that the birds were using absolute methods of evaluation.

Comparative methods of evaluation are theoretically much more likely because foraging decision-making is a Bayesian feedback process. This means an animal builds a subjective interpretation of the costs and benefits of options and combines which informs the animal’s present choice. The cues that animals use to evaluate the differences between options are still unknown and remains one of the great problems of decision-making. Moreover, the neurobiological bases of foraging decision-making are almost unknown and in order to investigate the existence of universal cognitive mechanisms for violations of rationality there is great need for these mechanisms to be investigated fully. At present, the evidence is too scant and the differences between taxa are too great in order to infer any general principals. Therefore, further studies into this phenomenon are imperative, specifically experimental methodologies that can offer analogous problems to different species.

Chapter 3

THE ENERGY BUDGET RULE AND FORAGING BEHAVIOUR OF RUFOUS HUMMINGBIRDS (*SELASPHORUS RUFUS*).

3.1 Introduction

Risk-sensitive foraging was developed in order to introduce environmental stochasticity into foraging models (Caraco 1980, 1981; Caraco *et al.* 1980; Real 1980, 1981). This was because initial models of animal foraging assumed mean rate of intake was enough to describe animal foraging behaviour (Stephens & Krebs 1986). However, in a stochastic environment, the long-term rate of energy intake can be different from the short-term rate. Therefore, animals may have evolved to pay attention to variance around the mean energy intake in addition to the mean itself. Risk-sensitive foraging can be defined as when an animal displays a preference for one of the two options with equal long-term means, but with different variances. Risk-sensitive foraging has been demonstrated in many species of animals (see Stephens & Krebs 1986, pp. 134-137; Kacelnik & Bateson 1996; Bateson & Kacelnik 1998; Bateson 2002b for reviews). However, the reasons for risk-sensitive foraging behaviour remain elusive.

Traditionally risk-sensitive foraging behaviour of animals has been explained through relationships that exist between the reward size and fitness. Many formulations of risk sensitive foraging use Jensen's Inequality to relate the shape function of fitness and reward size to the animal's behaviour (see Smallwood (1996) for a wider discussion of Jensen's Inequality). Figure 3.1 indicates that the fitness benefits of choosing between two options: constant (indicated by solid vertical line and C the x-axis) and variable (indicated by dotted vertical lines and V_L [low payoff] and V_H [high payoff] on the x-axis). The variable option is theoretically meant to have the same long-term average as the constant option. That is, supposing that the forager is unable to differentiate between variable options prior to sampling them. Figure 3.1 diagrammatically explains Jensen's Inequality and how it is applied to risk-sensitive foraging problems. Assume that the constant option delivers four food items and the variable option delivers either two or six food items for the low and high variable options respectively with equal probability. On occasions where fitness is a decelerating function of reward size (Fig. 3.1a), then it would be predicted that animals should be risk-averse. This is because if an animal chooses the variable option (V_L or V_H), then the fitness costs of randomly picking V_L are greater than the benefits of selecting V_H. Therefore, the net fitness gain for choosing the constant option is greater than for choosing the variable options. Conversely, if fitness is an accelerating function of reward size then risk-proneness is predicted (Fig. 3b). This is because the fitness payoff obtained from V_H is greater than the loss of fitness that accrues through picking V_L. If the fitness function were linear, then there would be no difference fitness outcomes. Therefore, animals ought to be indifferent as predicted by classical normative models of foraging (Schoener 1971; Pyke *et al.* 1977; Pyke 1984; Kamil & Roitblatt 1985; Stephens & Krebs; Morrison *et al.* 1990). The supposed non-linear relationships between fitness and reward size is at the heart of most fitness-based risk-sensitive foraging models. One hypothesis that assumes a non-linear relationship between energy intake and fitness is the energy budget rule (EBR).

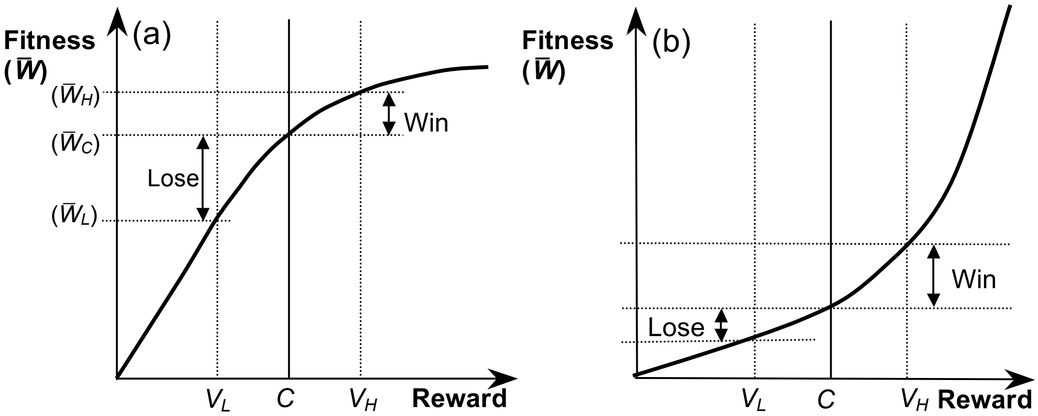


Figure 3.1. The dependence of risk-sensitive behaviour on the relationship between fitness and the reward size. (A) In situations where fitness ($\bar{W}$) is a decelerating function of the reward size, $\bar{W}_V = \Sigma\ (W_H) + \Sigma\ (W_L)/\ N < \bar{W}_C$ meaning that the constant option (C) ought to be chosen more often than the variable option in order to minimise losses associated with choosing V_L (risk-averse). (B) When $\bar{W}$ is an accelerating function of reward size, $\bar{W}_C < \bar{W}_V = \Sigma\ (W_H) + \Sigma\ (W_L)/\ N$ meaning that the variable option ought to be favoured as this strategy maximises gains (risk-prone) (modified from Bateson & Kacelnik 1998, p. 303).

The EBR was first formalised by Stephens (1981) after the verbal description of Caraco *et al* (1981). Stephens presented the problem from the perspective of a small bird trying to minimise the risk of starvation during a short winter day (Fig. 3.3). He argued that a bird had to reach a critical amount of energy reserves (R) by the end of its available foraging time. If the bird had N foraging periods before the end of the day, it had to ensure its reserves at the end of the day were greater than or equal to *R*. If the bird had the choice of two foraging options, one being with a constant reward, the other with a mean equal to the constant, but with variation around the mean, it should choose the option that maximises the probability of reaching *R* by the end of the day. Therefore, if the bird made a choice at N as to which patch to feed from for the rest of the day; it would use its reserves at time N, plus the sum of the rewards it would reap from each patch, minus the energetic costs of foraging from N until dusk. If the bird knows that its reserves at N are sufficient to reach *R* by the end of the day if it continues to forage in the constant patch, then it has a positive energy budget (scenario 1, Fig. 3.3). On a positive energy budget, the bird does not need to take risks in order to reach *R*. Alternatively, if the constant reward is insufficient to allow the bird to reach *R* the bird has negative energy budget (scenario 2, Fig. 3.3). This means, the bird needs to choose the risky option in the hope that it will experience a run of good luck, which will take its reserves onto a positive energy budget. There have been many studies of the

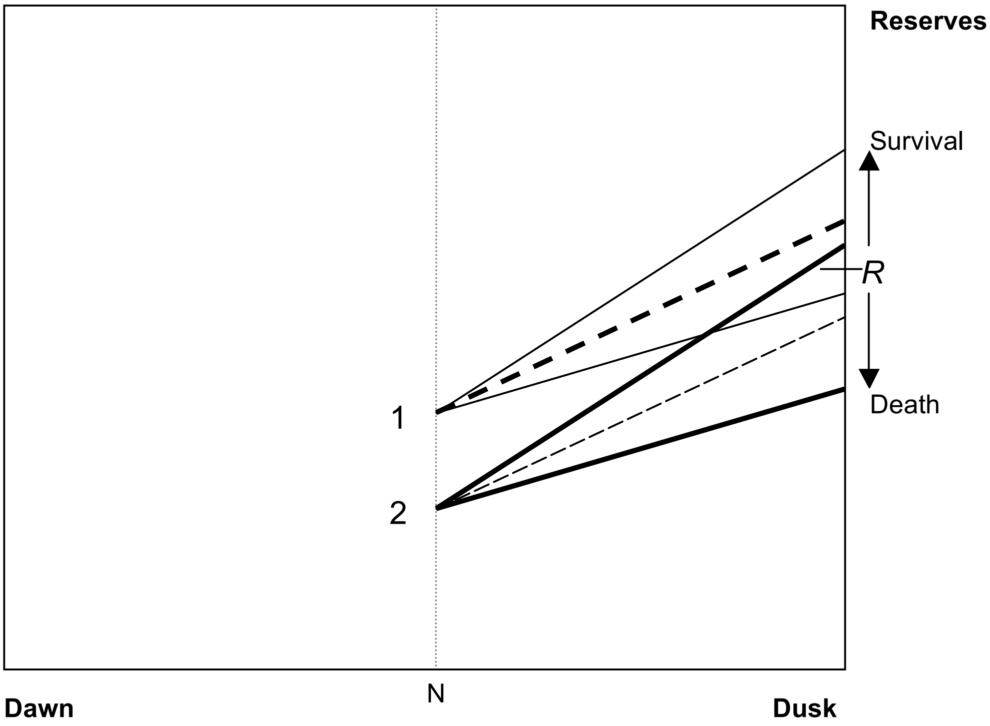


Figure 3.3. Over the course of a day's foraging, an animal needs to find enough food in order to survive until the next morning (the point *R*). If by time$_N$, a bird has had an average amount of success, then it is likely to reach *R* if it continues to eschew risky behaviour (the thick dashed line scenario 1). However, if it had a run of bad luck resulting in low energy reserves (point 2 by time$_N$), then it will be unable to reach *R* through exploiting the constant option. Hence, the animal can only reach *R* by adopting a risk prone foraging strategy (the thick solid line in scenario 2) (Illustration modified from Bateson & Kacelnik 1998, p. 309).

EBR and many have shown behaviour consistent with the EBR. However, many other studies have failed to find any evidence of the EBR (see Kacelnik & Bateson 1996; Bateson & Kacelnik 1998; Bateson 2002b for reviews).

Table 3.1 outlines the results from studies of the EBR in relation to changes in their energy budget and how it affects preferences for variance in amount. Generally, there is too much variability in results between studies for the EBR to be considered a general explanation for risk-sensitive foraging behaviour. The most convincing demonstration of the EBR remains the study of Caraco *et al.* (1990) who used temperature to manipulate the energy budgets of dark eyed juncos (*Junco phaenotus*). They demonstrated that the birds were risk-prone after being kept at 1˚C whereas they

Table 3.1. A summary of studies that have studied that have studied the changes in energy budget with regards to variability in amount.

Species*	Type of study	Result[†]
Insects		
Bombus spp.[2]	Manipulated colony reserves of energy	~
Bombus occidentalis[1]	Manipulated colony reserves of energy	+
Apis mellifera[3]	Manipulated colony reserves of honey	–
Xylocopa micans[4]	Energetic values of rewards varied to alter EB	–
Fish		
Rhoderus sericus[5]	Prior deprivation altered to manipulate EB	+
Spinachia spinachia[6]	Prior deprivation altered to manipulate EB	+
Cichlasoma octofaciatum[7]	Prior deprivation altered to manipulate EB	–
Birds		
Junco phaenotus[8]	Laboratory manipulation of EB	+
—————[9]	Temperature used to alter EB	+
Junco hyemalis[10]	Laboratory manipulation of EB	+
Zonotrichia luecophrys[11]	Laboratory manipulation of EB	+
Dendroica coronata[12]	Two groups subjected to different treatments	+
Coereba flaveota[13]	Laboratory manipulation of EB	+
Columbia livia[14]	Laboratory manipulation of inter-trial interval	–
Zonotrichia albicollis[15]	Laboratory and on a probably negative budget	–
Parus atricapillus[16]	Field study that manipulated the foraging costs	–
Cyanocitta cristata[17]	Laboratory manipulation of EB	–
Sturnus vulgaris[18]	Laboratory manipulation of energy budget	+
Mammals		
Rattus norvegicus[19]	Amounts of food delivered per unit of time	~
—————[20]	Number of forced trials before experimental trials	–
—————[21]	Number of trials in a session altered in order to alter EB	~
—————[22]	Mass manipulation in order to alter subjects EB	~
—————[23]	The amount of work varied which might have altered EB	~
Sorex araneus[24]	Length of inter-trial interval altered to manipulate EB	+
Macroscelides probiscideus[25]	Many methods of manipulation of EB	~

***References:** 1. Carter & Dill, 1990, 2. Carter 1991, 3. Banschbach & Waddington 1954. 4. Perez & Waddington 1996, 5. Young *et al.* 1990, 6. Croy & Hughes 1991, 7. Roche *et al.* 1998, 8. Caraco *et al.* 1980, 9. Caraco *et al.* 1990, 10. Caraco 1981, 11. Caraco 1983, 12. Moore & Simm 1986, 13. Wunderle *et al.* 1987, 14. Hamm & Shettlworth, 1987, 15. Tuttle *et al.* 1990, 16. Barkan 1990, 17. Clements 1990, 18. Reboreda & Kacelnik 1991, 19. Leventhal *et al.* 1959, 20. Battalio *et al.* 1985, 21. Kagel *et al.* 1986, 22. Ito *et al.* 2000, 23. Kirshenbaum *et al.* 2003, 24. Barnard & Brown 1985, 25. Lawes & Perrin 1995.

[†] Results: – Result not consistent with EBR predictions; ~ results partially consistent with EBR; + results fully consistent with EBR.

were risk-averse after being kept at 19°C. Table 3.1 summarises the results from 26 studies that have experimentally attempted to alter the energy budgets of individuals in order to observe changes in risk-sensitive foraging behaviour. Of these 25 studies, 10 studies have reported results consistent with the EBR and six studies have results that have partially supported the EBR. By partial support, I include studies where the behaviour fits with the predictions when in one state but not in another. For example, Kirshenbaum *et al.* (2003) found that rats (*Rattus norvegicus*) were risk-prone on negative energy budgets, but indifferent at other times meaning that the result whilst subjects were in negative energy budgets was consistent with the EBR. There also seems to be differences in the results obtained from different taxonomic groups. For example, comparing the results of studies from birds and mammals indicate that data from birds tends to more strongly support the EBR. One reason for these differences may be differences in body size between the mammals and birds.

Bateson and Kacelnik (1996, 1998) have reviewed the risk-sensitive foraging literature and one factor that they found that did seem to be important was the relationship between body size and switches in the risk-sensitive behaviour of animals They found that smaller species might be more likely to show changes in risk-sensitive behaviour that are consistent with the EBR. Small species may be more susceptible to changes in food availability because they cannot store as much of their future energy needs as body fat compared with larger species. Therefore, the time horizon for regulating fluctuations in food availability is smaller for smaller animals (Houston & McNamara 1993). The body sizes of birds that have been used in studies of the EBR are generally smaller than mammals which could help explain these differences between taxa.

There are a number of problems with the EBR, which were discussed by Bateson and Kacelnik (1998, pp. 311-314). One of the most important was the model of Stephens (1982), which assumed that animals could make only one decision at the beginning of a foraging period regarding which food option they chose. They were then bound to this option for the rest of the foraging period. Although Stephens (1982) was able to make his point clear using this method, animals are unlikely to behave in this manner. A more realistic scenario is likely to be closer to the SDP models of Houston & McNamara (1999, pp. 88-115). In these models, foraging periods are broken up into many shorter periods where an animal chooses among its foraging option at the beginning of each foraging iteration. The decisions at each foraging iteration are based on three variables: (1) the amount of time that is left in the foraging period; (2) the animal's energy reserves that it has when making the decision; and (3) the amount of reserves that are required to guarantee survival into the next foraging period (Fig. 3.2).

Risk-sensitive foraging theory provided new insights as to how animals might deal with variability in their foraging environment and provided the catalyst of a new state based approach to foraging (Houston & McNamara 1999). However, it generally neglects to integrate many other aspects of behaviour that may also impact on an animal's foraging behaviour. There have been attempts by zoologists to apply ideas of risk-sensitive foraging to social foraging problems (e.g., Ekman & Hake 1988; Wu & Giraldeau 2005) and sex-based foraging differences (Wright & Radford, unpublished manuscript). Recently, Hurly (2003) published a twin threshold model in which he stated that animals might have two thresholds which indicate switch points for both starvation and reproduction. His model predicted that birds are likely to eschew risk when the mean intake was lower as variable options were more likely to lead to starvation. However, as mean intake increased, birds were predicted to become more risk-prone as the probability of starvation decreases. This is because less variable options may be insufficient to provide enough energy to attain a second threshold (the reproductive threshold). This means accepting some risk is the only option which will provide the possibility of obtaining enough energy reserves to reproduce. He used this hypothesis to explain the risk intermediate choice behaviour that he observed in rufous hummingbirds (*Selasphorus rufus*).

Hummingbirds are a good species in which to study the effects of climate on the foraging decisions as they are small (mean mass of males=3.22 g, range: 2.9-3.9 g, N=22 [Johnsgard 1997]) and have among the highest mass specific metabolic rates for any vertebrate species (Suarez *et al.* 1990; Suarez 1992). It has also been found that the amount of time that different individuals spend foraging can vary greatly, with a range of between 5-23 percent (Wolf & Hainsworth 1971). These differences in foraging behaviour may be the result of differences in habitat quality, weather conditions, or the behaviour of subjects. Hummingbirds also exhibit a high degree of sexual dimorphism with males often appearing very different from females. The males also conduct species-specific courtship flights when they are visited on their territory by females. During these courtship flights, males climb to about 15 metres in the air and then go into a very steep dive and arcs out of the dive as he approaches the ground (Hurly *et al.* 2001). At the bottom of this arc, he makes an auditory wing buzz or vocalisation before making a series of wobbles associated with probable wing noise. He then stops near the female for up to 2 sec before he begins his ascent for another flight which can be repeated as many as 5-8 times. These flights are likely to put a lot of stress on blood vessels in the body and it is thought that males almost lose consciousness at the bottom of the arc as the blood pressure increases in the brain (Johnsgard 1997). These flights are also likely to be energetically costly due to the speed the male reaches in the arc.

Finally, there have been a number of studies that have previously examined the risk-sensitive foraging behaviour of rufous hummingbirds (Stephens & Paton 1986; Waser & McRoberts 1998; Hurly & Oseen 1999; Biernaskie *et al.* 2002; Hurly 2003). Generally, these studies have found that hummingbirds are risk-averse under most conditions (i.e., they prefer constant options) although indifference to volume variance has also been recorded (Bateson *et al.*, unpublished manuscript). Finally, Hurly (2003) presented data that birds preferred intermediate levels of risk when they were choosing between three options. This he took to be evidence for his twin-threshold model. Rufous hummingbirds are thought to be a particularly suitable species in which to study risk-sensitive foraging because their physiological and ecological characters appear to correspond with the basic assumption of the model for a small homeotherm living in an energetically stressful environment (Caraco 1980; Stephens 1981; Stephens & Charnov 1982; Stephens & Krebs 1986).

3.1.2 Experiment introduction

The EBR has only received limited empirical support (Caraco *et al.* 1980, 1990) and there only been one field study that has found support for the EBR (Carter & Dill 1990; Table 3.1). Carter and Dill (1990) found that when the colonies of bumblebees (*Bombus occidentalis*) were supplemented with nectar, that they became more risk averse. Barkan (1990) attempted a field-based study of the EBR in black-capped chickadees (*Poecile atricapillus*) and failed to find any evidence that birds were being risk-prone or that the birds were ever on a negative energy budget. Caraco *et al.* (1990) was able to alter the energy budgets of birds through keeping them at different temperatures. I attempted to recreate this finding using the natural temperature variation throughout the day.

The aim of this experiment was to test predictions of the EBR in a field-based experiment. These predictions are that birds have different energetic demands brought about by changes environmental conditions. These changes in environment can change the bird's metabolic rate and thus, the birds' energy budget. The volume of flowers has been shown to be an important factor in flower selection by hummingbirds (Gass & Sutherland 1985). Therefore, I compared the birds' preferences for artificial flowers with a constant volume of nectar (probability of a 25 μl reward=1) versus flowers with a variable volume of nectar, but with the same mean (probability of a 10 μl reward=0.5, probability of a 40 μl reward=0.5). These volumes are much larger than those that birds would normally encounter when foraging on natural flowers, but similar volumes have been used in previous studies of risk-sensitive foraging in hummingbirds (e.g., Hurly & Oseen 1999; Hurly 2003).

In order to do this, I conducted choice trials on 5 males during three time periods throughout the day (early morning, mid-day, and the evening) and at different points in the season that were separated by about two weeks. I did this in order to establish if there were any differences in their preferences that might be due to some factor that correlates with the time of day or season (e.g., ambient temperature). By choosing times of the day with the most variation, I assumed that the natural variation in temperature would cause differences in the birds' metabolic rates. This could in turn, cause changes in the birds' preference for the variable and constant flowers. I also examined the relationship between flower preference and possible seasonal changes due to changes in breeding or migratory status. During these observation periods, data was collected on the number of courtship flights made by males so I could examine if was a relationship between the number of J-flights and their flower preferences and rates of energy consumption. This data was collected in an attempt to examine the interaction of potentially costly behaviours and the decisions made by individuals.

I predicted that birds should become more risk prone when they should theoretically be most energetically stressed. These times are likely to be during the morning when the ambient temperature was at its lowest and because they may have been recovering from their nightly fast. I also predicted that the birds should be more energetically stressed earlier in the season compared with later in the season. This is because the ambient temperature increases in the late spring at the field site. Finally, I predicted that if behaviours are reliant upon the energy budget, then there should be a relationship between the expression of costly behaviour and the level of risk-prone behaviour with the risk-proness increasing after J-flights and other expensive behaviours.

3.2 Methods

3.2.1 Study species and site

The subjects were five wild male rufous hummingbirds observed between 5 June and 7 July 2004 at Westcastle Valley in southwestern Alberta, Canada, in the eastern range of the Rocky Mountains (49 29 N; 114 25 W, elevation 1400 m). The habitat was mixed arboreal forest interspersed with clearings of grasses. Rufous hummingbirds are one of the widest ranging of the North American hummingbirds reaching southeastern Alaska in the summer. They over winter in Mexico south to Guerrero and northern Oaxaca. The adults feed on nectar from various flowers supplemented with small insects and fruits (Johnsgard 1997).

3.2.2 Experimental apparatus

The artificial flowers were 20 holes (10 mm deep x 3.5 mm diameter) that had been drilled into a Plexiglas plate (280 mm x 215 mm x 12 mm) at equal distances from one another (52 mm). The flowers could hold 125 μl of liquid and were marked with coloured paper reinforcement rings to indicate if flowers were constant or variable. The Plexiglass plates were attached to a metal spike that could be easily driven onto the ground. These spikes held the plates at about 700 mm from the ground surface and at a 45° angle from the ground.

3.2.3 Training

During mid-May, commercial hummingbird feeders containing 14% sucrose solution (mass of solute/mass of solvent) were placed in potential territories to attract males. When males started defending feeders they were individually marked by spraying their breast feathers with a small amount of nontoxic coloured, waterproof ink (see Hurly & Oseen 1999 for details). These ink marks started fading after 4 weeks and were completely faded by the time males begin to migrate by the end of July. Between early June and early July 2004, I collected data during three periods of the day (morning, noon, and evening).

Birds were trained to feed from Plexiglas sheets in a gradual process that started with lowering the feeders to about 1.5 m above ground level and introducing a small Plexiglas block covered in red tape with yellow flowers around the wells. The corolla of the flower was indicated by a yellow coloured paper reinforcement ring. The Plexiglass block matched the colours of the base of the feeder and it gradually was moved onto the feeder. With each feeding visit the bird made, more of the tape was removed until the no tape remained. Once birds were feeding from the small block, it was then attached to the large Plexiglas sheet that were used in the experiment as the birds continued to visit the feeder. The small block was kept on the larger sheet until the birds were successfully feeding from the large sheet without hesitation, whence the small block was removed. This training process normally took between 3 and 6 hr. Each step progressed when the bird fed without hesitation after a change. Three of the birds that I used were trained using this technique by other people for use in other experiments prior to their use in this experiment.

3.2.4 Forced trials

At the start of each cycle (early or late in season), the birds were presented with forced trials where they were presented with Plexiglass plates containing only single flower types (constant or variable). I alternated the plate so that the birds had a bout with each flower type every second forced bout. This continued until the birds had sampled 30 flowers of each type. This allowed the birds to have prior knowledge of the flowers before the commencement of the experiment where both flower types were presented simultaneously on the same plate. Once the bird had experienced 30 of each flower type in forced trials, the experiment started.

3.2.5 Experimental protocol

Artificial flowers were presented to birds on single Plexiglas plates with 20 wells drilled into them and that had coloured paper reinforcement rings around the edge to indicate the corolla of the flowers. Each colour (purple or orange) was randomly assigned into one of two reward types (constant or variable) with the 10 constant wells containing 25 μl of 20 % sucrose solution. The variable wells contained either 10 μl or 40 μl of 20 % sucrose solution with equal probability. The association of flower type and colour varied between individuals, but was kept constant for each individual. I checked the preferences for constant flowers in relation to the flower colour in order to check for colour biases. If there had been a bias for one colour over the other, it would have been selected at a disproportionately higher rate than the other would have been. There was no evidence to suggest a preference by birds for one colour over the other when choosing between flowers (constant flower colour, proportion of flowers chosen that were constant: bird 1=orange, 0.435; bird 2=purple, 0.572; bird 3=orange, 0.541; bird 4=purple, 0.459; bird 5=orange, 0.552). The flowers were arranged in a random manner on the plate that was determined prior to the observation session. Each pattern was used for four foraging bouts and was rotated in a 90° clockwise direction after each foraging bout. This was to ensure individual birds did not learn where the more profitable flowers were situated. This was important because I assumed that individual's decisions were based on the amount of variation in each flower type.

The plates were set up in clearings near where the male's feeder was situated in order to aid observation. I observed the birds with binoculars from a distance of approximately 5 m from the plate and observed their behaviour while speaking onto a Dictaphone. I then transcribed the choice sequence of the bird and the temperature to the nearest 0.1° C later. After a foraging bout, I evacuated the remaining sugar water using a syringe and refilled each well using a micro-pipette with its corresponding volume and rotated the plate 90°.

The birds' choices were observed over three periods throughout the day: the morning (0600-0900 hr Mountain Standard Time [MST]), the afternoon (1200-1500 hr MST), and in the evening (1800-2100 hr MST). These times were chosen to maximise the temperature differences throughout the day and to observe the birds close to the beginning and the end of the foraging day. There were significant differences in the temperatures at the three times that I chose ($\bar{X}$ ± SE: morning=9.9 ± 0.76°C, afternoon=16.3 ± 0.38°C, evening=14.1 ± 1.13°C; repeated-measures ANOVA: $F_{2,8}=30.18$, $P=0.0002$). Pair wise comparisons revealed that the morning temperatures were significantly lower then both the afternoon (Tukey's HSD: $P<0.01$) and evening temperatures ($P<0.01$), but that there was no difference between the afternoon and evening temperatures ($P>0.05$).

I aimed to observe 60 drinks per individual for each 3 hr period of observation; however, on some occasions this was not possible due to poor weather or inconsistent bird behaviour. Individuals were observed for about 4 days in total, which I split into 2 cycles. Each cycle lasted about 2 days and were either early or late being separated by about 2 weeks from the end of the first choice cycle to the start of the second choice cycle ($\bar{X}$ ± SE=14.2 ± 0.23 days). The climate at this latitude and altitude meant that it was significantly warmer in the later cycle compared with the early cycle ($\bar{X}$ ± SE: early cycle=11 ± 1.25 ºC, late cycle=16.1 ± 0.57 ºC, paired *t*-test: *t*=-3.74, df=4, *P*=0.0201). At the beginning and end of each observation session, I recorded the weather conditions and temperature (to the nearest 0.1 °C). Immediately after each bird's foraging bout, I also recorded the temperature in order to incorporate this into the foraging analysis.

3.2.6 Behaviour

During the training and experimental trials I recorded the occurrence of three behaviours that might be related to state: (1) courtship flights (J-flights), (2) shuttle flights where males hover in front of females before attempting to copulate with them, and (3) intraspecific and interspecific aggressive interactions. I recorded courtship flights as this behaviour may have been associated with significant risk and energetic cost and so might have been related to the birds' energetic state. I assumed, the other two behaviour types would be more dependent upon extrinsic factors such as the presence of potential mates or competitors. When I observed the presence of any of these behaviours I also noted the time and the temperature.

3.2.7 Calculation of energy consumption

I calculated the level of energy consumed per flower by using the equation from Hurly & Oseen (1999). This allowed me to calculate the amount of energy that the birds obtained from each flower type. I could then used these values to calculate the amount of energy obtained by birds in each foraging bout by multiplying the number of each flower type sampled with its energetic contents. I then standardised this amount to amount of energy consumed per experimental session or per hour depending on the analysis type.

3.2.8 Statistical analysis

I pooled the foraging, behavioural, and energy data by the time of day, day within observation cycle, and observation cycle. I calculated the proportion of the constant flowers consumed by calculating the number of constant flowers consumed in a data collection period. I then arcsine square root transformed this data and performed a linear mixed model (LMM) analysis on the data. With LMMs, the observational units are assumed to be collected into clusters over which random effects (intercepts in all models presented here) vary. Fixed effects are those explanatory variables that are associated with the entire population or with certain repeatable experimental treatments. Therefore, I treated time of day, day within cycle, and cycle as factors. Random effects are associated with individual experimental units drawn at random from a population (Pinheiro & Bates 2000) and govern the variance-covariance structure of the response variable. Therefore, temperature and subject were included as random variables, which meant the model would control for differences in temperatures and individuals in the analysis. The significance of variance explained by between subject effects was calculated using Walds *Z*-test.

For the behaviour and energy measures, I calculated the frequency that the different behaviours were expressed as a function of time in order to standardise time between data collection periods. I analysed the resulting values against the pooled data set I had used in the main analysis of the LMM with the behaviours and subjects as random variables. I pooled the data for these analyses as none of the fixed factors were significant in explaining the variance.

I chose to use LMMs because they enable the use of unbalanced data sets and the incorporation of data collected from multiple individuals, but utilising a within subject approach. I used the least significant difference technique to test for pair wise differences between means after being corrected for multiple comparisons using the Bonferroni method (α/n).

Akaike's information criterion (AIC) was used to choose the best model when models had different sets of parameters. AIC was calculated for each model as:

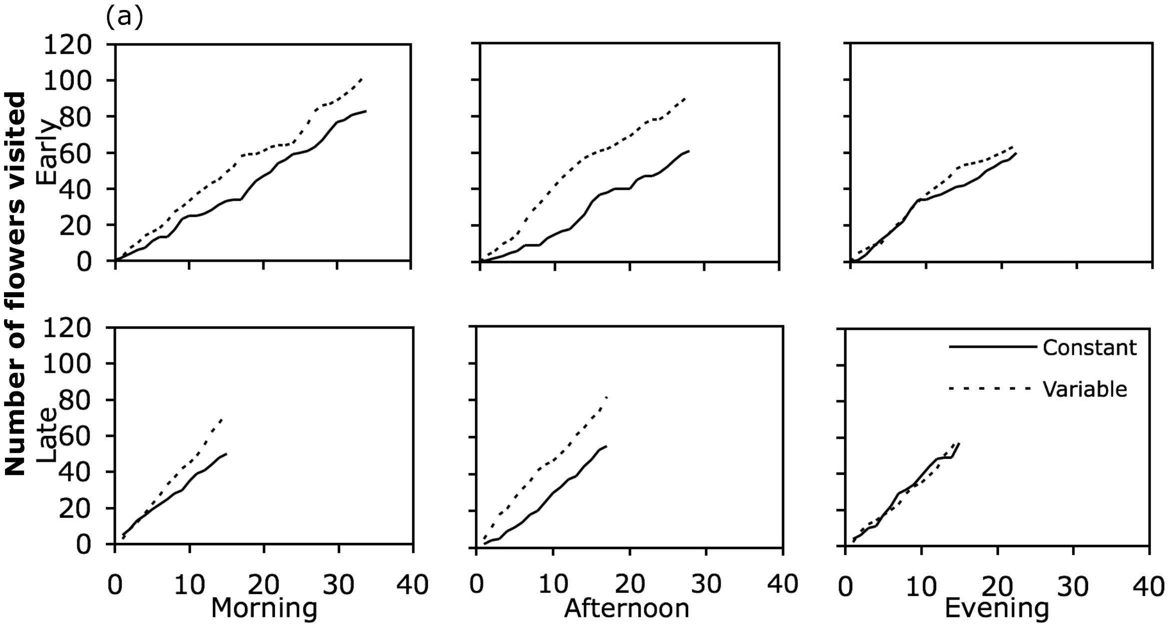
$$-2(\text{log-likelihood}) + 2 \times p$$

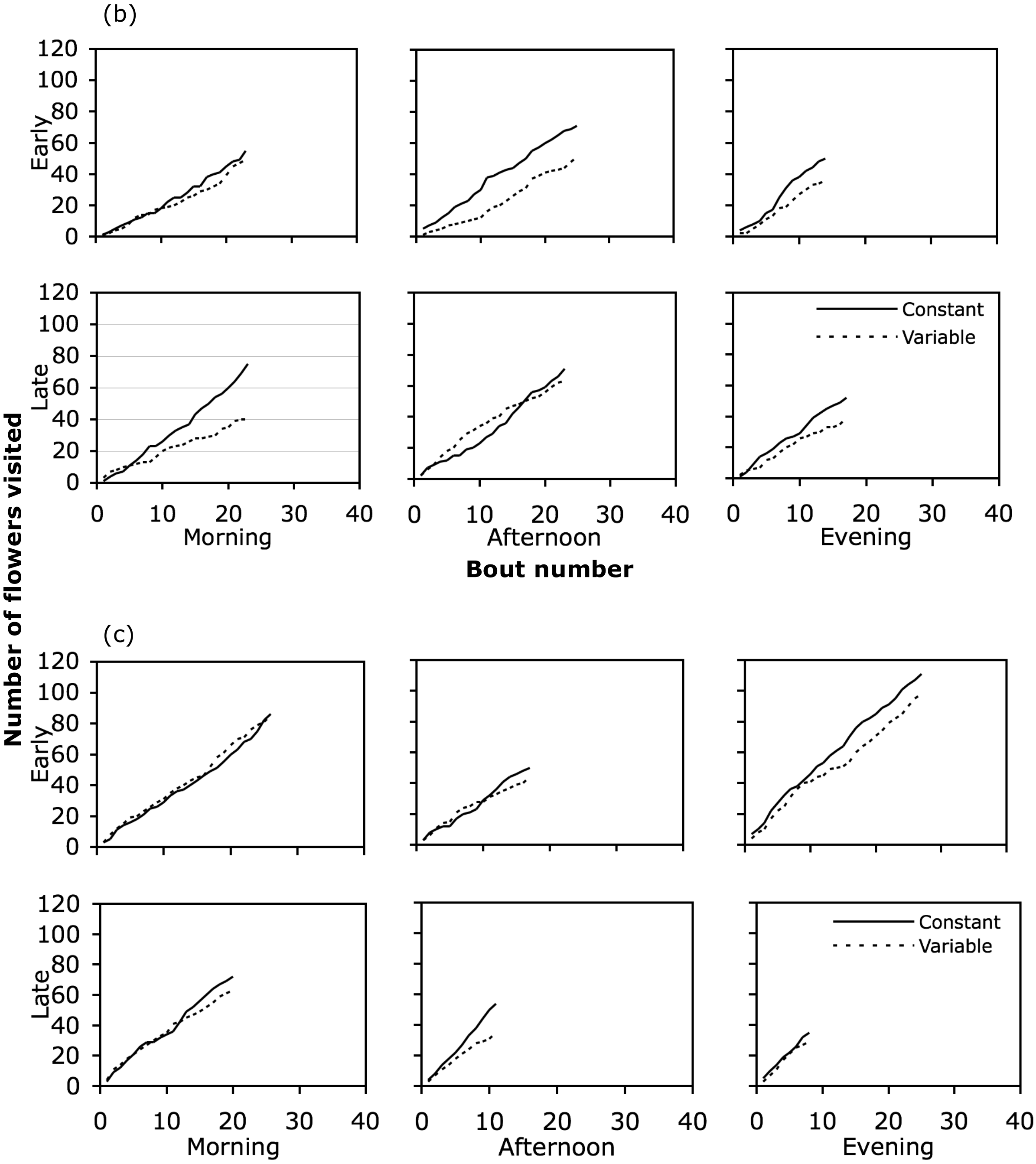
where p is the number of parameters estimated in the model. AIC therefore represents a measure of the explanatory power of the model discounted by the number of parameters that have gone into its construction; a lower value indicates the 'better' model. Finally, I calculated the variance components for the factors and their interactions using restricted maximum likelihood, REML (for an intuitive overview of REML, see Lynch and Walsh 1998). All calculations were made using SPSS (versions 12.1, 14.0, and 15.0) for PCs and (version 11.0) for Mackintosh computers.

3.3 Results

3.3.1 Raw data

I plotted the cumulative totals of the variable and constant flowers sampled by each individual bird during the morning, afternoon, and evening trials in relation to foraging bout number. I also divided the data by observation cycle so data could be compared between the early and the late seasonal data. The data indicates that although there may be some trends at a specific time of day or season, that these trends are not consistent within individuals or between individuals (Fig. 3.4). However, given I was looking for changes in risk-sensitive foraging behaviour based on environmentally induced changes to the subjects' energy budgets, this is not a problem if these inconsistencies are related to changes in temperature and energy budget. The birds visited the boards a median of 126 times (range: 85-156) and sampled a median of 689 flowers (range: 656-842).




(b)
Early
Late
120
100
80
60
40
20
0
0
10
20
30
40
Morning
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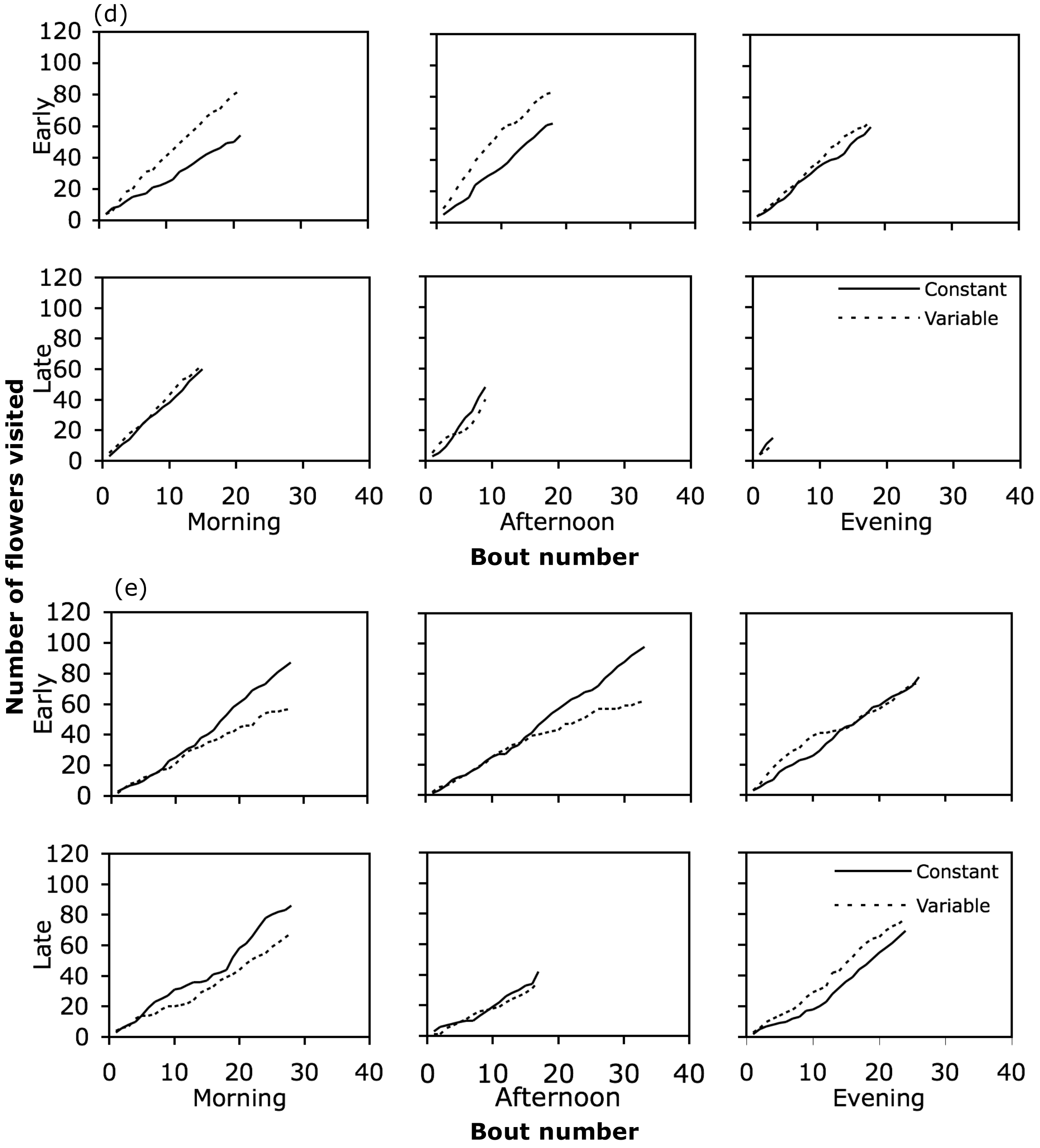

Figure 3.4. The cumulative frequencies of the flower choices per foraging bout in relation to the time of day and time of season. The data are presented for each individual bird (a-e).

3.3.2 Risk sensitivity of the group

The birds did not display any consistent risk-sensitive behaviour as there were no differences for the proportion of constant choices for cycle (LMM: cycle ($F_{1,47}$=0.027, P=0.87), day ($F_{3,47}$=2.102, P=0.113), time of day ($F_{2,47}$=0.210, P = 0.811) or any interaction (cycle*day: $F_{3,47}$=0.332, P=0.802; cycle*time of day: $F_{2,47}$=0.147, P=0.864; day*time of day: $F_{6,47}$=0.739, P=0.621; and cycle*day*time of day: $F_{4,47}$=0.132, P=0.970; Fig. 3.5). While there was no relationship between the number of constant flowers consumed and diurnal or seasonal effects, there might have been significant effects between individuals. However, there was no evidence of between subject differences in the analysis. I tested for differences in behaviour between individuals and found that variance between individuals accounted for only 17.6 % of the total variance of about 44.5 which was not statistically significant (Wald *Z* test: *Z*=1.045, *P*=0.296). The other 82.4 % of variation was accounted for by within individual variation and error.

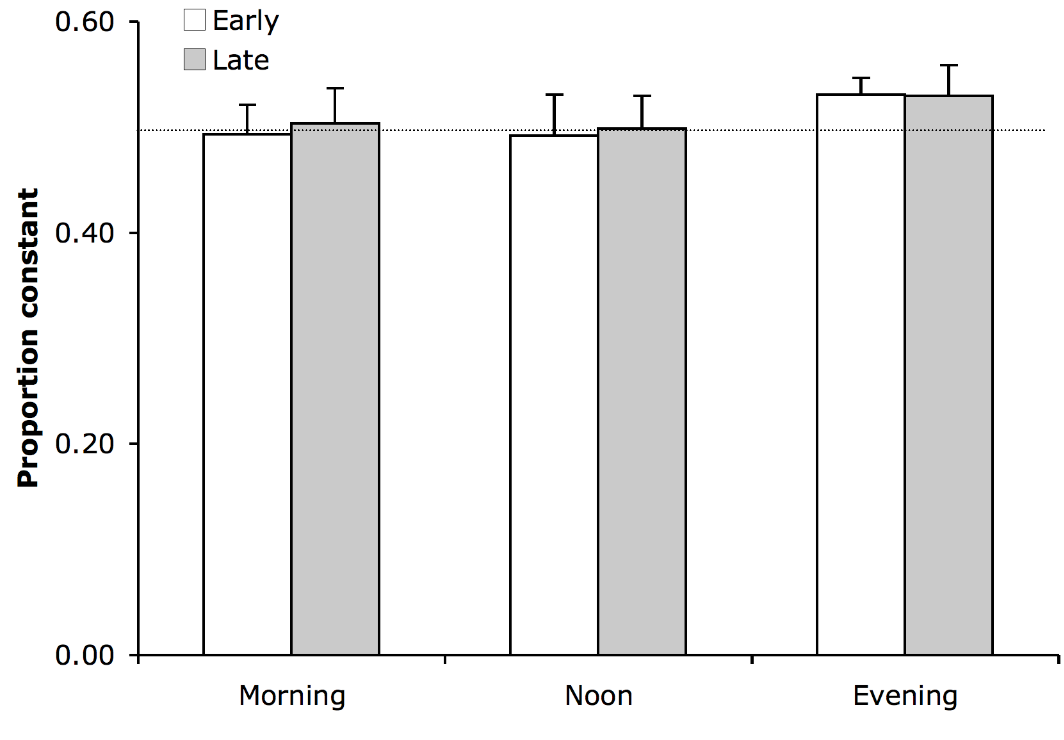

Figure 3.5. The mean proportions (+SE) of flowers chosen that were constant in relation to time of day and experimental cycle. The dotted line indicates 0.5, which is what would be expected if birds were choosing flowers at random.

There were 10 of each flower type offered to birds in each foraging bout and on some occasions, the birds did choose more than 10 flowers. Therefore, it is possible that as a foraging bout continued, that the birds choices may have been force towards the less favoured option. One way of examining this effect, is to examine a sample of choices that the birds made which might have been less affected by this effect. One way of doing this is to compare the first-choice preference for a flower type with the overall preference for the same flower. When I did this for the constant flowers, it was clear that there was little difference between the overall rate of attack and the preference for the first flower ($\bar{X}$ ± SE: first choice=0.510 ± 0.043, overall preference=0.512 ± 0.027; paired *t*-test: *t*=-0.0612, *P*=0.9534, df=4). Moreover, visual inspection of the birds' preferences for constant flowers in the first six choices indicated that the each individual, had a range of about 0.1 (Fig. 3.6). There was also little change in slope which might be expected if birds were being forced to choose flowers of the other type. These data indicates that birds' preferences were relatively constant within bouts and so this did not become a problem until later in within each foraging bout.

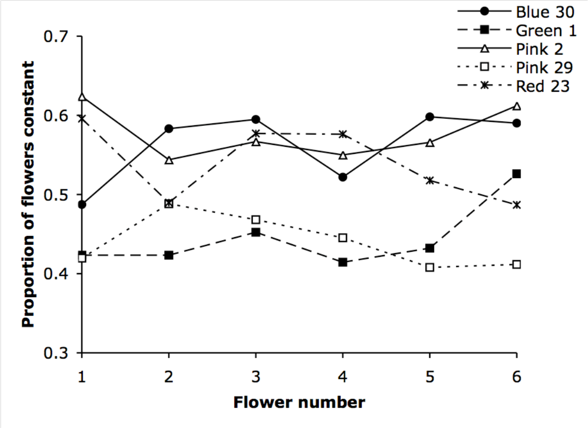

Figure 3.6. The individual preferences of birds for the first six flowers sampled in a foraging bout.

Finally, I conducted a correlation of the proportions of constant flowers out of the total number of flowers sampled against the proportion of variable flowers that were high. The relative number of high volume variable flowers compared to low volume flowers that birds' consumed was high, this could have had significant effects on birds' overall preferences. This is because if a bird consumed a relatively high number of high volume flowers compared to low volume variable flower, this could positively distort the bird's subjective evaluation of the payoff obtained from variable flowers. Therefore, a bird consuming relatively more high volume variable flowers than the low volume flowers might lead to birds favouring the variable option. Therefore, I examined this possibility by comparing the proportion of the total number of flowers consumed that were constant volume with the total number of variable flowers that were high volume. If this relationship were a factor in birds' preferences then it would have been expected that the higher the proportion of variable flowers sampled that were high volume, then the higher the preference for variable would have been. The median proportion of variable volume flowers sampled that were high volume was 0.53 (range: 0.47-0.57). When I compared the mean proportion of the flowers chosen that were constant for each individual against the proportion of variable volume flowers that they sampled that were high volume, I found that there was no relationship between these two measures (Spearman's rho=-0.308, N=5, *P*=0.614). Therefore, there is little evidence to suggest that birds could differentiate between high and low volume variable flowers. Moreover, there was no relationship between the proportion of total flowers sampled that were constant and the proportion of variable flowers that were high volume.

3.3.3 Effect of temperature

Throughout the experiment, there was a large range of temperatures recorded (minimum=-0.9 °C, maximum 30.4 °C). The results from the LMM above indicated that there was no effect of day, day within experimental period, or cycle on the birds' preferences. This meant that I could analyse the data set without including these factors in the analysis. Therefore, I constructed a LMM to test the effects of temperature on the arcsine square root transformed proportions of foraging choices that were constant. I also calculated the mean temperature during the observation session using all the temperatures collected at each foraging bout as well as the temperature at the beginning and the end of the observation session. I included subject and temperature as random variables. I found that the temperature had no effect on the proportion of constant flowers chosen (LMM: $F_{4,64}$=0.518, P=0.723). I constructed a similar model with energy consumed per hour as the dependent variables and with temperature and subject as or the amount of energy consumed per hour ($F_{4,67}$=1.603, P=0.184) by the birds. I also found that there were no differences between individuals for both the proportion of constant flowers chosen (Wald Z test: Z=0.163, P=0.871) or for the hourly rate of energy consumption (Z=0.401, P=0.688) that could be explained by temperature.

3.3.4 The relationship between behaviour and foraging choices

Theoretically, an animal's behaviour should be linked with its energetic state because an animal needs energy in order to perform behaviour. Hence, it might be possible to detect changes in the proportion of constant flowers chosen and the amount of energy birds used per hour, based on the bird's activity. I constructed LMMs of the average hourly rates for each behaviour collected during of the different observation sessions. I included the rate of behaviour as a random variable and subject as random subject variables. Table 3.2 summarises the results of these analyses and shows that there were no significant relationship between the behaviour of the birds and the proportion of constant flowers consumed or the amount of energy consumed.

Table 3.2. The test statistic results of the bird's behaviour on the proportion of constant flowers chosen and the amount of energy consumed per hour both within subjects (*F*) and between subjects (*Z*). All *P*≥0.3926.

	Proportion of flowers constant			Energy consumed/hr		
Behaviour	df	*F*	*Z*	df	*F*	*Z*
Feeding bouts/hr	4, 64	1.042	0.085	4, 67	0.264	-[a]
J-flights	4, 64	0.539	1.025	4, 67	0.216	0.013
Aggressive flights	4, 64	1.021	1.160	4, 67	0.156	-[a]
Shuttle flights	4, 64	0.643	1.204	4, 67	0.19	-[a]

a. The calculation of test statistic was not possible due to lack of variation.

3.4 Discussion

The results of this experiment found no evidence of switches in risk-sensitive foraging behaviour in relation to changes in behaviour or temperature. Moreover, none of the behavioural or environmental factors that I recorded, significantly explained the lack of variation in the data. The failure to find any risk-sensitive foraging behaviour of any kind was surprising given that hummingbirds are a seemingly ideal species in which to study this behaviour. This is because rufous hummingbirds are small homeothermic animals living in an energetically stressful environment (Stephens 1981; Stephens & Charnov 1982) and several studies have also found risk-aversion in this species given similar choices (Stephens & Paton 1986; Waser & McRobert 1998; Horley & Oseen 1999; Biernaskie *et al* 2002; Hurly 2003).

There are a number of reasons why the hummingbirds might not have displayed a consistent risk-sensitive foraging patterns within and between individuals. First, it is possible that the birds had not learned the difference between flowers at the start of the experiment. There are differences in the way that birds were trained compared with other studies which might have contributed to them not learning the relative values of the flowers. For example, some authors did not give hummingbirds forced trials, instead starting the experimental trials, once birds were feeding from the Plexiglass boards (Hurly & Oseen 1999; Hurly 2003). Interestingly, the only other study that has used forced trials also found that the birds were risk indifferent for variance in nectar volume (Bateson *et al.*, unpublished manuscript). However, the method of forced trials of single flower types ought to have increased the speed of learning if theories of associative learning are correct (e.g., Rescorla & Wagner 1972). Moreover, the number of flowers that birds sampled in this study were over double that were sampled in previous studies of hummingbird risk-sensitive foraging behaviour (e.g., Hurly & Oseen 1999; Hurly 2003). Another experimental difference was that I collected my data in approximately three-hour blocks separated by three-hour breaks. This might have affected the learning of birds as interruptions might allow the bird to forget the colour-reward associations.

Another explanation for the lack of preference might be due to the lack of costs for switching between flowers making it unprofitable for birds to discriminate between flowers. This is because with both flower types on the same board, if the bird got a poor flower, then it may have exploited the flower next to it and so on until it had met its energetic needs. Other authors have realised this problem and set different flower types apart with varying levels of success (e.g., Waser & McRobert 1998; Biernaskie *et al.* 2002; Bateson *et al.,* unpublished manuscript). However, having both flower types on the same Plexiglass board has also been used to successfully generate risk averse foraging preferences (e.g., Hurly & Oseen 1999; Hurly 2003). The data from this study supports the possibility that birds were not discriminating between the constant and the variable flowers. This risk-indifference was consistent irrespective of the behaviour of the bird or the climatic conditions at the study site. The birds' foraging behaviour indicated that they may have been choosing indiscriminately as there was no cost to feeding from as many flowers it took in order to fulfil their energetic needs. The average number of flowers that birds fed from in this study was 6.31 ± 0.46 flowers per visit as opposed to 5.1 ± 0.2 flowers (Hurly & Oseen 1999) and 4.8 ± 0.2 flowers (Hurly 2003) per visit in the two studies that utilised similar methods.

Another aspect of this study that supports the possibility that the birds may have indifferent rather than untrained was that there was no relationship between temperature and the amount of energy consumed per unit of time. This tends to indicate that despite the extreme differences in temperature experienced by birds, that their intake in energy from the experiment did not change. It is possible that this was because the birds were supplementing the diets from natural sources. It has also been noted in a study conducted on Norway rats (*Rattus norvegiscus*) that subjects were indifferent when there were few costs to switching between foraging options. In this study, the authors observed Norway rats under two conditions: (1) where they could choose only one foraging option and (2) where they could sample both options. It was found that the rats displayed risk-aversion when could only choose a single option in a trial, but were indifferent when they could sample both available options (Roche *et al.* 1997).

In this experiment, birds visited the Plexiglass plate and sampled flowers without replacement meaning that as the number of flowers sampled increased, the bird's 'preference' was funnelled towards 0.5 (Fig. 3.7). Therefore, feeding from a large number of flowers may make indifference more likely. One way around this funnelling effect of the bird's preferences maybe to examine their first choices in order to see if they deviate significantly from their overall preference. When I conducted a simple analysis of the first choices, there was no evidence to suggest that the birds' preferences for the first choices were different from their overall preferences for constant flowers. Moreover, there was little evidence to suggest that there were any large deviations in the preferences of individuals for at least the first six choices. This indicates that preferences within individuals did not differ significantly from their overall preferences throughout a foraging bout. This method has been used by other authors who have identified this problem (e.g., Bateson *et al*. 2003), but is problematic because it may misrepresent the overall preferences of birds.

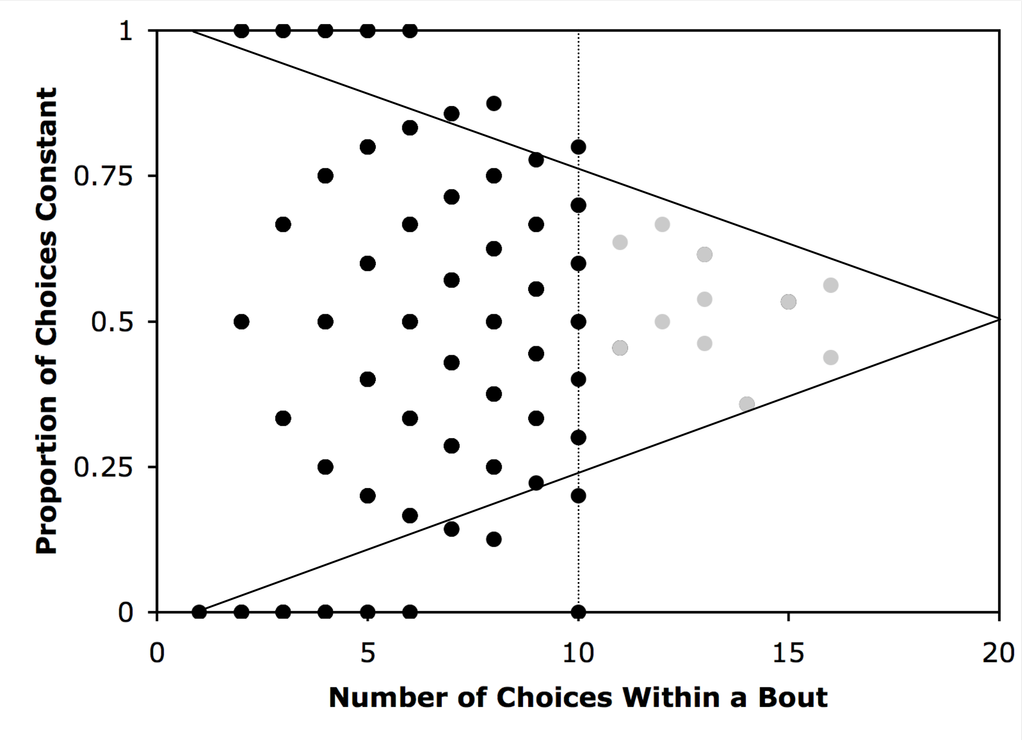

Figure 3.7. The funnelling effect of continued sampling within bouts as the birds continued to forage. As the number of flowers birds sampled within a bout increased, the probability that the birds' choices for each flower begin to approach 0.5 increases. This is because the birds were sampling flowers without replacement. Hence, it was possible for birds to exhaust all of a particular flower type if they sampled 10 or more flowers meaning all subsequent flowers sampled had to be sampled were the non-sampled flower type (marked with a dotted line). The points to the right of the dotted line (coloured grey) indicate the point where we see a dramatic decline in the ranges of proportions of variable and constant flowers sampled.

There are a number of different ways in which hummingbirds may have been able to mediate their use of bodily energy stores: (1) torpor, (2) micro-habitat selection, and (3) behaviour modification. Torpor is a form of regulated hypothermia and is an adaptive response in animals that face short day lengths, variable food supplies, low temperatures, and poor or scarce food supplies (Pravosudov & Lucas 2000; Welton *et al.* 2002). Nocturnal hypothermia may involve depressions in body temperature of up to 10 °C resulting in an energy saving of as much as 33 percent in parids (Budd 1972; Haftorn 1972; Chaplin 1974, 1976; Reinertsen & Haftorn 1983). In hummingbirds, the drop may be even more extreme with temperatures falling by as much as 20 °C and their metabolic rates falling to 5% of their normal value (Hainsworth & Wolf 1970; Wolf & Hainsworth 1972; Carpenter 1974; Withers 1977; Krüger *et al.* 1982; Hiebert 1990). The amount of energy lost by a homeothermic animal is proportional to the difference between the body temperature and the ambient temperature. As the ambient temperature falls, the difference becomes greater meaning that more energy is lost to the environment. One way to minimise the energetic losses to the environment is to lower body temperature, which reduces the amount of energy lost through radiation of heat. There are costs associated with going into torpor and these costs may prevent the more widespread adoption of torpor as an energy saving mechanism. Those species do that utilise torpor only tend to during times when energetic crisis seems inevitable (Hiebert 1992; Pravosudov & Lucas 2000; Welton *et al.* 2002). In this experiment, it was unknown which nights birds went hypothermic. However, hummingbirds are known to regularly utilise torpor as an energy saving mechanism and so torpor may have acted to reduce the impact of low temperatures on the birds' energy budgets in this experiment.

I measured temperature when the birds visited the feeders and it is highly probable that the temperature that was incorporated into the model was different from the temperatures experienced by the males. Birds are known to exploit favourable microclimates that may be subject to higher ambient temperatures than surrounding areas and to reduce wind chill in exposed areas. For example, mountain chickadees (*Poecile gambeli*) are known to select microclimates that ameliorate the effects of wind (e.g., Grubb 1975, 1977, 1978; Kessel 1976) or by selecting resting areas that have high temperatures or are exposed to direct sunlight (Wachob 1996). Rufous hummingbirds at the study site were seen often to perch at prominent points in their territories that had very high exposures to sunlight. These points may have had higher temperatures than the temperatures that I recorded in the shade, about 1.5 m off the ground. Hence, although the temperatures I included in the analysis were likely to be indicative of the general environmental temperature, they may have borne little relation to those experienced by the hummingbirds. This could be another of the factors that contributed to the birds' indifference for flowers of either kind.

Finally, birds may have been able to reduce their levels of energy expenditure by reducing their investment in energetically expensive behaviours. It has been found in other hummingbird species that birds will reduce energy expenditure in environments where they have become stressed (Fernández *et al.* 2002). I measured behaviours of birds in an attempt to correlate this with energy consumption and foraging preferences and found that there was no relationship. In hindsight, this is not surprising for two reasons. First, I was not able to watch birds continuously for long time-periods. This meant that the number of times each behaviour was observed may have been grossly under reported in my results. Second, the behaviours I recorded while costly, may be less common than other behavioural patterns and so might have been swamped with regards to the effects they may have had on the birds' energy budgets. For example, hummingbirds spend a lot of their time flying and hovering while feeding (Wolf & Hainsworth 1971; Pyke 1981) and so these behaviours may use a lot more energy than the occasional J-flight or aggressive interaction. Therefore, it is not surprising that the behaviours I recorded were not associated with changes in foraging behaviour because my method was probably too crude.

There has been much effort in trying to demonstrate the energy budget rule and although there is some evidence to suggest that, it is possibly a factor in risk-sensitive foraging behaviour. One possible explanation for this is that animals in natural situations may rarely experience energy budgets which are so dangerously negative that the only way to survive would be to exploit a variable option and hope for a run of extremely good luck. Birds in a natural situation are likely to have food available and there is a general relationship between foraging effort and level of energy intake (Stephen & Krebs 1986). Moreover, the longer a run of luck is required to last, the less likely a risk-prone strategy will be in increasing the level of intake above the constant foraging option. This is because the payoff from a risk-prone strategy and a risk averse strategy will approximate one another more closely with increasing time.

Although I have not given much space to discussing ecological, behavioural, or life-history approaches to examining foraging behaviour, the study of foraging is now more integrative of these factors than older models of foraging behaviour (e.g., risk-sensitive foraging models). We now are able to integrate a much greater number of constraints and factors into foraging models to yield more realistic predictions (e.g., Mangel & Clark 1986). However, it is only comparatively recently that researchers have attempted to include other aspects of behaviour, ecology, or life-history which might be important into risk-sensitive foraging theory (e.g., McNamara *et al.* 1991; Merad & McNamara 1994; Hurly 2003). One aspect that has consistently been ignored in risk-sensitivity is the risk of predation. While the prospect of aerial predation may seem remote in the laboratory environment, animals may be adapted to handle variability in food sources under the threat of predation. It is has become clear, in birds at least, that predation pressure can play a significant role in the evolution of mass regulation strategies (Lima 1986; Houston & McNamara 1993; Bednekoff & Houston 1994; McNamara *et al.* 1994). Therefore, predation could significantly affect a bird's risk-sensitive foraging strategy.

Given all of the possible confounding variables that could influence the results of tests of the EBR it is surprising that as much evidence for the EBR has been accumulated. The majority of the studies listed in Table 3.1 have not controlled for many, if any, ecological, behavioural, or life-history factors that could potentially affect the results of experiments. Moreover, many of the studies have studied the EBR devoid of any direct approximation of the subjects energetic state. This naivety in the approach to the study of the EBR in general, has hindered our understanding of how animals deal with variation in foraging options behaviourally. Many of the experiments listed in Table 3.1 may have found more support for EBR if they had been conducted with a better understanding of how animals manage their energy reserves and the relationship between energy consumption and behavioural expenditure.

3.4.1 Conclusions

Clear doubts remain over the explanatory power of the EBR in explaining risk-sensitive foraging behaviour. However, the ideas of risk-sensitive foraging are being applied to different biological problems (e.g., Ekman & Hake 1988; Wu & Giraldeau 2005). This observational study was based on the assumption that climatic differences could cause changes in birds' energy budgets and these changes could then affect the birds preferences for the one or other of the flower types offered. The observed indifference of birds towards the two flower types illustrates some of the general problems associated with tests of the EBR. There were a number of possible reasons for the failure of birds to show any risk-sensitive foraging behaviour. The EBR is an intuitively elegant and attractive idea, but in order to make progress in testing this hypothesis we need to design experiments that incorporate measures of subjects energetic state along with behavioural assays of their foraging preferences that present problems of stochasticity to animals in biologically meaningful ways. Initially, this will probably mean a return of these kinds of studies to the laboratory. However, the increasing statistical and technical sophistication of ecological research means that tests of the EBR may be feasible in the field within the next ten years.

Chapter 4

LITERATURE REVIEW OF APOSEMATISM AND MIMICRY

Aposematism and mimicry comprise a body of work is now considered amongst the classic examples of adaptation, mutualism, and evolutionary deceit. This makes aposematism and mimicry popular examples of the evolutionary process at work, although many aspects of aposematism and mimicry theory remain relatively poorly tested. Moreover, the field has relied upon ideas that have remained relatively unchanged for over a century. It is only recently, that theoreticians have revisited these original models and finding that aposematism and mimicry may operate in vastly different ways than had been previously thought. It is the aim of this review to provide a brief overview of the theoretical and empirical research of aposematism and mimicry. Following this, I will consider the role that predators play in the predator-prey interactions in systems with defended prey. I conclude that educated predators could have a far greater role in the evolution of aposematic and mimicry than has previously been considered.

4.1 Aposematism

Aposematism is an evolutionary paradox because it provides an interesting example of an adaptive trait along with a theoretical problem of how it evolved. Therefore, aposematism has traditionally been, and continues to be, of great interest to evolutionary biologists. Aposematic species are unprofitable (e.g., chemically defended, protected by morphological traits, or difficult to capture) and advertise their unprofitability to predators through conspicuous warning signals (e.g., bright colouration, smells, sounds, or conspicuous behaviour [Poulton 1890]). Many predators show innate aversions to aposematic species whereas other species need to learn the prey’s noxious properties (Schuler & Roper 1992; Mappes *et al*. 2005).

4.1.1 Early history of the study of aposematism

Wallace (1867) was the first modern biologist to suggest that it might be beneficial for animals with chemical defences to advertise their unprofitability to predators. Charles Darwin had drawn his attention to the problem of bright insect larvae in a letter (Darwin 1872). Darwin knew that larvae were not sexually mature and that there was no way of externally differentiating sexes apart. Therefore, sexual selection could not account for the bright colouration of some caterpillars. Wallace (1867) realised that it would be beneficial for animals with secondary defences to advertise this fact. Conspicuous colouration is one method of advertising prey unprofitability. He also predicted that brightly coloured larvae should always be rejected as food when encountered by predators, and that cryptic larvae should be devoured whenever encountered.

Poulton (1890) first used the term aposematic, which he took from the Greek, “apo” meaning away and “sematic” meaning sign. He also reviewed the evidence for aposematism and found numerous examples of predators having aversions towards brightly coloured, chemically defended species of insect larvae and imagos which supported Wallace’s prediction (Poulton 1887, 1890). For example, he cited the magpie moth (*Abraxas grossulariata*) larvae as being a conspicuous example of Geometridae, which are generally cryptic. He noted that, “all observers agree that [predators] either refuse this species altogether, or exhibit signs of the most intense disgust after tasting it” (Poulton 1890, p. 169). He then outlined a number of other traits that were strongly linked with aposematism such as sluggishness (p. 175), conspicuous behaviour (such as absence of hiding or escape behaviours) (p. 169-170), and gregariousness (p. 170). However, Poulton also noted the inconsistency in behaviour of predators when faced with defended prey (1890, pp. 180-181).

Beddard (1895) also noted inconsistencies finding that on one day a predator would not consume a defended prey only to consume it the next. However, early studies were generally poorly controlled observations. Hence, any number of factors that could have accounted for these observations. For example, these observations were made on unmarked predators, meaning that the authors may have been basing their observations on multiple individuals ([Weir 1869, Butler 1869, both in Darwin 1872, pp. 498-501]; Poulton 1887, 1890; Judd 1899; Marshall 1902; Pocock 1911; Carpenter 1921; Jones 1932, 1934; Carrick 1936). The lack of consistent predator behaviour led to scepticism by biologists and led them to question whether chemical defences were effective at deterring predators. This led to two divergent schools of thought as to how effective aposematic signals were at deterring predation.

The adherence of early biologists to the idea of complete avoidance was not compatible with many of the early observations of predator behaviour. In these studies, it was observed that aposematic signals were sometimes effective in reducing predation, although not always. For example, Marshall (1909) observed many cases of kestrels (*Falco tinnunculus*) and garden warblers (*Sylvia borin*) consuming chemically defended butterflies that they normally would not consume. Swynnerton (1915) noted that birds were more likely to consume chemically defended prey (such as butterflies of the Acraeinae and Danainae sub-families) earlier in the day when compared with their diets later in the day. Other biologists questioned how chemical defences could evolve at all. For example, McAtee (1932) proposed that predators were unselective and consumed prey in accordance with their abundance in the environment (the principle of proportionate predation). Cott (1940) published a voluminous tome in which he reviewed the evidence of the effectiveness of warning signals. He found that although there was ample evidence to suggest that predators sometimes consumed defended prey, this was likely to be due to increased hunger. These exchanges between McAtee and Cott made it clear that for aposematic signals to have a selective advantage, they only needed to reduce the rate of predation of the prey species rather than extinguish it completely. Now that this problem was sufficiently explained, biologists began thinking about the advantages of being aposematic.

Ronald Fisher (1958, first published in 1930) published one of the great books in evolutionary biology, *The Genetical Theory of Natural Selection*. In it, he considered the evolution of chemical defences and made two important points. First, he reiterated that chemical defences evolve in order to increase the probability of the defended species surviving. Second, he realised that attacked individuals would stand a high chance of dying. This was a problem because if selection operates at the level of the individual, then how can aposematism be of benefit to an individual that has been attacked and killed. Fisher knew prey animals such as caterpillars often aggregated. For example, butterflies often lay their eggs in clumps meaning that aggregated prey might be related. Therefore, individuals that are consumed by predators may still have indirectly benefited from their own death by kin selection. They may be educating predators as to the noxious qualities of the prey species including their siblings. Hence, if the defence is noxious enough, the predator will then reject those visually similar (and related) prey that are concentrated in the area, thus increasing the inclusive fitness of the depredated siblings (Hamilton 1964).

With these problems supposedly solved, biologists began to focus more on other aspects of insect colouration such as industrial melanism and mimicry. However, there were some important studies in aposematism such as those of Brower (1958a) who showed that Florida scrub jays (*Cyannocitta coerulescens coerulescens*) quickly learned to avoid attacking chemically defended butterflies. By the start of the 1970s, predator avoidance learning of aposematically coloured prey had been demonstrated many times in birds, anurans, lizards, and cephalopods (squids, cuttlefishes, and octopi) (Edmunds 1974, pp. 63-65). Since this time, the breadth of the diversity of aposematic signals unearthed is huge and although insects are the most well studied aposemes, there are numerous examples from other taxonomic groups (Edmunds 1974; Ruxton *et al.* 2004).

Most of the research that has been outlined above, has concentrated on visual aposematic signals. This might have reflected a human bias in the taxa we choose to study or might be an actual trend among animals (Pough 1988). Other sensory modalities have been investigated such as sound (Dunning *et al.* 1991), odour (Eisner and Grant 1981), and behaviour (Srygley & Chai 1990). It has also been discovered that predators may have evolved multi-sensory aposematic signals that may act to increase the effectiveness of the signals (reviewed by Guilford & Rowe 2001). These multi-modal signals may be more effective because they are harder for mimics to fake. To fake one trait might be quite simple, as the many examples of mimicry suggest, but to fake two or more traits may be an evolutionary bridge too far. Alternatively, one aposematic signal may increase the attention that potential predators pay to other signals thus increasing the effectiveness of multi-modal signals (e.g., Lindström *et al.* 2001b; Rowe 2002). However, as Poulton (1890) pointed out aposematism can be a suite of signals that are behavioural as well as morphological. Therefore, bright colouration along with slow flight can also be considered a multi-component signal.

Aposematism is generally considered a trait associated with animals (e.g., Ruxton *et al.* 2004); however, it has recently been shown that trees might also signal their unpalatability to herbivorous insects through the brightness of their autumnal displays (Hamilton & Brown 2001). This fits with the definition of an aposematic signal, although it has not been discussed as such yet. For this to happen, research into whether plants with bright foliage colouration also produce high amounts of plant secondary metabolites (PSMs) and whether signals deter their herbivorous “predators” (Schaefer

& Rolhausen 2006). If this idea were to bear fruit, then it would reinvigorate the field of aposematism because it would force us to examine our preconceptions of what is aposematism.

4.1.2 The evolution of aposematism

4.1.2.1 Problems in the evolution of aposematism

Fisher's (1930) kin based argument for the benefit of aposematic signals to individuals was an elegant hypothesis which was widely accepted by biologists. The adaptive significance of aposematism in reducing rates of predation in prey is obvious when it is fixed in the population, but the road to fixation may be far from smooth. This problem made biologists think about the initial evolution of aposematism and which trait evolved first; chemical defences or warning signals (Guilford 1988). If chemical defences evolved before warning signals, there is a problem in getting the signal to fixation in a population from a single mutant. One indication that this might be a problem is that there are many chemically defended cryptic species (Edmunds 1974). This is because if a single aposematic mutant were to appear in a population, it would most likely be eaten before its cryptic conspecifics. Therefore, it would perish before having the opportunity of contributing genes to the next generation (the problem of the lone mutant [Speed 2000, 2001]). However, this could also argue for the evolution of chemical defences before warning colouration.

Brodie and Agrawal (2001) have suggested that it is possible that a mutation for aposematism could occur in the germ line of females, thus conferring the aposematic mutation to all of her offspring. A weaker form of the lone mutant argument applies to a group of aposematic individuals. A group of individuals that is small, would pay a high education cost because it is likely a large proportion of individuals would need to be consumed in order to educate the predator. Hence, the smaller the mutant population, the higher the cost of carrying the conspicuous mutation because the probability of educating the predator population before extinction of the colour mutation decreases with declining aposeme population size (Speed *et al.* 2000; Lindström *et al.* 2001a; Speed 2003). Speed (2003) called this the "problem of the advantaged minority." Both the problem of the lone mutant and the problem of the advantaged minority are in effect the same problem. This is because as a population of mutants increases, there is a critical level of penetration at which the benefits of carrying the mutation outweigh its costs.

One suggestion that favours the maintenance of rare aposematic morphs for long enough for them to become established in a population is what Mappes *et al.* (2005) called wariness. They grouped the processes of neophobia and dietary conservatism into this more general term. Neophobia–as its name implies–is an aversion to novel food items whereas, dietary conservatism is the reluctance by members of a population to include new food items in their diet. These factors are thought to be important in promoting the fixation of aposematism in populations because predator wariness may allow aposematic mutants enough time to build up in numbers to avoid extinction in the course of predator education (e.g., Marples *et al.* 2005).

Poulton (1890) realised that animals living in heterogeneous environments could be cryptic in one part of the environment, but aposematic in other parts of their environment. It is possible that phytophagous insects could have evolved aposematic signals in order to increase their crypsis. For example, Papageorgis (1975) suggested that *Heliconius* butterflies might be partially camouflaged in flight through a flicker effect (although this has been disputed by Benson 1982). An alternate view is that differently coloured plant parts could evolve in order to expose green "cryptic" herbivorous insects to insectivorous predators (Lev-Yadun *et al.* 2004). This means that bright leaf colouration could favour the evolution of brightly coloured herbivorous insects as an attempt to remain cryptic. Endler (1984) found that the more heterogeneous the background was, the more moths would match a random sample of background. This also can be interpreted to mean that the more heterogeneous an environment, the larger number of colour morphs it can support. Therefore, aposematic signals could have evolved because of an interaction with the environment in which the animal finds itself and the selection pressure exerted by predators (Speed & Ruxton 2005). If bright colouration evolved before the acquisition of chemical defences for some other purpose, such as intra-specific communication (e.g., sexual selection, Jiggins *et al.* 2001), then the initial evolution of warning signals is less of a problem (Mallet & Singer 1987). The evolution of chemical defences will always be beneficial to animals possessing them if they are brightly coloured for some other purpose. Whatever the reason for how aposematic traits get their foothold in a population, once they are established, they will tend to become fixed in a population if they are advantageous.

A final point to note is that there would also seem to be a problem in macro-mutations for massive changes in wing colouration. Many authors believe such saltational jumps are improbable based on a probabilistic argument (e.g., Dawkins 1976). However, it has been found that the genes for wing patterns in Lepidoptera may be highly linked and thus, behave in a manner similar to a single gene (Clarke *et al.* 1968; Clarke & Sheppard 1971; Turner 1984). These 'supergenes' may produce large changes in phenotypes allowing bridging of the valleys of adaptive landscapes to new adaptive peaks that would otherwise be non-adaptive to cross. Therefore, it is possible that large adaptive phenotypic shifts can take place in butterflies for wing patterns and colouration, which means that the evolution of warning colouration does not need to be a gradual process (but see also Lindström *et al.* 1996).

4.1.2.2 Modern refinements of theories for the evolution of aposematism

Two hypotheses have been proposed to explain the evolution of aposematism. The first is that the benefits of aggregation accrue to populations of individuals because of the reinforcement of the negative stimulus received when the initial aposematic prey item was attacked. Many later models incorporated Fisher's kin-based model which suggested that the death of one or more members of a population could enhance the survival of the remaining group to such an extent, that it could generate higher levels of fitness for aposematic individuals compared with the non-aposematic conspecifics (Turner 1975; Harvey *et al.* 1982). The model of Harvey *et al.* (1982) is important since they found that the survival of rare aposematic forms could be favoured under a number of conditions. First, evolution of aposematic morphs would be favoured if they were not too brightly coloured. Second, if aposematism accelerates the rate of learning, then it will evolve because fewer aposematic prey would be sacrificed to predator learning compared with non-aposematic prey. Finally, they predicted that if the number of prey families in each predator's territory were low, this would mean that in relative terms, that the occurrence of conspicuous mutants in one family would mean that the appearance of aposematic mutants might not be as rare as previously thought. This would be especially true if a mutation were to occur in the germ line of the parents thus conferring the mutation to all the offspring in a brood (Brodie & Agrawal 2001). Guilford (1985, 1988) argued against the kin selection idea in favour of "green beard" effects. His argument was that as long as the phenotypic expression of aposematism and its genetic linkage were high, then aposematism did not need to be explicitly based on kin selection. While this is true, the closely related individuals are most likely to have high rates of linkage between different alleles.

The second hypothesis is that there may be higher levels of survival accruing to defended individuals upon being attacked by predators. There have been some laboratory-based studies on the effectiveness of prey aggregations at reducing the levels of predation of aposemes. Alatalo and Mappes (1996) found that when prey were aggregated, great tits (*Parus major*) learned to avoid in aggregations of aposematic prey more quickly than when prey were solitary. However, Tullberg *et al.* (2000) criticised their analysis on two points. First, Alatalo and Mappes included the undefended prey in the analysis of the adaptive significance of aposematic colouration. When undefended prey were not included in the analysis, there was no significant difference in mortality of solitary and aggregated prey they (Tullberg *et al.* 2000) confirmed in an experiment. Second, the pattern observed in the Alatalo and Mappes data may have been the result of behavioural differences of birds towards cryptic defended and undefended prey. Birds may have been attacking the whole aggregation when prey were undefended, but attacking only one prey when they were in a defended aggregation before resuming the search for undefended prey. Again, Tullberg *et al.* (2000) confirmed this effect in a separate experiment. The results of Tullberg *et al.* (2000) therefore raise valid doubts as to the increased efficacy of aposematic signals in promoting predator learning in aggregations. Riipi *et al.* (2001) tested the benefits of aggregation in association with the differences between defended and undefended prey. They found that when the visual distinctiveness between defended and undefended prey was low; there were few learning effects throughout successive trials and that there were few benefits in forming groups. However, when the visual distinctiveness between the defended and undefended prey increased, rates of learning resulting in lower rates of predation for the defended prey that were more distinct and aggregated. This suggests that there was a synergistic effect with distinctiveness and increased group size which enhanced predator learning and the prey survivorship in later trials. These studies provide evidence of the benefits of aggregations and suggest that the models need not be kin-based. A kin-based view is the most parsimonious, in the early evolution of aposematism, but is not essential. However, if the basis of aposematism is mutation based, then the mutation is likely to be restricted to kin early in its evolution. The analysis of Tullberg *et al.* (2000) is a good reminder of the need to be clear in analysing results.

The second explanation for the evolution of aposematism is based on one of the assumptions that Fisher made in his initial kin-based model. This assumption was that aposematic prey were probably killed when they were attacked by a predator. This has been challenged because it has been found that attacked individuals can often survive attacks from predators and so may accrue direct benefits of their aposematism (Järvi *et al.* 1981, Wiklund & Järvi 1982, Wiklund & Sillén-Tullberg 1985; Marples *et al.* 1994). For example, Järvi *et al.* (1981) compared the rates of attack by great tits (*Parus major*) and release of swallowtail caterpillars (*Papilio machaon*) and mealworms (*Tenebrio* sp.). They found that aposematic prey that were attacked were released more regularly than mealworms and that they did not sustain fatal injuries. However, this experiment had some flaws which may have increased the difficulty of interpretation of results. For example, birds were wild caught meaning it was possible that the birds may have not been naive to swallowtails. This body of research shows that it is probable that aposematic prey can survive attacks from predators, but they still run a higher risk of injury or death compared with if they were not attacked. Another problem with this finding is that it does not solve the problem of the initial evolution of aposematism.

A final point to consider is that there may be some form of abatement effect whereby aposemes have a higher rate of detection, but suffer a lower probability of being attacked when compared with non-defended aposemes (Krause & Ruxton 2001, pp. 13-16). Hence, the overall mortality of an aposeme may be lower than a cryptic counterpart. Overall, there is growing evidence for post-attack survival of aposematic prey, although this argument does not explain the evolution of aposematism. It is possible that individual survival models could hasten the spread of aposematic signals in a population by reducing the initial costs of possessing the aposematic signals. However, questions remain how predators might be able to detect chemical defences of prey without killing them (e.g., Kassarov 1999; 2003; but see also Skelhorn & Rowe 2005).

There are a number of other explanations for the evolution of aposematic colouration. For example, it has been suggested that aposematism may have evolved in order to accentuate the differences between defended and undefended species (Turner 1975, 1977; Beatty *et al.* 2004). This effect may elicit a neophobic response in predators towards novel colouration (Coppinger 1969; Shettleworth 1972; Greenberg 1990; Schuler & Roper 1992). However, it is also possible that novelty could make the prey look different from the search image that predators had evolved to attack meaning that the predators may attack imperfect mimics at greater rates (Dukas & Ellner 1993; Dukas & Clark 1995). It is also possible that the higher costs associated with searching for cryptic undefended palatable prey could make brightly coloured defended prey attractive propositions under some circumstances (Dukas 1998 pp. 97-102). However, this trade-off is likely only to occur among species and does not explain the initial evolution and survival advantage of aposemes.

Poulton (1890, pp. 170-175) also identified many behavioural traits in prey that may act as signs of unprofitability. Two of the traits he listed were prey aggregation and sluggishness. Prey aggregation also is consistent with Fisher's (1930) hypothesis of selective advantage through kin selection. However, aggregations may also cause predators to attack prey more forcefully than they would attack a similar prey when encountered singly (Gamberelle-Stille & Tullberg 1996; Skelhorn & Ruxton 2006). This effect may also increase the effectiveness of the aposematic signal because it may allow the predator to ingest sufficient prey so it gets sick (Mathews 1977; Guilford 1991). It might be the case that optimal group size may also be related to the average level of defences delivered by prey with more potent prey requiring smaller aggregation sizes to withstand predator attention. The sluggishness of prey at escaping predators was also identified by Poulton (1890) as a possible aposematic cue. More recently, it has been found that in diurnal Lepidopterons can reduce their risk of predation by fast and evasive flight (protean defence, Humphries & Driver 1970). The development of protean defence is dependent upon increased investment in thoracic flight muscles. Therefore, species that adopt protean defence may be constrained in the size of their reproductive organs. In Neotropical butterflies, species that contain fewer defences have higher thoracic masses and lower abdominal masses than defended species (Srygley & Chai 1990; Marden & Chai 1991). Moreover, when these measurements were related to estimated flight speed, the thoracic width to body length ratio was the best predictor of flight speed (Chai & Srygley 1990; Srygley & Dudley 1993). This is one of the first problems in the aposematism and mimicry literature that have been treated as an evolutionary trade-off.

4.1.3 Automimicry/Browerian mimicry

Traditionally, theoretic models of aposematism have not dealt with variations in the levels of chemical defences that might exist within a species. This changed when Brower *et al.* (1967a) reported a laboratory population of monarch butterflies (*Danaus plexippus*) raised on cabbage (*Brassica oleraceae*) were palatable to blue jays (*Cyanocitta cristata bromia*) at all stages of the butterflies' life cycle. Butterflies that had been raised on milkweeds (*Asclepius curassavica*) caused the same birds to vomit.

Given that oviposition sites are chosen by laying females, this research raised the possibility that the intra-specific levels of defence may vary (Brower *et al.* 1967a; 1968).

Automimicry (also termed Browerian mimicry after Brower *et al.* [1967] [e.g., Pasteur 1982]) has been confirmed in chemically defended species both in laboratory-based studies (Brower *et al.* 1984; McLain & Shure 1985; Malcolm & Brower 1989; Ritland 1994; Tullberg *et al.* 2000) and the field (Brower & Moffitt 1974; Brower *et al.* 1975; Cohen 1985; Eggenberger & Rowell-Rahier 1991; Bowers & Williams 1995; Pasteels *et al.* 1995; Moranz & Brower 1998). Most of the variation in the levels of defensive chemicals has been found to be due to geographic variation in the levels of defences that the prey species contain (Edmunds 1974). Therefore, these differences are possibly caused by micro- and macro-geographic differences in nursery plant abundance. The mechanisms for these geographic variations remain obscure, although there is some evidence that there is a genetic component to these differences. For example, it has been noted that con-specific monarch butterflies from different regions of Africa raised on the same nursery plants contained different levels of defensive compounds (Edmunds 1974). However, intra-specific variation in the levels of secondary metabolites produced by nursery plants might cause differences in the dietary intakes of secondary metabolites (e.g., Bowers and Stamp 1992). Some of the variance in diets of larvae may be under direct female control because females choose the plants on which they lay their eggs.

These studies while important in documenting the possible existence of intra-specific mimicry, they do not unequivocally demonstrate the costs and benefits to the models and mimics in this system. The existence of automimics presents a problem for evolutionary models of aposematism because if mimics are fitter than models, then why does aposematism exist? This is an interesting problem and the answer may be related to sexual selection and the costs and benefits of models and mimics in relation to predation and fitness.

4.2 Mimicry

Species are known to resemble aspects of their environment and often attempt to resemble their backgrounds in what is known as background matching or crypsis. However, this is not the only form of deception in which animals engage (see Edmunds 1974; Ruxton *et al.* 2004 for reviews). Often, animals evolve signals that mimic the behaviour or form of unrelated species; a phenomenon called mimicry. Mimicry can take two forms with it being either aggressive or defensive.

Aggressive mimicry occurs when a predator or parasitic species resembles a non-threatening form in order to remain undetected by potential victims of predation or parasitism (Ruxton *et al.* 2004, pp. 172–174). For example, bluestreak cleaner wrasse (*Labriodes dimidiatus*) face similar problems to their evolution as aposematic species. This is because this species performs a dangerous behavioural task where they are at high risk of being consumed by larger client fish. Initial evolution of this mutualism is problematical because large piscivorous fish would tend to consume a conspicuous small fish swimming in front of them (Trivers 1971). Another problem is how this behaviour can be maintained when cheating may be more profitable (Axelrod & Hamilton 1981; Dugutkin 1997). This system is parasitized by mimics (e.g., bluestriped fangblenny fish, *Plagiotremus rhinorhynchos* [Côte & Cheney 2005]) that use the same visual and behavioural signals as cleaner fish in order to get close to client fish that they aggressively attack. Therefore, mimics of cleaner fish face similar evolutionary problems as in some cases of defensive mimics because what do mimics do once they become too successful? As is often the case with defensive mimics, it appears that the fangblennies are polymorphic and the different polymorphisms use different behavioural strategies (Côte & Cheney 2005). Defensive mimicry is when mimics resemble models in order to gain protection from predators. Although there may be similarities between the evolution of aggressive and defensive forms of mimicry, this thesis deals with defensive mimicry. Therefore, I will concentrate the focus of my review to this area.

Traditionally, there were only two forms of defensive mimicry considered by biologists: Batesian mimicry and Müllerian mimicry. Both theories were published in the nineteenth century (Bates 1862; Müller 1879) and have been influential in shaping how we think about aposematism. More recently, many of the assumptions of both forms of mimicry have been challenged by theoretical and empirical findings that indicate that there is more to mimicry than meets the eye.

4.2.1 Batesian mimicry

Batesian mimicry is the resemblance–visual or otherwise–between undefended mimics and a chemically defended model (Bates 1862). Species that share the same warning signal are said to comprise a mimicry system or complex (or a ring when the number of species is large). The relationship is parasitic with the mimic benefiting from defences of model, but not contributing anything to the reduction of predation directly. The rate of predatory attacks on models is determined by three main factors: (1) the frequency of mimics in relation to models; (2) the strength of the models defences; and (3) the abundance of undefended prey. Each of these factors has been empirically verified (Ruxton *et al* 2004, pp. 147-152).

Evidence from field studies suggests that when Batesian mimics outnumber models, the advantages of mimicry decline and the mimicry system may begin to break down. Sheppard (1959) found that in species of African mimetic butterflies, their accuracy in matching the models pattern declined as the model became less common. Moreover, in cases where a mimic's range extends beyond that of the model's, the rate of predation increases for the mimic in parts of its range where models are not sympatric (Ritland 1998; Pfennig *et al.* 2001). Many Batesian mimics are also allochronous, emerging after the models, meaning that the models bear the entire cost of predator education (Waldbauer 1988 a,b). However, these lines of evidence for the negative effects of mimics are circumstantial and more direct observations of predator foraging behaviour are preferable to test this assumption.

Brower (1960) tested the effectiveness of a Batesian mimicry system by varying the frequencies of mimics in the system that was being depredated by European starlings (*Sturnus vulgaris*). She found that the effectiveness of the models at deterring predation decreased concomitant with increasing mimic frequency. Similar results have been observed in amphibians (Huheey 1980), mammals (Nonacs 1985), and birds (Avery 1985) as predators. Another approach has been to use imperfect mimics to test the effectiveness of mimics at higher mimic frequencies. Imperfect mimics are those whose visual signals do not match those of the models' perfectly. Imperfect mimicry has been proposed to be a response to the breakdown of a mimicry system when the frequency of the mimics becomes too high (Sheppard 1959, Brower 1960). However, there are other explanations for imperfect mimicry such as prey species simultaneously resembling more than one model species (Edmunds 2000).

Many studies have investigated the relationship between the effectiveness of a models defence against different frequencies of imperfect mimics. In these studies, similar results to those obtained using perfect mimics have also been found (Duncan & Shappard 1965; Pilecki & O'Donald 1971; Lea & Turner 1972; Goodale & Sneddon 1977, Avery 1985; Nonacs 1985; Lindström *et al.* 1997). For example, Lindström *et al.*

(1997) studied the effectiveness of models' chemical defences at preventing predation by great tits (*Parus major*) with changes in mimics frequency. They found that the rate of mortality for both models and mimics increased as the frequency of models declined. This effect is likely to be due to the rate at which the mimics were encountered in relation to the model, which would reinforce the aposematic signal or the dilution effect of increasing the relative abundance of mimics. This is because predators are likely to exert a stronger predator pressure on models compared to mimics as they generally comprise a larger proportion of the population in model-mimics systems (Nur 1970; Huheey 1984; Turner 1987). Lindström *et al.* (1997) also tested the effectiveness of the mimicry system in relation to the potency of the level of chemical defences of the model. They found that the models and mimics generally sustained lower mortality rates with more potent models, which is consistent with many other studies (Duncan & Sheppard 1965; Alcock 1970; Pilecki & O'Donald 1971; Goodale & Sneddon 1977, Ritland 1994). Learning has also recently been shown to be faster in more potently defended prey compared with less well defended prey types (Darst *et al.* 2006; Skelhorn & Rowe 2006a) which could influence the rates of attacks on the mimicry system.

Predation on a mimicry complex is also related to the abundance of alternative prey in the system (Nonacs 1985; Lindström *et al.* 2004). This is because visually distinct undefended prey will always be preferred to mimics since this reduces the chances of attacking a model. This assumption has a long history in the biological literature. For example, Carpenter and Ford (1933) noted that predators propensity to attack chemically defended prey depends on the availability of alternative prey, which has since been supported in many theoretical treatments (Holling 1965; Emlen 1968; Dill 1975; Luedeman *et al.* 1981; Getty 1985; Kokko *et al.* 2003; Sherratt 2003; Sherratt *et al.* 2004). However, there is comparatively less empirical evidence of the effects that alternative prey have on the effectiveness of Batesian mimicry systems (Nonacs 1985; Hetz & Slobodchikoff 1988, Lindström *et al.* 2004). In a recent paper Lindström *et al.* (2004) demonstrated the rate of predation on model-mimics decreased when the alternative prey were at higher densities. This effect is because alternative prey will be exploited before prey from model-mimic systems. This effect is ultimately related to the predator's energetic state as at higher alternative prey densities, predators are able to find a larger proportion of their energetic needs from alternative prey meaning they do not need to take unnecessary risks by attacking the mimicry complex.

4.2.2 Müllerian mimicry

Müllerian mimicry is the resemblance of two or more chemically defended species in their warning signals (Müller 1879). The model is an arithmetic argument based on spreading the costs of predator education among more than one species. This argument is that if it takes a fixed number of prey items to educate a predator of prey species noxiousness, then this places a burden on the defended species. Consider two or more species that are both defended and that resemble one another. Each prey type will pay the costs of predator education in relation to their overall proportionate contribution to the prey population sharing the warning signal. So rather than the education cost being borne by a single species, the adoption of a common signal of unprofitability means that the cost will be borne by all species sharing the signal.

Traditionally, it has been thought that the evolutionary process resulting in Müllerian mimicry was convergent. This raises the question of which species is the model and which species is the mimic? This question is not new, and was raised by Müller himself (Müller 1879). More recently, Mallet (2001) speculated that the relationship might be more one sided with one species being selected to resemble an aposematic sympatric species. Mallet (2001) called this an advergent process rather than convergent because convergence occurs when two species are selected to converge upon a single form–often allopatrically. Models of Müllerian mimicry also assume that predators will attack a fixed number of defended prey before they will learn to completely avoid them (Müller 1879; Joron & Mallet 1998; Mallet & Joron 1999). Species of the mimicry system receive a benefit based on the dilution of the effects of predation that each species experiences (Turner 1987). Therefore, the selection pressures of each species due to predation will decrease, but as is the case with the evolution of new aposematic species, selection for a rare novel morph may be anti-apostatic (i.e., predators will select against novel morphs). Therefore, the selection for the mimicry system is likely to be stabilising and it is predicted that all mimicry between Müllerian co-mimics be monomorphic (Turner 1984; Turner 1987; Endler 1991).

A number of studies have tested if rare defended forms suffer a selective disadvantage when compared with more common defended species when their appearance is similar to the model or when it is novel. Benson (1972) altered the wing colouration of a sample of *Heliconius erato* butterflies by staining a red patch on the forewing black. Another sample were sham controls that had an equal sized area of black wing stained black, but leaving the overall pattern of the wing intact. The two groups were then released into an area that contained many other species that were known co-mimics and the numbers of manipulated butterflies seen at roosting sites were counted. Butterflies were released in two years and in the first year, the butterflies with novel patterns tended to be resighted less often. In the second year, there was no significant difference in resighting between the two groups. However, Benson (1972) found that the amount of wing damage suffered by altered individuals was higher in both years. Another study conducted on two allopatric races of *H. erato* in Peru found that when the morphs were translocated to new sites within the normal range of their race, they suffered lower rates of predation than when they were translocated to sites where their race was uncommon (Mallet & Barton 1989). However, the areas of high predation tended to correspond with areas of high jacamar (*Galbula* spp.) abundance and the highest disappearance of butterflies tended to occur early in the experiment. This indicates that the major cost paid by the new forms was paid early in the experiment and thus may have been associated with predator education. This indicates that predators might have been very quick to form aversions to the initially less familiar species (Mallet & Barton 1989). This observation helps explain the results of Benson (1972) in that predators were already educated for one species, but uneducated regarding the novel species.

Although there was a disadvantage to the novel morph in the previous experiments (Benson 1972; Mallet & Barton 1989), a better test would be to release the two novel morphs to a predator that has not experienced either morph previously. Kapan (2001) did exactly this, releasing two novel morphs (one morph abundant and one rare) at two different densities. He found that the less common morph of butterfly was resighted less often than would have been expected, and that a smaller proportion of the population was resighted at low release densities. This indicates that the predators learn to avoid the novel forms quickly despite their initial selective disadvantage and that a smaller proportion of the population are sacrificed at higher release densities. Finally, Langham (2004) found that rufous-tailed jacamars (*Galbula ruficauda*) tended to attack novel *Heliconius* species butterflies more than they did with familiar morphs after have had previous experience with all butterfly morphs. The periods between presentations ranged from four to 429 days. This suggests that specialised butterfly predators may utilise specific pattern recognition to identify potential prey. Moreover, these studies together indicate that the predators stabilise the selection of the colour morphs of mimicry systems.

4.2.3 Recent developments in mimicry theory

The traditional concept of Müllerian mimicry has survived remarkably well, but this is mainly due to a lack of empirical studies that have tested the models. The predictions that the relationship between co-mimics will be mutualistic and that a fixed number of prey will be required to educate a predator seem biologically unrealistic. The number of prey that is required to educate a predator will be dependent on a large number of factors. From the predators' perspective there are the factors such as its energetic state, and the state of its mate and its offspring which all influence the bird's likelihood to attack defended prey. The intraspecific and interspecific differences in toxin concentration and type may have an important bearing on how the prey's defences are perceived. These factors are all confounding factors that have only begun to be addressed in an experimental manner. However, the increasing use of integrative experimental methods has the potential to reinvigorate the study of aposematism and mimicry.

There have been a number of models devoted to Müllerian mimicry and these have been summarised by Ruxton *et al.* (2004, pp. 164-171, pp. 202-205). These models differ in their predictions based on the assumptions they make. The models all generally agree that when two species are equally well defended, that the two distasteful species will gain from using common signals. However, it has long been suggested that the levels of defence may not be equal in Müllerian mimicry complexes (Wallace 1871; Nicholson 1927). More recently, biochemical assays of different populations and species of co-mimics have catalogued the range of defences and show high degrees of natural variation among species (Blum 1981; Pasteels *et al.* 1983; Nishida 2002).

As experimental investigations of mimicry have become more sophisticated, the models of Bates and Müller have look more unlikely in their pure forms. Brower *et al.* (1968) proposed the idea of a palatability spectrum, where for the first time, edibility was considered as a continuous variable. At one end, prey may be completely edible and consumed almost all of the time. At the other end, prey may be very noxious and almost never consumed. Brower *et al.* (1968) also raised the possibility that defended prey could be used as a food resource when undefended food items became rare.

Therefore, the palatability spectrum was important as it provided the possibility that birds might consume defended prey regularly as part of their diet.

The palatability spectrum also has implications for how Müllerian mimics affect overall effectiveness of an aposematic signal. If Müllerian co-mimics were defended with different toxins, then predators may find one prey type more palatable than the others may. Speed (1993a) developed a model which considered this effect in Müllerian mimicry systems. He found that if a predator could detect differences in the defences between co-mimics, then predators ought to eat more weakly defended co-mimics. This could lead to more weakly defended co-mimics increasing the rates of attacks on the better defended co-mimics. Therefore, the relationship between the co-mimics could be parasitic, thus making the relationship between co-mimics similar to the relationship of models and mimics in Batesian systems. Indeed, Speed (1993a) dubbed this effect quasi-Batesian mimicry although it remains a highly debated prediction. If quasi-Batesian effects do occur, then this questions the traditional treatments of Müllerian mimicry stating that co-mimics act as mutualists. For example, Turner *et al.* (1984) predicted that each attack on a defended prey must lower the likelihood of future attack toward zero regardless of its levels of defence. The rate at which a species reduces the rate of attack is dependent on its defences meaning that highly defended species will reduce the rate more quickly than moderately defended prey.

The prediction of the parasitic nature of quasi-Batesian mimics on the models, means that the effectiveness of the models' chemical defences are dependent on the abundance of mimics. Therefore, systems are thought suffer the lowest rate of predation when the frequency of the quasi-Batesian mimics are low. Quasi-Batesian relationships between prey of differing levels of defence and frequency have not been well studied to date.

Speed *et al.* (2001) conducted an experiment where pastry baits were left outside for garden birds to attack them. They used five prey types in the experiment: edible controls, models, mimics, model controls, and mimic controls. The models and mimics were visually indistinguishable from one another, although the models had more quinine hydrochloride and mustard powder added compared with the mimics. The model controls had the same level of defence as the models and were visually distinct from one another. The mimic controls had the same defences as the mimics and again, were visually distinct. The experiment lasted for 40 days which was split into two phases (20 days each) that differed in how many mimics were put out each day. The results suggest that: (1) that the mimics had a parasitic effect on the effectiveness of the models, (2) the predators displayed partial preferences for the defended prey, and (3) that the models were depredated more when the numbers of mimics was higher. These results indicated for the first time that supposed Müllerian mimics could behave in a Batesian manner. These effects have been supported–although not strongly–in two later studies (Lindström *et al.* 2006; Ihalainen & Mappes 2007).

Quasi-Batesian mimicry is an exciting development in mimicry theory because it also offers a biologically more realistic way of thinking about Müllerian mimicry systems. Generally, Müllerian mutualisms will only occur when all co-mimics if co-mimics defences are perceived as being equal or if both co-mimics are so toxic that predators learn to avoid prey after one attack for each co-mimic species. While these systems occur (e.g., poison arrow frogs), they are likely to be the exception rather than the rule. Therefore, it is highly likely that relationships that were thought of as being mimetic, are more likely to be parasitic like Batesian systems at least under some conditions.

4.3 The role of predators

4.3.1. Predator learning

Traditional models of predator learning through associative learning state that chemical defences should eventually extinguish predator attacks. The direct route by which animals are thought to learn the association between warning colouration and chemical defences is through associative learning. The most prevalent theory of associative learning is that of Rescorla and Wagner (1972), although there have been several other theories of associative learning proposed (see Pierce & Bouton 2001 for a review). The Rescorla-Wagner model predicts that a predator learns to associate the unconditioned stimuli (US)–the defence–with the conditioned response (CS)–the colour signal. The model predicts that the continued experience of negative stimuli (US) will lead to extinction preference for the source of the US.

Associative learning is dependent on a number of factors for it to be effective. For example, the principle of contiguity states that the effectiveness of the association between the US and CS will be less effective if they are separated by long time-periods (Wasserman & Millar 1997; Mazur 2006). However, studies have found that rats can form aversions to food with a separation in the CS and the US by over 24 hours (Garcia *et al.* 1966; Etscorn & Stephens 1973). This seriously questioned the assumption that the temporal gap between CS and US could be no more than a few seconds (e.g., Kimble 1961, p. 165). It is now considered that taste aversions in many animal species can occur when the CS-US interval is several hours long. It has also been established that this associative learning is not dependent on any aftertaste that might fill the lag between ingestion and illness (Mazur 2006, p. 103). For example, herbivorous browsers may have delays of many hours between ingestion of plant material and experience of illness caused by PSMs (e.g., Belovsky 1982; Provenza 1995; Alm *et al.* 2002; Bergvall & Leimar 2005; Marsh 2005; Bergvall *et al.* 2006). Yet, they are able to learn to avoid forage which are higher in PSMs.

Recently, a new model (Yearsley *et al.* 2006) of diet selection based on post-ingestive feedback was proposed which was based on ideas such as Shannon-Weaver entropy (Shannon & Weaver 1949). In this model, they hypothesised that the time lag between ingestion of prey and post-ingestive consequence can affect the information that a forager can derive from its diets. Their model yielded a number of straightforward predictions: (1) decreasing time between ingestion of food and the onset of its post-ingestive consequences will increase the speed and accuracy of association; (2) the effectiveness of learning is less effective when the continuous foraging experience with a single food type is shortened; (3) decreasing the relative frequency of a food type decreases the probability of correctly associating the food with its post-ingestive effect and thus increases the probability of erroneous associations forming; and (4) increasing the number of food types available to a forager has a weak effect on the accuracy of association when food abundance in held constant (Yearsley *et al.* 2006). However, the use of Shannon-Weaver entropy in biological systems has been criticised by some authors because it supposes that reducing ambiguity is invaluable to animals in itself (e.g., Dall *et al.* 2005). They argue that reducing ambiguity I only advantageous if it increases fitness. In the case of consuming chemically defended prey, being more certain about the level of a potential prey's defence could have definite fitness advantages.

4.3.2 Psychological aspects of signal design

Predator psychology has come to prominence more in the understanding of the effectiveness and evolution of aposematic signals. This means that prey might be selected to exploit properties of predator psychology that may enhance the formation and maintenance of memories. The psychology of the predator is likely to be very important in the evolution of aposematism and mimicry. This was noted early last century by Von Uexküll (1909, quoted in Thorpe 1979) in his notion of the *Umwelt*–the sensory world in which each species lives. Von Uexküll's concept was that the anthropomorphic viewpoint precludes understanding of another species' behaviour. He taught that each species experiences the world in a unique way and the experience is determined by the animal's sensory abilities. For example, the discovery of avian UV vision in the early 1990s brought about a revolution in how we think about avian vision and questioned traditional approaches in testing prey and mate choice preferences in birds (Bennett & Cuthill 1994; Bennett *et al.* 1996). However, it is also important to realise that there may be relationships between species, which can lead to generalisations and increase our basic understanding. This is the approach used by those seeking to understand the evolution of aposematism and mimicry from the perspective of predator psychology (e.g., Endler 1978, 1988; Endler & Mappes 1994).

It has been shown in birds that they have unlearnt aversions to specific colours and colour patterns of artificial prey (see Schuler & Roper 1992 for a review). Generally, black, red, and black-and-yellow striped prey items can generate avoidance in birds. Conversely, items coloured green, yellow, and half-black/half-yellow induce little or no avoidance in avian predators (Schuler & Roper 1992; Speed 2000). The strength of unlearnt avoidance can also vary with age as has been found in great tits (Lindström *et al.* 1999). Therefore, aposematic signals could be favoured because they exploit unlearnt biases in the predators psychology. However, it is clear that some colours are more effective at influencing the learning and memorability of prey defences.

Shuler and Roper (1992) reviewed warning signals and how they increase predator learning in birds. They identified a number of factors that might aid in predator learning and retention of prey aversion. For example, the conspicuousness of signals against the background has been noted to aid learning of prey aversive qualities. Predators are quicker to learn the aversive qualities of prey that have colours that contrast with those of the background compared with cryptic prey (Gittleman & Harvey 1980; Gittleman *et al.* 1980; Roper & Wistow 1986; Riipi *et al.* 2001). A number of other properties have been identified which might lead to accelerated learning of prey defences: (1) novelty; (2) distinctiveness; and (3) contrasts with the background (reviewed extensively by Shuler & Roper 1992; Ruxton *et al.* 2004). The same effect of colour has also been noted with memorability of the prey properties. For example, Roper and Redston (1987) tested the memorability of red or white beads that were, or were not, coated in methyl anthranilate. The subjects (domestic chickens, *Gallus gallus*) were then tested after different intervals to estimate how well they remembered the beads. The authors found that as the time between training and retesting increased, the number of pecks in the retest session increased. Red beads also suffered fewer pecks than white beads. This study has been critiqued because the birds might have been able to detect methyl anthranilate odours. This was later confirmed by Marples and Roper (1997), which was the first demonstration that odours can be aversive for birds. However, other factors might also have influenced the results. First, birds might associate the colour white with the colour of faecal matter. This might lead to a bias in the rate of pecking. Birds often are seen to peck at white leg bands more frequently they do at other colours and white is often eschewed as a band colour for this reason. Therefore, the use of this colour could have also interfered with learning. Second, figure 4 in the paper (Roper & Redston 1987) indicates that there were differences in the rates of attack for the different bead colours during training due to the level of colour matching with the background. This means that there could have been differences in the level of attention that the chicks paid with each bead type. The white beads were pecked about 0.75 the rate of the red beads meaning that subjects might have been less motivated to remember this colour (given they were less motivated to attack it). Therefore, the retrials should have been corrected for this bias. If this were done, many of the differences in peck rates for the different coloured beads might have been extinguished. Although this was not a particularly convincing study, there is growing evidence that properties of visual aposematic signals do increase the rate of learning and retention of learned aversions at least in avian predators (Ruxton *et al.* 2004). Most of this research has concentrated on studying signals in one sensory modality. It may often be the case that aposematic signals are expressed as a suite of signals given in many sensory modalities.

In the case of mimicry, the selective factor that has led to two or more prey species sharing the same warning signal is probably predation. Therefore, Müllerian mutualisms may be the result of selection driving one species to look like another by predator selection. Herrera (1985) suggested that frugivorous birds might have biases for aposematic prey given the similarity in colouration between aposematic fruits and also because frugivores have the metabolic machinery for dealing with greater amounts of secondary metabolites in their diets (see also Gamberale-Stille & Tullberg 2001). This is interesting because it suggests that rather than specific colours being aversive *per se*, the context in which the colour is seen is also important. This suggests that it is possible that colour pre-dispositions will be much more pervasive than previously thought as many sexually selected traits are similar in colouration to aposematic signals.

4.3.3 How does prey toxicity affect predator attack decisions

Recently optimal toxicity of cane toads (*Bufo marinus*) was modelled to determine the conditions favouring the evolution of chemical defences (Longson & Joss 2006). The model predicted a number of patterns in the evolution of toxicity. First, the authors predicted that toxicity should decrease with production cost of toxins (both direct costs and indirect costs). Second, they predicted that toxicity should decrease with decreasing resource availability. Third, they predicted that increases in life-span would favour increases in the level of toxicity of prey. Finally, they predicted that animals ought to adopt a strategy of being highly toxic in order to back-up the threat of defence. However, the interspecific and intraspecific differences in prey defences indicate that prey are often not as highly defended as is possible.

The amount of protection gained from a chemical defence is still likely to be positively related to the potency of their defence (e.g., Turner *et al.* 1984; Leimar *et al.* 1986; Speed 1993a). However, the effect that of the strength of chemical defence has at deterring predation is still poorly studied. Alcock (1970) found that the speed at which black-capped chickadees (*Poecile atricapillus*) learned to avoid food was related to absence of food, bad taste, or the addition of an emetic compound (quinine). The food that the birds learned to avoid first was the one with added quinine. Recently, Darst *et al.* (2006b) also found that domestic chickens were quicker to learn to avoid more potently defended species of poison dendrobatid frogs. Skelhorn and Rowe (2006a) had a similar result in naïve domestic chickens which learned to avoid more potently defended artificial food quicker than less potently defended food. However, most research into the predation of chemically defended prey has focussed on the effect of defence potency on the rates of learning.

Observational studies have also shown that birds are able to discriminate among different aposematic prey (Jones 1932, 1934; Brower *et al.* 1963; Chai 1986; Sargent 1995, Pinheiro 1996). However, the main problem with these studies is that it is unknown if the birds learn the differences in between each prey type or generalise from one prey type to another. This means that it was unknown if birds were basing their choices on the levels of defences contained in the prey. The level of defensive compounds that a chemically defended prey animal contains may vary by a great deal both within an aposematic species or between defended co-mimics utilising the same visual signal.

4.3.4 Do educated birds continue to consume chemically defended prey?

In the 1940's, it came to be accepted that birds would sometimes consume chemically defended prey due to hunger (Cott 1940). The role of hunger was recognised earlier than this as a possible factor mediating predators' choices (Poulton 1890; Swynnerton 1915). For example, Swynnerton (1915) suggested that diurnal birds might be more likely to consume chemically defended prey early in the morning because of their nightlong fast.

However, this view changed with the development of new models. The Rescorla-Wagner model of associative learning predicts that the consumption of any chemically defended prey should eventually lead to an extinction of preference for chemically defended prey (Rescorla & Wagner 1972). However, it was noticed that predators continued to occasionally consume defended prey items. The continued consumption of chemically defended prey was thought to be due to predator errors such as the effects of imperfect memory (e.g., Turner *et al.* 1984; Servedio 2000). However, Skelhorn and Rowe (2005, 2006b) recently demonstrated that predators can form consistent and repeatable partial preferences for chemically defended prey. Given that the education period for a predator is likely to be short in comparison to the entire lifespan of the predator, this raises the possibility that educated predators may be a more significant factor in the evolution of aposematic traits than naïve predators. There are a number of hypotheses for the continued consumption of chemically defended prey: (1) birds may have been consuming chemically defended prey as they were energetically stressed; (2) birds consumed them as they had a specific dietary need; and (3) the birds were consuming chemically defended prey to self-medicate the effects of parasites or disease. In this thesis, I will primarily explore the potential relationship between numbers of prey consumed and manipulations of individual birds' energetic states.

4.3.5 Physiological state of the predator

Other authors have suggested that predator behaviour could change over time and that they might attack Müllerian mimicry systems at times of nutritive need (Marshall 1908; Holling 1965; Dill 1975; Sheppard 1975; Benson 1977). Recently, there have been a number of unconventional theories of Müllerian mimicry that also predict that predators might continue to consume moderately defended prey at times of energetic stress (Huheey 1976; Owen & Owen 1984; Speed 1993a, 1999; MacDougall & Dawkins 1998; Speed & Turner 1999, Sherratt 2003; Sherratt *et al.* 2004). This is because although defended species might contain toxins, they also contain nutrients that are useful to the predator during times of need (Speed 1993b).

The levels of toxins that predator has ingested will affect a predator's physiological state. This idea was suggested in a number of early studies stating that animals may attack and consume defended prey at times of energetic stress and that animals may strategically utilise these food items in times of nutritive need (Brower *et al.* 1968; Speed 1993b). This idea formed the basis of what came to be known as the palatability spectrum (Brower *et al.* 1968; Turner 1984, 1987). Later, the saturation theory (Mallet & Joron 2000; Mallet 2001) stated that predators might consume moderately defended prey regularly in their diet. This hypothesis predicted that predators should consume prey until they reach a point where their system becomes saturated with toxins and the predator cannot risk consuming more defended prey.

Most recently, stochastic dynamic programming (SDP) models of the state-based consumption of chemically defended prey have predicted that predators should have a critical value of toxins below which they can consume chemically defended prey (Kokko *et al.* 2003; Sherratt 2003; Sherratt *et al.* 2004). Above the critical value, the defended prey is prohibitively expensive to consume and so should be eschewed. There is growing evidence that predators are able to strategically manage their levels of body toxins (John Skelhorn, Pers. Comm.; unpublished data) and this offers an exciting new branch of research in the future.

Many predatory species are adapted to the defences of their prey at a physiological level. These adaptations may indicate a long evolutionary history of predator-prey interactions. For example, black-headed grosbeaks (*Pheucticus melanocephalus*) have been shown to be able to consume large numbers of monarch butterflies in their Mexican wintering sites, which suggests that they may be physiologically adapted to detoxify cardiac glycosides (Fink & Brower 1981; Brower & Calvert 1985; Brower 1988). These examples provide evidence that predators can overcome the defences of prey and utilise them at times of nutritional need.

A final consideration for predators attacking chemically defended prey may be that they use the defended prey as a way to self-medicate when they are ill. Various species of animal have been observed to consume unusual food items when they are sick that are capable of improving health (Garber & Kitron 1997; Engel 2002; Huffman 2003) although the cause and effect have only been disentangled recently (e.g., Villalba *et al.* 2006). For example, avian geophagy has been recorded in many species in the tropical and sub-tropical regions by many species of frugivorous birds (Munn 1994; Pryce 1994; Gilardi & Munn 1998; Diamond *et al.* 1999; Cooper 2000; May 2001; Gilardi 2003; Low 2003; Symes & Marsden 2003; Brightsmith & Aramburú 2004; Symes *et al.* 2005). The reasons for geophagy are still debated, but one strong hypothesis is that clays are consumed in order to bind PSMs, which are often found in high concentrations in fruits (Gilardi *et al.* 1999). Therefore, it is possible birds are able to mediate the effects of distasteful and toxic plant compounds thorough the ingestion of clays. It is also known the starlings collect samples of green material that they place in their nests that may release volatile compounds, which may help control the numbers of sucking lice in their nests (Lozano 1998).

These observations (that birds may be able to at some level self-medicate) raise the possibility that birds may consume chemically defended prey in order to mediate the effects of parasites or pathogens. For example, some insects contain quinones (e.g., tenebriod beetles [*Palembus ocularis*], contain hydroquinone [Wahrendorf & Wink 2005]) which are also effective agents against malaria (Fotie 2006). This means that birds could increase their ingestion of insects containing chemical defences as a method of reducing numbers of microorganisms in their systems. However, this strategy may entail risk on the birds part because if there are intra-specific differences in the concentrations of the defence chemicals, this might lead to poisoning if the birds were to consume too many of the defended prey. Therefore, birds may resort to consuming plants before insects in order to self-medicate. However, this is an interesting idea worthy of experimental and theoretical investigation.

4.4 Conclusions

Aposematism and mimicry have been receiving much theoretical and empirical consideration recently (see Ruxton *et al.* 2004 for a review). This has led to many new hypotheses and ideas regarding the evolution of aposematism and mimicry. One of these new hypotheses is the toxin-energy trade-off, which has recently been formalised using SDP models (Kokko *et al.* 2003; Sherratt 2003; Sherratt *et al.* 2004). This is one of the first trade-offs in the field of aposematism and mimicry to be explored theoretically and the models yield many seemingly straightforward predictions, but others that seem at first counter-intuitive. In the following chapters, I will test the energy-toxin trade-off and examine some of the less intuitive predictions of the models. In doing this, I hope to provide empirical data in support of the new SDP models which will hopefully lead to a re-evaluation of the way we view predators and their behaviour towards aposematic and mimetic prey.

Chapter 5

EDUCATED PREDATORS STRATEGICALLY TRADE-OFF THE COSTS AND BENEFITS OF CONSUMING CHEMICALLY DEFENDED PREY

5.1 Introduction

The observation that animals can learn to avoid aposematic prey is supported by much data across a broad range of taxa (birds [Mostler 1935; Cott 1940; Brower 1958a,b,c; Brower & Brower 1962; Papageorgis 1975; Hensel & Brodie 1976; Schuler & Hesse 1985; Bowers & Farley 1990], fish [Kruse & Stone 1984], reptiles [Sexton 1964; Boyden 1976; McLain 1984; Terrick *et al.* 1995], molluscs, [Darmaillacq *et al.* 2004], and insects [Berenbaum & Miliczky 1984; Bowdish & Bultman 1993; Kauppinen & Mappes 2003; but see Rashed *et al.* 2005]). However, recent experiments have found that animals may continue to attack chemically defended prey after learning is complete indicating that birds develop partial preferences for chemically defended prey (Skelhorn & Rowe 2005; Skelhorn & Rowe 2006b). These findings therefore, raise questions over the models of aposematism based on associative learning.

Two recent stochastic dynamic programming (SDP) models that have attempted to capture predatory behaviour in order to predict evolutionary outcomes have assumed that attack rates on defended prey will increase as the energy reserves of a predator decrease (Sherratt 2003; Sherratt *et al.* 2004). Currently there are no data showing that manipulations of a predator's energetic reserves affect its foraging decisions on defended prey. Observational studies suggest that predators increase their attack on defended prey when palatable prey are rare (Swynnerton 1915; Cook et al., 1969). Other studies have found that food deprivation periods can increase a predator's motivation to attack defended prey (in birds [Chai 1986], lizards [Sexton *et al.* 1966], copepods [Williamson 1980], molluscs [Gillette *et al.* 2000], and insects [Gelparin 1968; Hileman *et al.* 1995]). However, these studies fail to show whether this behaviour results from strategic decision-making caused by reductions in energetic reserves. Moreover, these studies have failed to directly measure the predator's energetic state and so strategic state-based consumption of chemically defended prey is yet to be demonstrated. This experiment specifically investigates the effects of a predator's energetic reserves on its consumption of chemically defended prey which will aid in understanding the selective forces exerted by educated predators on defended prey.

5.1.1 A note on stochastic dynamic programming (SDP) models

Stochastic dynamic programming (SDP) modelling is a computational technique which has been used to model many behaviours that follow a periodic cycle. Its origins lie in operations research and economics (Bellman 1957; Nemhauser 1966; Mitchell 1972; Bunn 1982). It was first discussed in discussed as a tool for the calculation of optimal behaviour sequences in the early 1970s (McFarland 1971; Sibly & McFarland 1976). More recently, with the increasing computational powers of personal computers, these techniques have led to many behaviours being extensively modelled (see Mangel & Clark 1988; Houston and McNamara 1999; Clark and Mangel 2000 for reviews). However, empirical study of model predictions has lagged behind their formation (Houston & McNamara 1999; Hutchinson & McNamara 2000).

Stochastic dynamic programming is a mathematical technique which is used to deliver optimal sequences of behaviour given the environmental constrains and the animal's energetic state. It works through dividing the period of time under consideration (e.g., a day), into a series of small time intervals. At the beginning of each of these time-intervals, the animal makes a decision on which behaviour it will adopt in the upcoming interval. This decision is based on the animal's body state at the end of the last interval and on a set of environmental constraints if the behaviour is time invariant (Nemhauser 1966). In most biological models, the animal's behaviour has to change with time because one behaviour is unlikely to be the best across all environmental conditions. Thus, the animal will choose from a set of behaviours in response to stochastic changes in the environment. The decision that an animal makes therefore will affect the probability of survival to the next time interval. The optimal behaviour is the one which maximises the probability of survival into the next interval whence, the animal makes its next decision.

Dynamic programming works through backward iteration from the end time, at which the state of the animal is known, to earlier time-periods to calculate the optimal behavioural policy. This policy is a rule specifying the optimal behaviour given the state of the animal at each time. Given the strategy, it is possible to follow an individual through time and calculate what decision the animal makes given its state and its likely state in the next period concomitant with each possible behavioural choice using conditional probability. This sequence of actions constitutes the behavioural routine (Hutchinson & McNamara 2000).

Recently, three papers (Sherratt 2003; Kokko *et al.* 2003; Sherratt *et al.* 2004) were published that have addressed predator behaviour towards chemically defended and mimetic species. These models were state-based models that utilised SDP methods to calculate how a predator (generally assumed to be an avian predator) will attack chemically defended prey and how this predation can drive evolution in aposematism and mimicry systems. The model of Sherratt (2003) was the first and modelled the state-based consumption of chemically defended prey. Sherratt (2003) predicted that predators should consume chemically defended prey at times of nutritive need. He also found that the rate of attack upon chemically defended prey should decline with increases in undefended alternative prey. Kokko *et al.* (2003) also formulated a state based model which investigated the effects of predation on Müllerian mutualisms and found that both mutualistic and parasitic relationships between co-mimics. Their algorithm was quite simple but included state variables for toxin load and energy levels. They also found that the predators' tendancy to attack chemically defended prey should decline with increases in bodily energy reserves and increases in the undefended alternative food. Finally, the model of Sherratt *et al.* (2004) examined the state-based foraging behaviour of birds but with the added state variable of toxin loads. They modelled the behaviour of birds when attacking a prey system that had prey with one defence compound and found the state-based relationship that was described in the other two models. They found quasi-Batesian effects in Müllerian mimicry and they also indicated that there may be quasi-Müllerian effects when the frequency of Batesian mimics increases. They also modelled a situation where birds were exposed to two prey types with different defensive compounds. They found that the two prey species may act mutualistically because the predator may be unsure of the co-mimics chemical defence. For the rest of this thesis, I will consider predictions drawn mainly from the models of Sherratt (2003) and Sherratt *et al.* (2004).

5.2 Methods

5.2.1 Study species and housing

Subjects were six male European starlings (*Sturnus vulgaris*) caught under licence from English Nature (Licence Nos. 19991381 and 20001512). Starlings are large ground-foraging insectivorous passerines. Males are larger than females with their masses ranging between 75-100 g in the wild (Feare 1984). Therefore, I used solely male in order to reduce potential variance in behaviour arising from sex differences in mass and mass regulation strategies. In the laboratory birds lose mass compared with free-living birds (due to muscular and visceral atrophy) and so the masses of the birds ranged between 70-85 g when allowed access to food *ad libitum*. Prior to the experiment, birds were housed in a larger, mixed-sex group in an indoor aviary. All the birds had previously been used in operant foraging experiments; however, they had not experienced defended prey in the laboratory, nor the stimuli or experimental protocol used in the current experiment. For the duration of the experiment, the birds were housed individually in wire mesh cages (450 x 750 x 450 mm, h x w x d) arranged in a single room such that they had visual and acoustic contact with each other. Cages were equipped with two dowel perches and two water bottles; birds had *ad libitum* access to water at all times. The birds were maintained under a 14:10 hour light/dark cycle produced by daylight spectrum bulbs. At the end of the experiment, birds were returned to the aviary for future use.

5.2.2 Mass manipulations

The experiment relied upon comparing foraging choices of subjects at free-feeding mass (Free-fed) and at a reduced mass (Restricted). Throughout the experiment, birds were caught each morning, when their intestines were empty (approximately 0800 hr Greenwich Mean Time [GMT]), and weighed (to the nearest 0.1 g [Ohaus Scout SC6010]). In order to provide an additional measure of condition, birds' furcular fat levels were visually assessed using a scale modified from Gosler (1996; 2004, pp. 111-113, see section 2.2.2). Fat scores were established before they were weighed so that experimenter knowledge did not influence the assessment of fat in marginal cases. To establish the birds' free-feeding masses at the start of the experiment, they received 40 g of pheasant breeder pellets a day, which was more than any bird had previously consumed in a 24 hr period. Once their weights had stabilised, I calculated each individual's mean mass over five days as its free fed mass, and from this I calculated the corresponding 95% free-feeding masses which birds were maintained at during the restricted phases of the experiment. To reduce the birds' masses to their restricted masses during the experiment (see below), I reduced their daily food intake, initially giving them 14 g, and then slowly reducing the daily ration according to each individual's mass loss. It took birds between 6-17 days to reach their restricted masses.

Once a subject had reached its restricted mass, it was maintained at this mass for four days (Fig. 5.1), before again being given 40g of food per day in order to return it to its free-feeding mass and so on, until the end of the experiment (Fig. 5.1). I repeated this process again allowing the birds' masses to return to return to their free-fed masses and then reducing them to 95% of their free-fed masses for the final experimental phase.

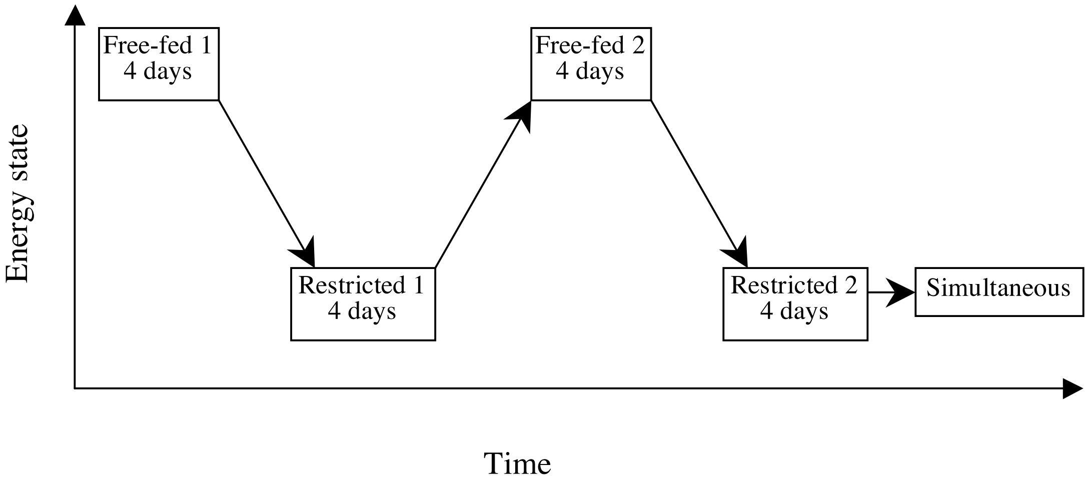

Figure 5.1. A schematic view of the experiment design. The experiment was split into four phases where their preferences for chemically defended prey were tested. Each phase consisted of four days where birds were fed 16 prey on each day (8 undefended and 8 chemically defended prey). I manipulated the energetic state of individuals so that in the first and third phases, birds had *ad libitum* food access; in the second and fourth phases, birds were kept on restricted diets. In the final phase, birds were given three simultaneous choice trials to test which cues they were using to inform their foraging decisions.

At the end of the experimental phase, I maintained the birds at their restricted masses and presented the birds with three days of simultaneous choice trials. The birds were then allowed to return to their free-fed masses which was accomplished through feeding birds 40g of food.

5.2.3 Prey

The prey were live mealworm larvae (*Tenebrio molitor*) which are a favoured food and so birds will persevere with training in order to obtain them. I used mealworms measuring approximately 20 mm in length and with an average mass of 0.114 g (SE ± 0.002 g, *N*=60). Therefore, a bird could hope to obtain about 1.8 g of mealworms if it consumed all 16 mealworms offered during the trial. The energetic composition of 1 g of mealworm is approximately 8.3 kJ (Hillstöm 1995). If it is assumed that starlings can extract about 0.85 of the total available energy from invertebrate food, which is about average for avian species (Harper *et al.* 2001), meaning that the net energetic gain obtained from 1 g of larvae is about 7.1 kJ. This means that birds would obtain about 12.8 kJ in energy if they chose to consume all 16 mealworms. Male starlings have been found to expend between 168 kJ to 272 kJ of energy daily (Feare 1984; Rickliefs & Williams 1984, Nagy *et al.* 1999). Hence, the mealworms would represent a small supplement while the birds had *ad libitum* access to food. However, their importance was greater when they were food restricted.

Birds were initially trained to eat single mealworms presented on a 38 mm diameter clear plastic Petri dish placed on the bottom of the cage. During the experimental trials, I used undefended and chemically defended mealworms. Before the start of each experimental trial, the undefended mealworms were injected with 0.02 ml water intra-orally, whereas the defended mealworms were injected with 0.02 ml of 2% quinine sulphate suspension intra-orally. To allow birds to distinguish between the two prey types, I placed coloured disks of paper measuring 42 mm in diameter under the Petri dishes to signal the level of chemical defence. I used three colour pairings (either pink and blue, orange and purple, or yellow and green) to signal the two prey types, and by reversing the colour-prey type association for half the birds, I ensured that each bird had a unique colour discrimination task.

5.2.4 Training

Birds were trained at their free-fed conditions, but were food deprived for two hours before the start of each daily trial. During training, each cage was moved to a new position in the room that was behind a white curtain that visually isolated the bird from both the experimenter and the other birds. The bottom of the curtain was level with the bottom of the cage so that the Petri dishes could be inserted and removed, via the cage door, without disturbing the bird. In order to observe the bird, I used a video camera connected to a television monitor that was placed where the focal subject could not see it.

As soon as the birds readily consumed the mealworms from the dishes, each bird was given a trial of 16 sequentially presented mealworms a day. At each presentation, a mealworm in a dish was placed in the middle of the front face of the cage next to the cage door. If a bird ate the mealworm, the dish was removed immediately, but if a bird failed to eat the mealworm, the dish containing the uneaten mealworm was removed after one minute. There was a three minute interval between each successive presentation. Once a bird had consumed five consecutive mealworms in a day, I introduced a disk of white cardboard underneath the dish. Once a bird had consumed five consecutive mealworms with white cardboard lids, I began the experiment.

5.2.5 Experimental procedure

Birds started the experiment subject to free-feeding conditions. After a two-hour deprivation period, they were each given a daily trial of 16 presentations as described above. Birds were given a series of eight undefended prey (signalled by a coloured disc of paper under the dish), and eight defended prey (signalled by a differently coloured paper disc). The sequence of prey was randomised within each block of four presentations so that there were two undefended and two defended prey every four presentations, ensuring that prey were equally distributed within the daily trial. As during training, birds had one minute to decide whether or not to eat a mealworm, and presentations were made every three minutes. I recorded if the mealworm was attacked and consumed and the latency to attack. The attack latency was the amount of time that elapsed from when I removed my hand from the cage until the bird's bill was seen to touch the prey item. Although the data was hand timed (and so subject to error), it was collected because it might indicate relative differences in times to attack, which could then be used to corroborate the preference data. I also recorded other behaviour such as bill wiping and head shaking to monitor any effects that the quinine had on the birds. All birds continued to eat the mealworms throughout the experiment.

I gave the birds daily trials of 16 presentations until they had acquired the discrimination task and ate more undefended mealworms than defended mealworms which took between 6 and 14 days to achieve. I defined acquisition as being when a bird's previous three days' choices significantly departed from random using a chi-squared test (chi-squared tests: χ^2 range =3.84-16.04, P all <0.05). Once a bird had learned to discriminate between the two prey types, I continued to collect data for four more days to provide the initial free-fed measure of discriminatory performance (Fig. 5.1). I then began to reduce the bird's mass for the restricted treatment. Once the birds had reached its restricted state, I collected data for another four days, before returning it to their free-fed state. I collected four more days of data with the bird at free-fed state before returning it for the final time to their restricted state and collecting four more days' data. This alternation of masses enabled me to rule out any order effects on an individual's decisions. I continued to collect data during the periods of mass change in order to look for correlations between mass and prey choice.

5.2.6 Simultaneous choice trials

While the birds were still in the restricted phase of the experiment, I gave them three days of simultaneous choice trials. Since defended prey consumption increased at lower masses, I needed to test whether this change resulted from a strategic decision by the birds to eat defended prey or was simply a loss of discriminatory ability. I also needed to establish whether birds had learned the colour signals, or were using other visual cues to differentiate between undefended and defended mealworms. The trials followed the same basic procedure outlined above, except that instead of a single mealworm being presented on each iteration of the daily trial, two prey types were presented simultaneously, and the birds were required to choose between them. Birds had a single trial of 16 presentations on each of three consecutive days. On the first day, birds were given a choice between the two prey types that they had experienced in the experimental trial (colour-quinine treatment). This treatment enabled me to test whether birds could discriminate between the defended and undefended prey at low body mass. On the second day, birds were given a choice between mealworms presented on their coloured backgrounds, but now all mealworms were injected with water (colour only treatment). This treatment allowed me to test whether birds could use the colour signals to distinguish between the defended and undefended prey. On the final day, I gave birds a choice between defended and undefended mealworms without the colour cues present (quinine only treatment). This allowed me to test whether birds could distinguish between undefended and defended mealworms in the absence of colour cues.

5.2.7 Statistical analysis

The latency to attack data was analysed using a linear mixed model (LMM), which is a powerful method of statistical analysis. LMMs allow data to be hierarchically arranged meaning that factors are nested within other factors. Moreover, it is a flexible method allowing the analysis of unbalanced experimental designs, (something not possible in a standard general linear model) and allows for repeated measures or inclusion of subjects as a variable in the analysis. In order to test the latencies I used the same set of variables for each of the analyses. I included the factors time within trial, day of phase, phase, and prey type as factors. Individual birds were included as a random subject variable. I compared contrasts within a factor using the least significance method (henceforth LSD method) which had been corrected for multiple comparisons using Bonferroni method (α/n). I used the Mixed procedure in SPSS to fit LMMs to my data.

Akaike's information criterion (AIC) was used to choose the best model when models had different sets of parameters. AIC was calculated for each model as:

$$-2(\text{log-likelihood}) + 2 \times p$$

where p is the number of parameters estimated in the model. AIC therefore represents a measure of the explanatory power of the model discounted by the number of parameters that have gone into its construction; a lower value indicates the 'better' model.

5.3 Results

5.3.1 The effects of food restriction on body mass of starlings

The manipulation of birds' body masses (Fig. 5.2a) and fat reserves (Fig. 5.2b) were successful. The average masses of the birds were significantly lower in the restricted phases than the free-fed phases of the experiment ($\bar{X}$ ± SE: free-fed=76.3 ± 0.96 g, restricted=71.4 ± 0.89 g; paired t-test: t=24.2, P<0.001, df=5; Fig. 5.2a). Birds also had lower furcular fat scores in the restricted phases compared to the free-fed phases (free-fed median=3, range (lowest-highest)=2-4; restricted median=1, range=1-1; Wilcoxon signed ranks test: Z=-2.23, N=6, P=0.026, Fig. 5.2b). I performed linear regressions on the daily masses and furcular fat scores for each bird and found that they were highly correlated (for all birds, all r^2>0.78, all P<0.001). These data indicate that the mass losses, during the restricted phases of the experiment, were due in large part to a reduction in body fat reserves.

5.3.2 The effects of body state on foraging decisions

There were no differences in the proportions of attacked prey that were then consumed with almost all prey that were attacked being eaten irrespective of the prey type ($\bar{X}$ ± SE: undefended=0.999 ± 0.001; mild=0.996 ± 0.006; paired t-test: t=0.7084, df=5, P=0.5103). Therefore, the rates of consumption can be taken as a good indicator of the rates of attack for the rest of this chapter. By the end of the training period (the final three daily trials), birds learned to discriminate between the undefended and defended mealworms ($\bar{X}$ ± SE: undefended=21 ± 0.68, defended=7.5 ± 1.34; all $\chi^2 > 3.84$, all $P<0.05$). During the experimental phases, birds continued to consume a high proportion of the undefended mealworms that were presented to them (see Fig. 5.2c). In both the free-fed and restricted phases of the experiment, birds consumed almost all of the undefended prey offered, eating slightly more of the undefended prey in the restricted phases than in the free-fed phases ($\bar{X}$ ± SE: free-fed= 60.8 ± 0.91 mealworms, restricted=64.0 ± 0.0 mealworms; paired t-test: $t=3.48$, $P=0.018$, df=5). This difference occurred due to slightly lower scores in the first free-fed phase, which may be indicative of the birds not having fully learned the task in this first stage of the experiment. However, the difference in the numbers of defended mealworms eaten between restricted and free-fed phases were far more striking (Fig. 5.2c). Birds ate significantly fewer defended mealworms in the free-fed phases than the restricted phases ($\bar{X}$ ± SE: free-fed=23.0 ± 4.94 mealworms, restricted mean=62.3 ± 0.84 mealworms; paired t-test: $t=9.36$, $P<0.001$, df=5).

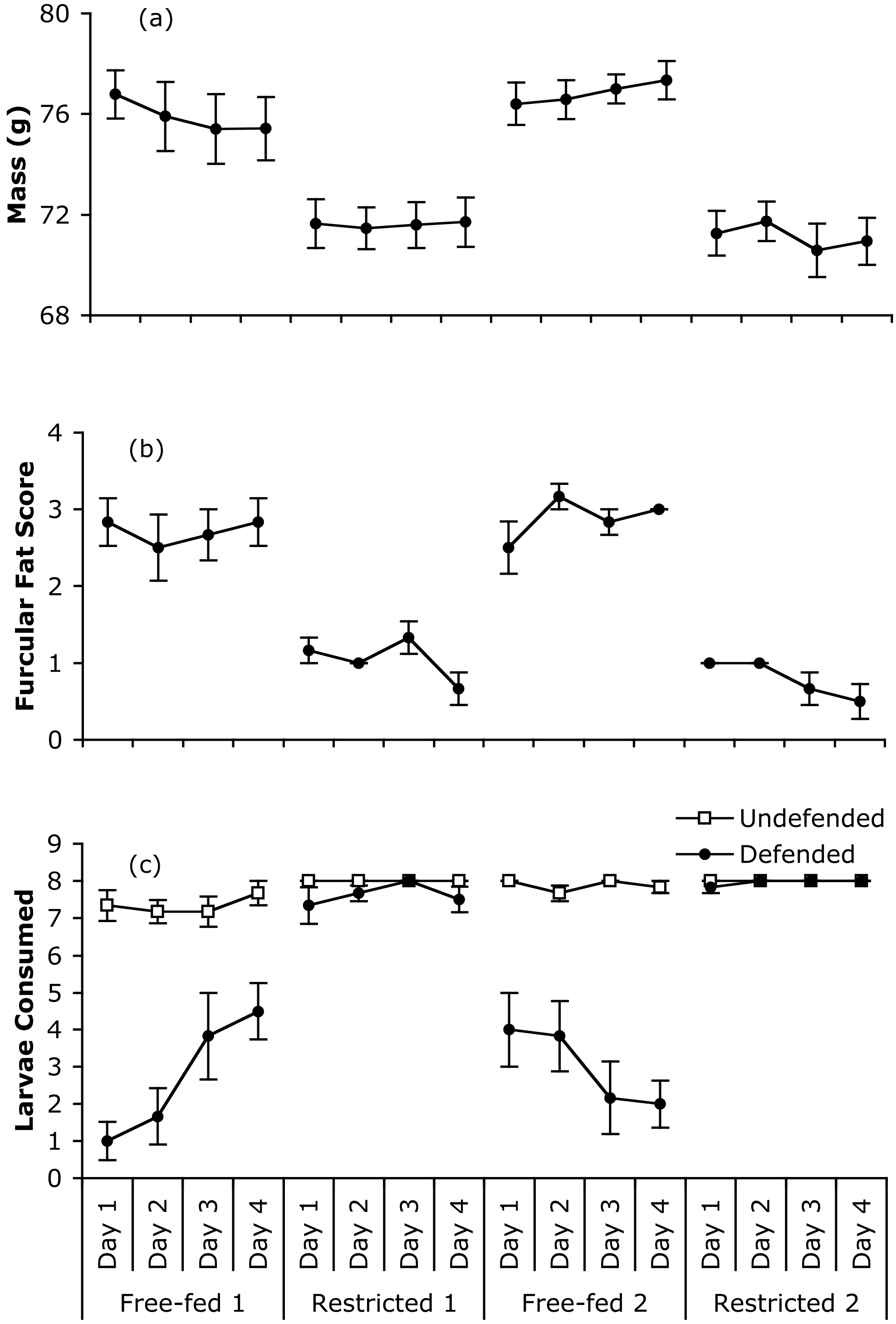

Figure 5.2. The daily means (±SE) of (a) mass, (b) furcular fat scores, and (c) numbers of defended and undefended prey eaten throughout the four phases of the experiment.

To control for relative changes in the numbers of undefended and defended prey consumed, I also compared the proportion of undefended prey eaten by dividing the number of chemically defended prey consumed by the number of undefended prey consumed. I arcsine square root transformed the resulting proportion to restore normality. I found that the proportion of defended prey consumed was significantly lower in the free-fed treatment than in the restricted treatment (paired t-test: *t*=20.5, *P*<0.001, df=5; Fig. 5.3).

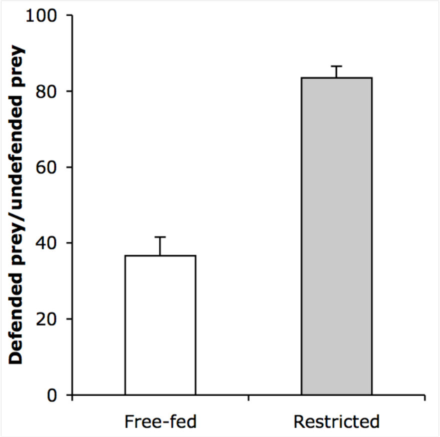

Figure 5.3. The mean proportion (+SE) of defended mealworms divided by the number of undefended mealworms eaten in each treatment.

During the free-fed phases of the experiment, the mean number of defended prey consumed by birds increased on successive days during free-fed phase 1 and decreased on successive days during free-fed phase 2 (Fig. 5.2c). To test whether these changes were related to small scale differences in daily masses, I plotted linear regressions to the number of defended prey consumed against their standardised mass for each bird during each free-fed phase and drew linear regression lines through the points. The standardised mass was the daily mass divided by the free-feeding mass calculated for each bird. In free-fed phase 1, four out of the six birds consumed fewer defended prey when they were heavier (signs test: *P*=0.34, Fig. 5.4a) whereas in free- fed phase 2, all six birds ate fewer defended prey when they were heavier (*P*=0.016, Fig. 5.4b). Therefore, the changes in the number of chemically defended larvae consumed in the free-fed phases could have resulted from daily changes in the birds' masses.

I plotted within-trial consumption of defended prey relative to the consumption of undefended prey (Fig. 5.5). By dividing the daily trial into four blocks, it allowed me to observe if there were any changes in the number of defended prey throughout the daily free-fed trials. I only used the free-fed trials as the relative consumptions of the defended and undefended prey types were approximately equal which would act to decrease the observable differences that were obvious during the free-fed trials. The number

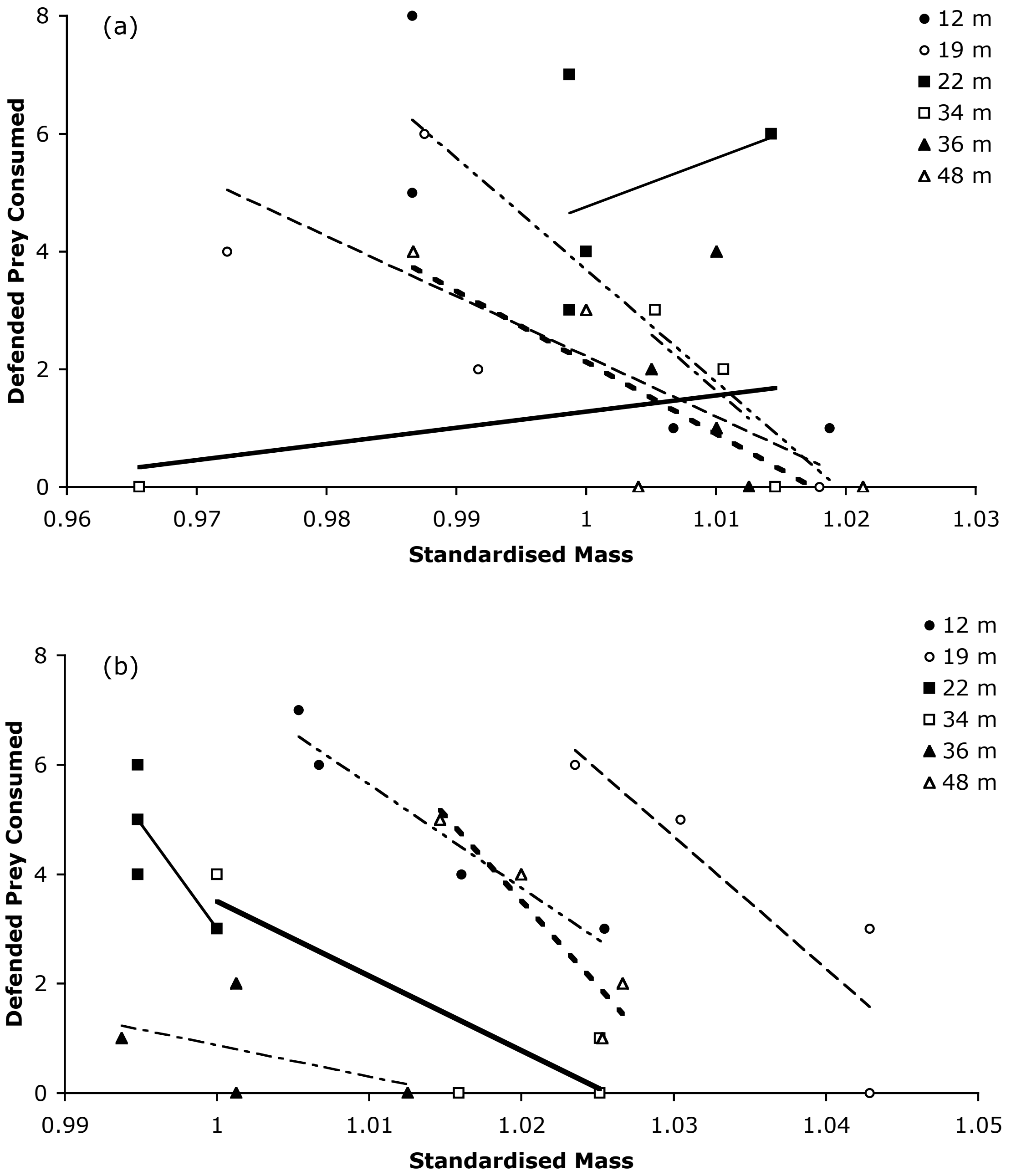

Figure 5.4. Regressions of the number of defended prey eaten on each day plotted against the standardised mass for each bird on that day during (a) the first free-fed phase, and (b) the second free-fed phase.

of undefended prey consumed throughout the trial did not differ between blocks (mean number of undefended prey consume in each quarter (±SE): first block=15.0 ± 0.52, second block=15.3 ± 0.33, third block=14.7 ± 0.42, fourth block=15.0 ± 0.44; repeated measures ANOVA: $F_{3,15}$=0.548, P=0.657, Fig. 5.5.). This meant, that I could use the arcsine square root transformed sum of the number of defended prey consumed divided by the number of undefended prey consumed. There was a significant decrease in the number of defended prey consumed relative to the number of undefended prey consumed as the trial progressed (Repeated measures ANOVA: $F_{3,15}$=21.888, P<0.001). Pair-wise comparisons revealed that the proportion of the first block was significantly greater than the proportions from the third block (LSD: P=0.006) and the fourth block (P=0.006).

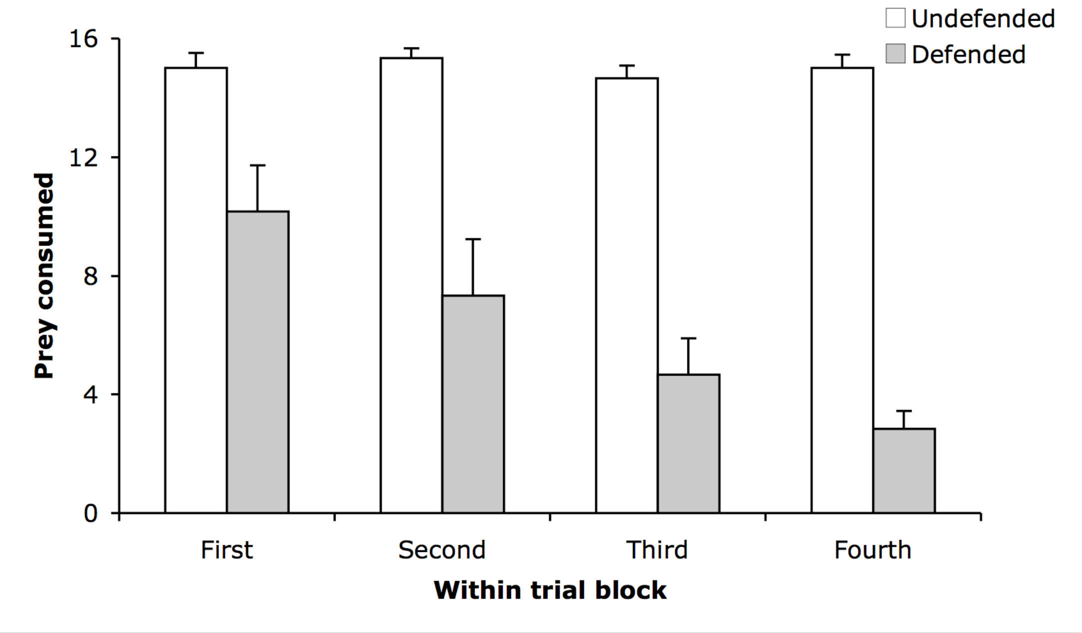

Figure 5.5. The mean (+SE) totals of each prey type consumed during daily trials during the free-fed phases of the experiment.

5.3.3 The differences in attack latency according to prey type and energy state

Throughout the experimental trials, I collected hand-timed latency data, which I analysed using two methods. These methods differed in how they treated occasions where birds did not attack chemically defended prey. First, I included non-attacks as a gap and performed statistics on this raw data (Fig. 5.6a). Using this method reflects the bird's behaviour and shows that although there is some overlap in the latencies between prey types, that the birds tended to be quicker to attack undefended prey. I constructed a LMM for the raw data (i.e., without 60 sec added for non-attacks) and including prey type, experimental phase, day within phase, and time within daily trial as fixed variables. Subjects were included at the highest level as a random subject variable and the latencies to attack were the dependent variables. I also included terms for all two-way interactions. The results indicated that there were significant differences between the two prey types (LMM: $F_{1,1247}$=29.993, P<0.001, Fig. 5.6a). Comparisons of the differences between means indicated that the latencies for the undefended prey were significantly lower than those for the defended prey (LSD: undefended-defended=-1.456, P<0.001). There were also significant differences in the latencies between the different experimental phases of the experiment ($F_{3,1247}$=62.909, P<0.001, Fig. 5.6a). Table 5.1 presents the results from the multiple comparisons of the contrasts that were computed using the LSD method. Generally, the results indicate that the latencies between restricted and free-fed phases are significantly different. However, there is also a significant difference in the latencies between the first and second food restricted phase of the experiment. Finally, there were no significant results for either day within phase ($F_{3,1247}$=1.503, P=0.212) or within trial prey order ($F_{7,1247}$=0.519, P=0.821). None of the two-way interactions included in the model were significant (all *P*-values greater than 0.1).

Table 5.1. Pair-wise comparisons for the differences in the estimated marginal means of the raw latencies to attack of the birds using the least significant difference (LSD) method, *P*-values were corrected using a Bonferroni adjustment (α/n).

Treatment (i)	Treatment (j)	Mean Difference (i-j)	Standard Error	df	P
Free-fed 1	Free-fed 2	0.706	0.411	1247	0.086
	Restricted 1	3.319*	0.387	1247	<0.001
	Restricted 2	4.554*	0.386	1247	<0.001
Free-fed 2	Restricted 1	2.612*	0.374	1247	<0.001
	Restricted 2	3.848*	0.373	1247	<0.001
Restricted 1	Restricted 2	1.235	0.335	1247	<0.001

For the second method, I attributed a value 60 seconds where prey were not attacked (Fig. 5.6b). I utilised this method as it has been used recently in a study to indicate the rate of learning (Darst *et al.* 2006). Another way of interpreting latency to attack would be to assume that the birds would eventually attack the prey, but enough time had not elapsed for the bird attack the prey. Hence, attributing 60 seconds could be interpreted as providing a conservative estimate of the actual latency to attack had the bird and the prey been left in the cage for longer (Fig 5.6b). This treatment of data yielded a different result. I constructed an identical LMM to the one above in order to analyse the data to which I had attributed 60 seconds to each non-attack.

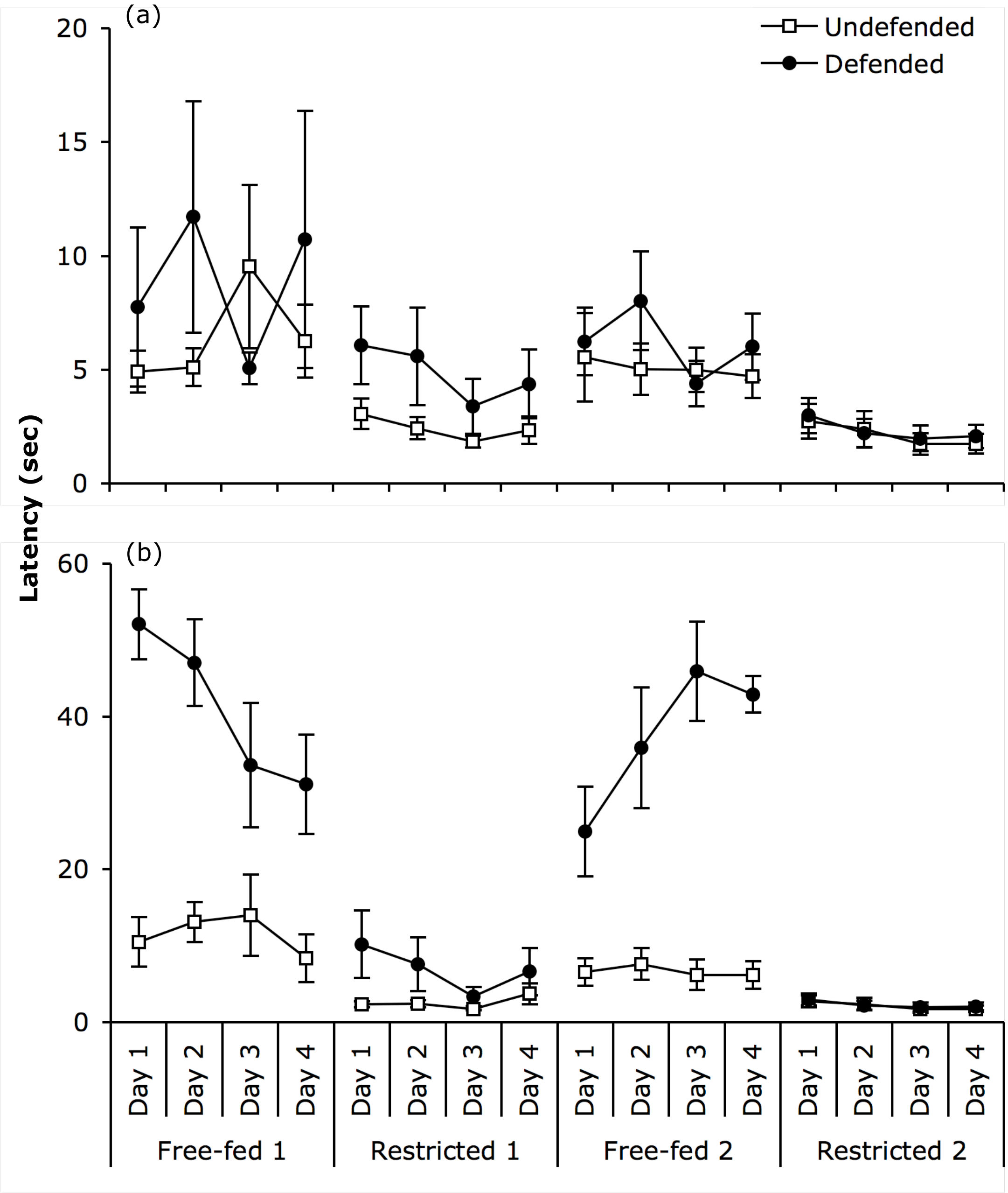

Figure 5.7. The daily means (±SE) of the latencies to attack in relation to day within treatment, treatment, and prey type when gaps when (a) non-attacks did not count and (b) when 60 seconds was used when prey not attacked.

Table 5.2. Pair-wise comparisons of the differences of the estimated marginal means of latency for the within trial order calculated using the LSD method (Bonferroni corrected).

Order (i)	Order (j)	Mean Difference (i-j)	Standard Error	df	P
Prey 1	Prey 2	-0.549	1.532	1280	1
	Prey 3	-1.577	1.532	1280	1
	Prey 4	-4.003	1.532	1280	0.257
	Prey 5	-6.929*	1.532	1280	<0.001
	Prey 6	-4.274	1.532	1280	0.15
	Prey 7	-6.298*	1.532	1280	0.001
	Prey 8	-7.392*	1.532	1280	<0.001
Prey 2	Prey 3	-1.027	1.532	1280	1
	Prey 4	-3.454	1.532	1280	0.688
	Prey 5	-6.379*	1.532	1280	0.001
	Prey 6	-3.724	1.532	1280	0.425
	Prey 7	-5.748*	1.532	1280	0.005
	Prey 8	-6.843*	1.532	1280	<0.001
Prey 3	Prey 4	-2.427	1.532	1280	1
	Prey 5	-5.352	1.532	1280	0.014
	Prey 6	-2.697	1.532	1280	1
	Prey 7	-4.721	1.532	1280	0.059
	Prey 8	-5.816	1.532	1280	0.004
Prey 4	Prey 5	-2.925	1.532	1280	1
	Prey 6	-0.271	1.532	1280	1
	Prey 7	-2.295	1.532	1280	1
	Prey 8	-3.389	1.532	1280	0.767
Prey 5	Prey 6	2.655	1.532	1280	1
	Prey 7	0.631	1.532	1280	1
	Prey 8	-0.464	1.532	1280	1
Prey 6	Prey 7	-2.024	1.532	1280	1
	Prey 8	-3.118	1.532	1280	1
Prey 7	Prey 8	-1.094	1.532	1280	1

Table 5.3. The effects of prey type, experimental treatment, within treatment day, and within trial prey order on the latencies of birds (α=0.05).

Source	df	F	P
Prey	1, 1280	446.035*	<0.001
Treatment	3, 1280	248.912*	<0.001
Day	3,1280	1.16	0.324
Order	7, 1280	7.194*	<0.001
Prey*Treatment	3, 1280	111.606*	<0.001
Prey*Day	3, 1280	0.304	0.822
Prey*Order	7, 1280	6.796*	<0.001
Treatment*Day	9, 1280	7.102*	<0.001
Treatment*Order	21, 1280	2.068*	0.003
Day*Order	21, 1280	0.294	0.999

Visually comparing the results between figure 5.6a and figure 5.6b it is clear that the two methods yield differing patterns of the effect of prey type and energetic state on the latencies to attack. It is likely that this practice would accentuate the differences between prey types because defended prey were left untouched more often than undefended prey. The results confirm this suspicion with the *F*-scores for the prey effect and the between phase effect being much greater (Table 5.3). Moreover, there was also a significant within-trial effect with the latencies decreasing as the trial continued (Table 5.2, Table 5.4). Table 5.4 lists the results of the LSD tests for differences between the mean latencies for each treatment. They show that the latencies between all four phases were significantly different from one another except for the two restricted phases (Table 5.2). Comparing the two methods, I believe that the first method which excludes the non-attacks gives a better indication of the actual latencies to attack despite the problem of unequal numbers of data points contributing to unbalanced data sets. This problem is overcome by using LMMs which are capable of handling unbalanced data sets.

Table 5.4. Pair-wise comparisons for the differences in the estimated marginal means of the latencies of the data using the least significant difference method, *P*-values were corrected using a Bonferroni adjustment (α/n).

Treatment (i)	Treatment (j)	Mean Difference (i-j)	Standard Error	df	P
Free-fed 1	Free-fed 2	4.199*	1.083	1280	0.001
	Restricted 1	21.499*	1.083	1280	<0.001
	Restricted 2	24.003*	1.083	1280	<0.001
Free-fed 2	Restricted 1	17.3*	1.083	1280	<0.001
	Restricted 2	19.804*	1.083	1280	<0.001
Restricted 1	Restricted 2	2.504	1.083	1280	0.126

I also conducted a regression analysis of the number of chemically defended prey consumed during the two free-fed phases of the experiment using the subjects's average latency as the independent variable. In order to calculate the daily mean latency, I calculated the mean of the median daily latency for each day of the free-fed phases. There was a significant relationship between the total number of defended prey consumed during free-fed trials and the latency to attack (r_s=-0.8857, *P*=0.05, r_s^2=0.6521, Fig. 5.7). This is an interesting finding as it indicates that a bird's tendency to consume chemically defended prey is related to the latency. If latency to attack prey was related to the levels of energetic reserves, it might be expected that the latency to attack would have been related to standardised mass. However, this was not the case and latencies were not correlated with the standardised mass (r_s=0.285, *P*=0.5836, r_s^2=0.081). So it is unknown if increases in latency allow the birds more time in which to make a correct attack decision, or if the differences are due to behavioural differences between individuals.

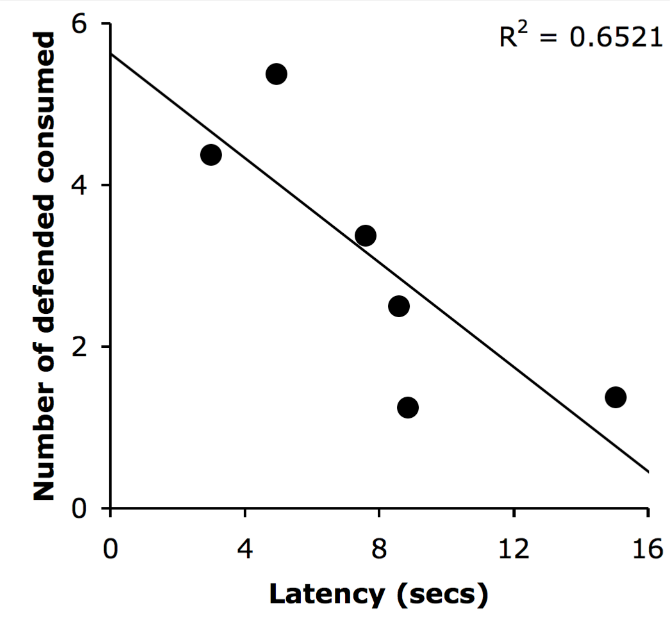

Figure 5.7. The mean number of chemically defended prey consumed on each of the daily trials during the free-fed phase of the experiment plotted against the mean of the daily median latencies.

5.3.4 Simultaneous choice trials

In order to confirm that colour was being used as the cue for decision-making, I conducted a series of three simultaneous choice trials. In the first trial, birds were given a choice between the two prey types that they had previously experienced in the experimental phase (colour-quinine trial). Birds were able to discriminate between the two prey types when they were presented simultaneously. Birds consumed significantly greater numbers of the undefended prey than the defended prey during these trials (paired *t*-test: t=7.79, P=0.001, df=5, Fig. 5.8). In the second trial, all mealworms were injected with water and presented with colour cues (colour only trial). Birds still preferred to attack prey with the undefended colour, confirming that they could use colour cues in the absence of any potential differences between quinine-injected and water-injected mealworms (t=3.83, P=0.012, df=5; Fig. 5.8). In the third trial, birds were given a choice between quinine-injected and water-injected mealworms in the absence of colour cues (the quinine only trial). However, birds were unable to discriminate between defended and undefended prey (t=0.19, P=0.86, df=5; Fig. 5.8). These results show that despite the birds having low levels of food availability and consequently low energetic states, they were able to select food according to its level of quinine contents by using the associated colour cues. Moreover, there were no other visual or olfactory cues that birds were able to use from the mealworms.

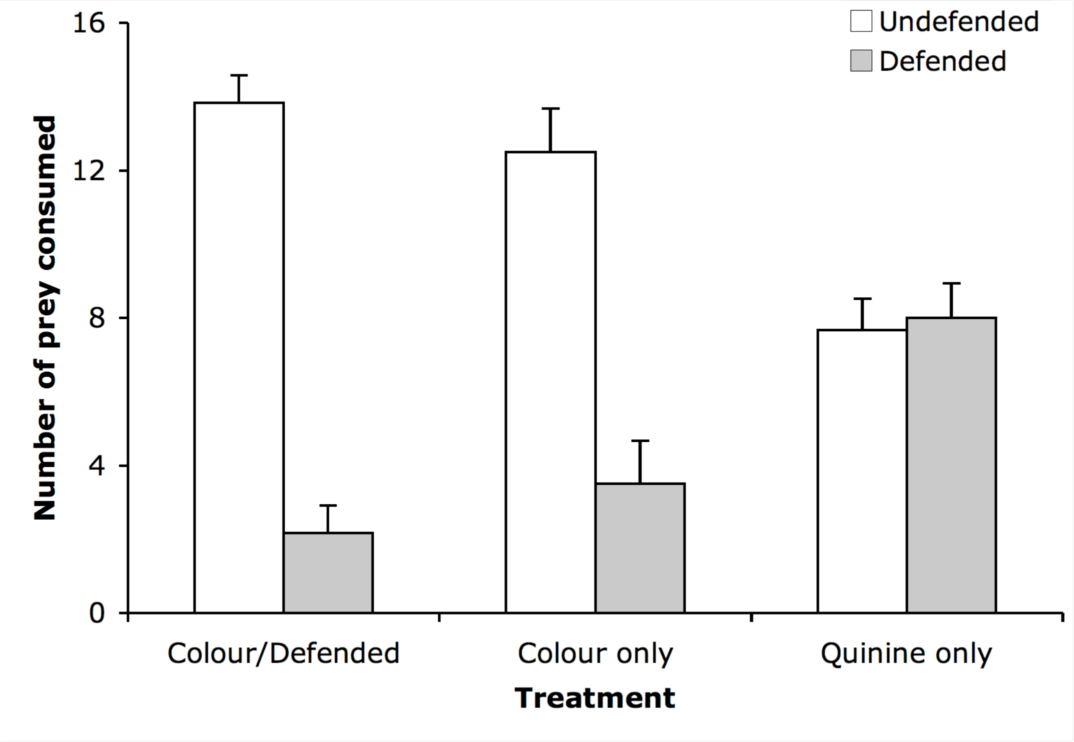

Figure 5.8. The daily mean number (+SE) of defended and undefended prey consumed during the three simultaneous choice trials.

5.4 Discussion

The results of this experiment demonstrate that energy reserves affect the proportion of chemically defended prey that birds consume. When food was restricted and birds' fat reserves and masses were reduced, they ate more of the defended prey compared with when they were free-fed. Small daily fluctuations in individual masses during the two free-fed phases also explained some of the variation in the numbers of defended prey attacked on each day. These results are consistent with previous observations and studies on a variety of predators (Swynnerton 1915; Sexton *et al.* 1966; Gelparin 1968; Chai 1986; Hileman *et al.* 1995; Gillette *et al.* 2000), and support the assumptions of recent SDP models of the state-based consumption of chemically defended prey (Sherratt 2003; Kokko *et al.* 2003; Sherratt *et al.* 2004).

My data also suggest that the birds may be able to strategically manage their intakes of quinine because the risks associated with over-consumption of toxins may be severe. The data indicate that as the daily trials progressed, the birds' consumption of defended prey declined. The SDP models of the state-based consumption of chemically defended prey indicate that animals ought to have a maximum body burden of toxins that they can ingest before they become prohibitively expensive (e.g., causing emesis or death) (Sherratt 2003; Sherratt *et al.* 2004). However, this assumption is supported by little empirical evidence in predatory vertebrates. The best evidence for vertebrate animals foraging to strategically manage body levels of toxins comes from herbivores foraging on plants containing secondary plant metabolites (PSMs). It has been found that herbivores do not forage at random and that they will consume plants containing PSMs, as long as they do exceed their metabolic capabilities to detoxify the toxins (the detoxification limitation hypothesis [Freeland & Janzen 1974; Marsh 2006]).

The results of this experiment can also be considered in the context of optimal diet theory. The problem I addressed could be thought of as being similar to animals increasing their diet breadth in response to energetic stress. For example, shore crabs (*Carcinus maenas*) will consume less profitable prey once they have exploited the favoured high profitability prey (Elner & Hughes 1978). Bluegill sunfish (*Lepomis macrochirus*) also become less discriminating in the size of prey they consume when prey becomes less abundant (Werner & Hall 1974). Many authors have also found that changes in the frequency of undefended prey changes the rates of consumption of chemically defended prey (e.g., Nonacs 1985). These results are due to a decrease in the capture rate of favoured prey and so that predators needed to increase their diet breadth to maintain their energetic reserves. However, state was not considered as a factor in these experiments. Some experiments have also included hunger as a factor that influences prey choice. For example, three-spined sticklebacks (*Gasterosteus aculeatus*) consume more of an unprofitable prey type when they are hungrier (Beukema 1968) as do toads (*Bufo fowleri*, Heatwole & Heatwole [1968]), carabid beetles (*Notiophilus bigattatus*, Ernsting [1977]), 15-spined sticklebacks (*Spinachia spinachia*, Kislaliogu & Gibson [1976]), and copepods (*Mesocyclops edax*, Williamson [1980]). Increases in the perceived risk of predation can also change the optimal food choices of animals so that they decrease their direct risk of predation (Sih 1993; Ydenberg 1998, Pp. 363-368; Caro 2005, Pp. 107-108). Therefore, the results from this experiment support previous foraging research into optimal diet theory.

The starlings also had significant differences in their attack latencies between the free-fed and food-restricted treatments. These differences in attack latency may have indicated the differences in the birds' energetic states and thus their increased motivation to attack when at lower states. Data from previous studies show that animals' latencies to attack prey are related to energetic state with latencies becoming shorter with increasing time from when they were last fed (Godin & Sproul 1988; Godin & Crossman 1994; Horat & Semlitcsh 1994; Moore 1994; Whitham & Mathis 2000). However, as is often the case with earlier studies of this kind, no direct measurement of predator energetic state was made. Hence, the data collected in this study show the distinct decrease in latencies associated decreases in energy reserves and perhaps, provides another behavioural measure of the birds' energetic state. An alternative explanation is that increases in attack times can reduce the probability of making recognition errors (Guilford 1985). However, the results from the simultaneous presentations would tend to argue against that interpretation since birds were able to reliably differentiate between prey types when they were energetically stressed.

I analysed the latency data in two ways: (1) I used the raw data that had missing values where birds did not attack the prey and (2) I attributed a value of 60 seconds to when a prey was not attacked. I would tend to favour the first method of analysis, but a recent paper by Darst *et al.* (2006) used the second method of attributing the maximum trial time (120 secs) on occasions where subjects did not attack the prey. When I treated my latency data in a similar manner, I found that this method accentuated the differences that existed between the different prey types. As the number of defended prey consumed declines, the latency to attack increases meaning that the latencies to attack in Darst *et al.* (2006) are not true latencies, but an index of prey consumption and latency combined. Therefore, it is disingenuous to suggest these data reflect the actual subjects latencies to attack because the subjects may have never attacked these prey for good reason. This reason is that ingestion of Dendrobatid frogs comes with significant risk of death meaning that the birds should have never attacked these frogs. Indeed, the one subject that did consume a poison frog (*Epipedobates bilinuis*) died three days after ingestion.

One explanation for birds eating more defended prey in the restricted phases was that it resulted from a reduction in their motivation to discriminate or an impairment in their cognitive ability to do so. However, the simultaneous choice trials at the end of the experiment when birds' food was still restricted confirmed that birds were able to discriminate between the defended and undefended prey based on their colour signals. Therefore, they had retained the association between the colour cues and the defence level of each prey type, and could use it to avoid defended mealworms when prey were presented simultaneously. Therefore, birds can have information about the chemical content of prey, but trade off that information against the nutritional benefits of consuming the prey. When birds have low fat reserves, the nutritional benefits of consuming the defended prey increased relative to the costs of ingesting the chemicals, which remained constant across the treatments.

The costs to predators of ingesting defence chemicals can occur through increased handling, malaise, or additional costs of processing toxins (e.g. Möstler 1935; Fink & Brower 1981; Gilardi *et al.* 1999). This experiment cannot quantify the cost of quinine to starlings, but given that it is potentially toxic to birds at very high doses (Alcock 1970), it seems likely that the chemical was having some post-ingestive effects. It is possible that birds are able to taste test the mealworms and evidence from later chapters indicates that birds can learn to taste the quinine in less than 10 seconds. This taste perception is likely to be a gustatory or trigeminal nervous response (Werner & Clark 2003). However, it is unknown how this information is integrated with nutritional information to inform decision-making, which is a complex problem in a varied diet (Yearsley *et al.* 2006). This experiment shows that birds can make these associations in sequentially presented prey, which is an implicit key assumption in many theoretical models of aposematism and mimicry (e.g. Speed 1993; Turner & Speed 1999; Sherratt 2003; Kokko *et al.* 2004; Sherratt *et al.* 2004).

One potential criticism of this study is that the manipulations of energetic state in starlings were not realistic, and may not lead to selection on defended prey in the wild. However, the mass manipulation of birds was biologically realistic as starlings' masses fluctuate by up to 15 grams throughout the year (Feare 1984). Although some of this mass change may be due to other morphological changes rather than simply just changes in fat deposits (Feare 1984), body mass has been shown to be a reliable indicator of fat levels in birds and a good indicator of an individual's energetic state (Blem 1990; Witter *et al.* 1995). Energetic state can be used as a short-term surrogate of reproductive success due to the difficulties involved in determining lifetime reproductive success in free-living iterparous species. Therefore, a manipulation where birds lost about five percent of their mass is ecologically realistic. This study is the first that has directly investigated the state based consumption of chemically defended prey within the context of SDP models. It is important to study how energy reserves affect behaviour by measuring both concurrently in the same individual (Mangel & Clark 1986, Hutchinson & McNamara 2000). This integrated approach, effectively allows researchers to partial out inter-individual variation and intra-individual variation that can be due to changes over time. This has previously been overlooked by those studies of how energy interacts with behaviour (e.g., Ekman & Hake 1990; Witter 1995; Thomas 1999).

When birds had free access to food, the number of defended prey that they consumed had a negative correlation with latency to attack. As the latency to attack increased, the birds decreased the number of chemically defended prey consumed. However, this was not related to the standardised masses of individuals. Therefore, this could mean that some birds could have higher tolerances to quinine or that some birds were more cautious than others were. Both hypotheses are possible as I did not obtain any physiological data from birds. If there are differences in personality, this could be an important possibility promoting the evolution of aposematism and mimicry and may help explain some of the variation in results from studies in dietary conservatism (Thomas *et al.* 2003, 2004; Marples *et al.* 2005).

The data in this experiment demonstrate that educated predators can change their rates of attack on chemically defended prey based on their energy reserves. This is consistent with other recent studies that have demonstrated that birds can form partial preferences for chemically defended prey (e.g., Skelhorn & Rowe 2005; Skelhorn & Rowe 2006b). The exact level of avoidance may depend on whether the birds can taste the defences and potentially the level of the chemical defence of prey (Skelhorn & Rowe 2006b), but also as this study shows, it is subject to change according to a predator's energy levels. This has implications for the evolution of warning patterns and chemical defences in both aposematic and mimicry systems.

First, warning signals may not just be designed to be easily associated with chemical defences (e.g. Gittleman & Harvey 1980; Guilford 1992), but may need to be effective for recognition by educated predators. This idea is not new; for example, it has previously suggested that warning signals could be designed to facilitate recognition or cautious sampling by experienced predators (Guilford 1985; Guilford 1994), or be more memorable (Speed 2000). However, the results of this experiment emphasise this by demonstrating that birds develop partial preferences for defended prey, meaning that there could be strong selection from educated predators. In addition, since partial preferences may change with changes in energetic state, selection on warning signals may vary across the year according to food availability or the birds' energetic needs. For example, migrating species may eat more defended prey in their pre-migration period, meaning chemical defences might be less effective at such times. Changes in the perceived trade-off between nutritional benefits and defensive costs may also explain seasonal changes in prey behaviour. For example, defended seven-spot ladybirds (*Coccinella septempunctata*) are solitary in the warm summer months but are often found in aggregations in the winter months (Majerus & Kearns 1989). An increase in attacks from avian predators when food is scarce and more valuable in the winter, could lead to individuals enhancing their survival chances from dilution or saturation effects of being in an aggregation (Lindström *et al.* 2001; Turner & Speed 2001).

Second, these findings indicate that the models of aposematism based on predator learning may be flawed (Rothschild *et al.* 1984; Guilford & Dawkins 1993; Speed 1993a; Speed & Turner 1999; Servedio 2000. This is because these models tend to be based on theories of associative learning (Rescorla & Wagner 1974; Pierce & Bouton 2001). Theories of associative learning predict that the continued experience of a negative stimulus (e.g., an electric shock or an objectionable taste) will lead to a cessation in attacks. Therefore, partial preferences for aposematic prey are inconsistent with these models.

Finally, many models of Müllerian mimicry are also built on the similar assumption of associative learning (e.g., Turner *et al.* 1984). This means that in cases of Müllerian mimicry it is expected that the rate of attack of Müllerian co-mimic will decline to negligible levels with increasing predator knowledge in a similar manner to aposematism. However, if predators can display consistent partial preferences for moderately defended prey over long time-periods, then this questions the basis of the mimicry models. Whether prey can be moderately distasteful and attacked at intermediate rates is important for recent debate concerned with the evolution of mimicry (Speed 2001; Joron & Mallet 1998). If the data reflect how birds deal with prey under natural foraging conditions, moderately defended prey may be commonplace, allowing for the evolution of quasi-Batesian mimicry, where a moderately defended co-mimic is parasitic on a more defended co-mimic (Speed 1993a). Moreover, given that prey acceptability changes according to the energy reserves of the birds, I also suggest caution interpreting partial preferences unless they can be married with subjects' energetic state.

Overall, the data from this chapter suggest a re-evaluation of our current approach to studying aposematism and mimicry in many ways. First, we need to consider more fully the role of educated predators in the evolutionary dynamics of aposematism. Second, it is perhaps time to develop a new approach to the study of aposematism in the wider context of optimal diet theory, and the potential benefits and trade-offs faced by foraging animals (Stephen & Krebs 1986; Yearsley *et al.* 2006). Finally, it will be interesting to further investigate how predators perceive defence chemicals, and whether they are able to make foraging decisions according to what they have learned about a prey's nutritional content and defence chemistry. The methods that predators use to assess the nutritional and toxic properties of prey are likely to be complex, but this study provides the first insight into the decision-making strategies that predators might employ in this process.

5.4.1 Conclusions

This study has for the first-time, established the relationship between a predator's energetic state and its propensity to consume chemically defended prey. These results are important because this is a previously untested assumption underpins the recent SDP models' reliance upon energetic state as a basis for decision-making. The results are also important because they demonstrate the role that educated predators might have in the evolution and maintenance of chemical defences in prey species. However, I believe that the importance of energetic state in the role in the consumption needs further investigation. Moreover, other aspects of the SDP models warrant further investigation (e.g., is there a critical burden of toxins that birds will stop consuming chemically defended prey). Food resources are important in determining carrying capacities of animals. However, the effects of food limitation are often difficult to determine especially in populations where demographic variables may take years to respond to changes in resource availability.

Chapter 6

THE RELATIONSHIP BETWEEN ENERGETIC STATE AND FORAGING PREFERENCES FOR THREE PREY TYPES WITH VARYING LEVELS OF CHEMICAL DEFENCES

6.1 Introduction

Laboratory studies of aposematism tend to be conducted on simple model systems, in particular having only a single undefended and defended species (e.g., Sexton *et al.* 1966; Williamson 1980; Gelparin 1968; Hileman *et al.* 1995; Gillette *et al.* 2000). These systems have provided valuable insights into the dynamics of the consumption of chemically defended prey. However, these models may be too simplistic with regards to the choices an animal may face in a natural environment. Free-living animals rarely have the choice between two food items that differ in a single attribute, and are likely to be faced with many different types of prey in the field. However, little is known about how animals choose among prey choices that differ in reference to their levels of chemical defences, nor how changing energetic state affects these choices.

The speed with which a forager learns the association between a food item and its noxious properties is thought to be dependent upon three factors: (1) the strength of the chemical defence, (2) the latency from the time of ingestion of the prey until perception of the prey’s associated defences, and (3) the number of food types ingested per unit of time (Leimar *et al.* 1986; Yearsley 2006). Leimar *et al.* (1986) suggested that the rate of learning could be related to the levels of defensive secondary chemicals contained in a prey item. Alcock (1970a) fed black-capped chickadees (*Poecile atricapillus*) three food types that differed in their energetic contents, taste, and inclusion of an emetic. There were three groups of birds which were presented with identical seed shells that covered nothing, half a salted mealworm, or half a mealworm with added quinine (an emetic). The birds pecked at the shells covering the quinine treated mealworms significantly less often and learned to avoid the shells when the mealworms on offer were quinine treated. This indicates that birds may learn the avoid more potently defended prey faster than if they were less well defended. However, Alcock's (1970a) study did not address how rates of pecking may have equated with actual prey consumption. Recently, Skelhorn and Rowe (2006a) demonstrated that naïve domestic chickens (*Gallus gallus*) learned to avoid food with more potent defences more quickly than weakly defended food.

Studying food preferences is complex when using multiple food types in free-living animals although this has not stopped some biologists from trying. For example, Chai (1986) fed two rufous-tailed jacamars (*Galbula ruficauda*) over 1,000 butterflies of 114 morphs. He found that birds were able to discriminate between defended and undefended butterflies. However, this study was poorly controlled and the results are hard to interpret since it was difficult to distinguish what cues the birds were using to distinguish between prey. Moreover, the birds were kept in different sized cages and the author even wrote that at times he hit cages with a stick when butterflies settled in order to get them to fly. This could have startled birds and stopped them feeding. Bowers and Farley (1990) fed seven different species of butterflies to four wild caught grey jays (*Perisoreus canadensis*) and found that birds learned the differences between undefended and defended species. However, the individuals were fed between three and six larvae of the seven species and the results were pooled making interpretation difficult. The number of presentations Bower and Farley (1990) used may not be enough to ascertain the natural rates of attack. Moreover, often studies of these types did not measure the levels of defensive compounds in the prey offered and so it was unknown if the level of prey toxicity was the main factor influencing the predators' foraging choices.

Recently, it has been found that European starlings will form partial preferences for moderately defended prey that persist after the birds have been fed large numbers of defended and undefended prey over long time periods (e.g., Skelhorn & Rowe 2006b, Chapter 5). These studies have found that birds may attack and consume chemically defended prey about twenty percent of the time at free feeding mass. Finally, it was unknown if the rates of learning and the rates of consumption corresponded with the actual levels of defence. Therefore, it is unknown if the birds were basing their attack decisions on the levels of toxin each prey consumed.

In this study, I was interested in whether or not birds were able to learn to differentiate between different prey with varying levels of chemical defences. I also manipulated the energy state of individuals in order to ascertain the pattern of consumption of chemically defended prey as birds lost energy reserves and regained them again. However, unlike the previous chapter, the consumption of chemically defended prey was assayed daily so I could examine the trend of state-based consumption of chemically defended prey more precisely. This is a more ecologically realistic way to investigate the how birds' preferences change with energetic state in since predators rarely lose 5 percent of their body mass suddenly (except during times of high energy use and low food intake such as during migration). Finally, this study is one of the few experiments where the protective properties of different levels of defence have been examined in distinct and identifiable prey. Moreover, it is the first time a study has attempted, in an integrated manner, to investigate how birds' foraging preferences for different defended prey may change in response changes in energetic state.

6.1.2 Experiment introduction

Recent models have formalised the defensive costs and economic benefits of consuming chemically defended prey (Kokko *et al.* 2003; Sherratt 2003; Sherratt *et al.* 2004). From these models, I made two predictions: (1) birds should consume prey according to their chemical defences, and (2) birds should include less well defended prey into their diets as they lose mass before they include more highly defended prey, and that this trend should be reversed as birds gained mass.

6.2 Methods

6.2.1 Study species and housing

The subjects were six wild-caught male European starlings (*Sturnis vulgaris*) caught under licence from English Nature (Licence Nos. 19991381 and 20001512). The birds were taken from their free-flight aviary and housed individually in wire mesh cages measuring 450 x 750 x 450 mm (h x w x d) with a metal drawer at the bottom, which could be removed for cleaning. The birds were subjected to a 14:10 hour light/dark cycle. The light was full spectrum light that simulates natural daylight. During the experiment, birds were fed pheasant breeder pellets and water *ad libitum*. When food was changed the remaining food was weighed and the amount of food consumed per 24 hr was calculated. At the end of the experiment, birds' masses and fat scores were monitored until they had stabilised and then they were put back into free-flight for future experiments.

6.2.2 Energetic state

The objective of this experiment was to examine the order of preference that birds display when offered three prey types with different levels of defence as they lost and gained mass throughout the experiment (Fig. 6.1). Birds were caught each morning (around 0900 hr British Summer Time [BST]) and their furcular fat levels were visually assessed using scale modified from Gosler (1996, 2004, pp. 111-113) before being weighed (to the nearest 0.1 g [Ohaus Scout SC6010]). This was done so my knowledge of the mass did not influence the assessment of fat in marginal cases. The birds were fed after weighing (between 0915-0930 hr (BST) either 40 g of food during training or a restricted amount of food which allowed the birds to gradually lose and gain fat throughout the experiment at about 0.5 g per day (mass change: $\overline{X}$ ± SE: losing mass=-0.48 ± 0.128 g, gaining mass=0.30 ± 0.037 g).

In order to make mass comparisons within the same individual, I first calculated an average mass of the individual birds whilst under experimental conditions. I calculated the mean mass of the birds on the last five days of training phase of the experiment which was then used to calculate a standardised mass that could be compared among individuals. The masses did not need to be standardised using tarsus or wing length as each bird was acting as its own standard for making comparisons.

My method of mass manipulation allowed birds to gain and lose mass in accordance to small changes in their levels of food availability. Figure 6.1 indicates the different phases in the experiment: a mass loss phase and a mass gain phase where birds had the amount of food restricted to allow them to slowly lose or gain mass. On the first day of the mass loss phase of the experiment, I fed birds their average amount of food that they consumed in each 24-hour period during the five days that I collected mass data, which was used to calculate the birds' free-feeding masses. I calculated the birds' average 24 hour food consumption by weighing how much food was left in the birds' cage (in the bowl and on the floor) to which I added a sixth of the mass of the food that was swept off the floor. For the rest of the mass loss phase of the experiment I calculated food as stated in the left hand column of Table 6.1. When I switched birds to the mass gain phase of the experiment, I switched to the right column to calculate the birds' food allocation. For example, during the mass loss phase, if a bird lost 1.2 g of mass in a 24-hour period in which it was fed 15 grams of food, the bird was fed 15.5 g of food [i.e., (15 – 0.5) – (–1.2)=15.7 g, which is rounded down to 15.5g]).

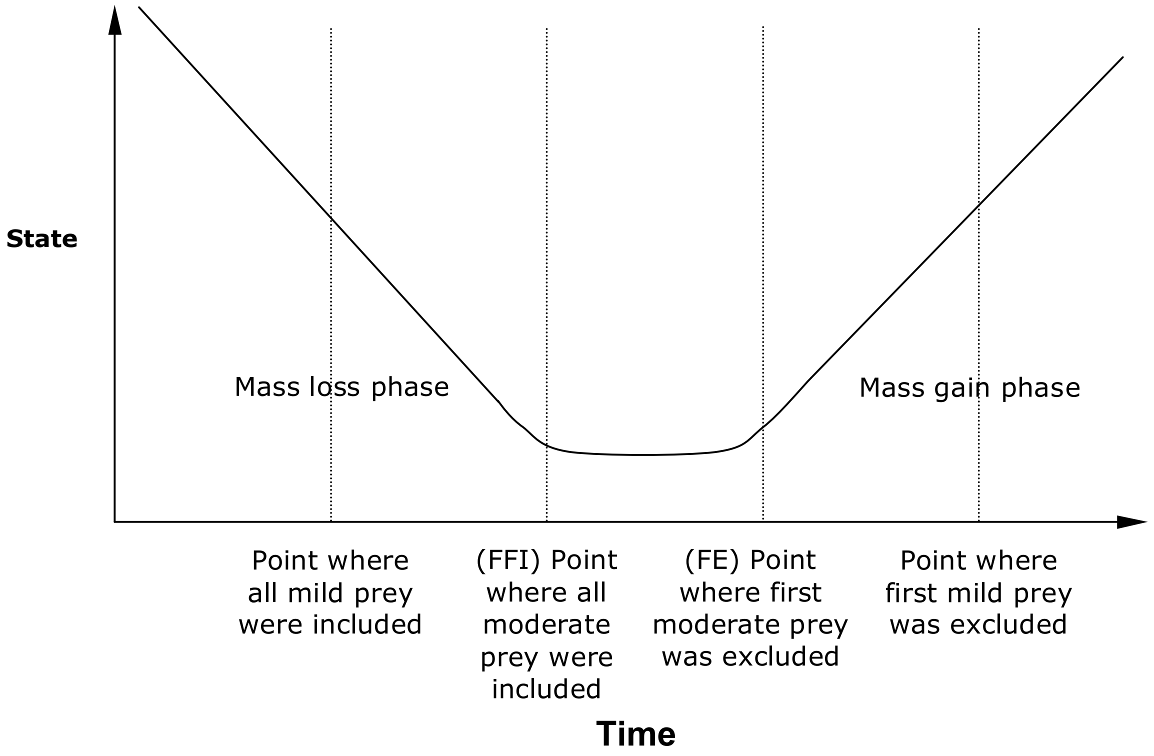

Figure 6.1. A schematic diagram of the experimental period. The experiment is split into two phases: a mass loss phase (left hand side) and a mass gain phase (right-hand side). The middle period of the figure where there is no change in state. This is a period of adjustment where birds' food amounts were adjusted in order to allow them to begin gaining mass again. FFI=first full inclusion of prey, FE=first exclusion of any prey item.

Once the mass manipulation stage of the experiment began, I continued to allow the birds to slowly lose mass until either (i) they were including all prey presented or (ii) their mass declined to 90 percent of their free-feeding mass. Once the birds reached this stage, I fed the bird its previous 24 hrs food ration plus the weight of its previous 24 hrs mass loss plus another gram of food. I then used the right column (mass gain column) of Table 6.1 to calculate the birds' food allocation on subsequent days so that birds begin gaining mass at about 0.5g of mass a day.

Table 6.1. The reactive adjustment method used calculating birds 24-hour food ration during the mass manipulation phase of the experiment. Changes in mass are also substituted with changes in food at a 1:1 ratio, but rounded to the nearest 0.5 gram of food.

	Experimental Period	
24 hr Mass Trend	Losing Mass	Gaining Mass
Start	Average bird's food intake	Previous days food + 0.5 g
All other days	(Previous day's food – 0.5 g) – mass change	(Previous day's food + 0.5 g) – mass change

6.2.3 Prey-preparation and training

The objective of the experiment was to examine the responses of birds to three prey types. Prey were *Tenebrio* sp. larvae (henceforth, mealworms) of approximately 20 mm in length (see section 5.2.3 for details). The three prey types were undefended larvae, mildly defended larvae, and, moderately defended larvae (see Table 6.2 for details of amounts and concentration of defences). The birds were trained to associate a different colour with each prey type and colours were balanced among individuals to spread the effects of possible colour biases throughout all prey types. The mealworms were injected with quinine or water in the same manner as in Chapter 5.

Table 6.2. The treatments of the three prey types used in this experiment.

Prey Type	Treatment
Undefended	0.2 mL water
Mildly Defended	0.2 mL 1 % quinine sulphate suspension
Moderately Defended	0.2 mL 3 % quinine sulphate suspension

On each day of training, birds were food deprived for two hours before the start of a training trial. Five minutes before the trial started, birds were moved in their cages to a new position within the room and left to habituate. They were positioned behind a curtain so that the other birds in the room could not see the subject and *vice versa*. The bottom of the curtain was level with the bottom of the cage so it could be removed without disturbing the bird. In order to observe the bird during each trial I used a video camera connected to a television monitor.

Mealworms were presented in small (38 mm diameter) Petri dishes, which were placed on the floor of the bird’s cage. I used my hand to place the Petri dish in the cage using the door cut into the from of the cage. The trial started once I had taken my hand from the cage. The birds had 1 min in which they could attack and consume the prey. If a bird consumed the mealworm, the dish was removed immediately. If the bird failed to consume the prey after one minute, the Petri dish was removed. This three minute cycle was repeated 18 times on each day. Once birds consumed five mealworms in succession, I introduced a disk of white cardboard placed under the dish to act as a background. Again, each bird was required to consume five larvae in succession before proceeding to the next step in training. It generally took the birds between one and two weeks before they would approach and consume prey without hesitation.

The next step in training was to introduce coloured paper disks underneath the Petri dishes to signal the different prey types. However, before this could be done, birds had to be habituated to the different colours. This was done by introducing the coloured paper disks under Petri dishes with undefended prey. I did this six times for each prey type and the order of colours was randomised in order to prevent possible learning effects forming in relation to the order of colour encounter. Once the birds consumed all 18 larvae I began using mealworms injected with quinine or water in a session so the birds could learn the colour associations between colour and defended prey. At the start of the first trial, I presented five undefended prey with their colour underneath so that birds would not cease attacking mealworms as might be the case if they consumed defended larvae during initial presentations. During the training trials, the birds were presented with 18 prey of the different prey types (six of each type). They were presented in a random order, but with the rule that two of each prey type had to be in each third of the presentation sequence. This was to ensure that each part of the daily trial had relatively equal probabilities of encountering each prey type. This randomisation rule was also used in the experimental phase of the experiment. The training sessions continued until the birds had displayed significant and consistent preferences for the three prey types during the proceeding three days (see section 6.3.1 for details). It took the birds an average of 21.0 ± 1.81 days ($\bar{X}$ ± SE) to learn the difference between the three prey types.

6.2.4 Experimental trials: mass manipulation and prey choice

The mass manipulation phase of the experiment had two periods: a mass loss and a mass gain period (Fig. 6.1). During the mass loss period, the birds' levels of consumption of different prey types were noted until all of the moderately defended prey were being consumed. The next two days after all the prey offered were consumed for the first time, I stabilised the birds' mass and observed if the bird continued to consume all the prey offered. If the bird consumed all prey on each of the three days, I called the first of the three days; the point of first full inclusion (FFI) because it was the first time the bird included all the prey in its diet (Fig. 6.1). Once birds reached FFI, their daily food ration switched to the mass gain period of the experiment (Table 6.2). Again, I aimed to allow birds to consistently gain mass at about 0.5 g per day (see section 6.2.2). As the birds continued to gain mass, the first day of any three consecutive days where any prey item was not consumed was called the point of first exclusion (FE) (Fig. 6.1). The FE was the considered the first day of the mass gain phase of the experiment. I used only these data because I was interested only in the pattern and consistency of the data as birds lost and gained mass and if their behavioural trends would be similar in both phases of the experiment. The period between FFI and FE was not considered in the foraging prey consumption analyses because including this data it would add unnecessary noise to the result. In order get the birds' consumption of all prey starting at full consumption in the mass gain period; I also included the two days prior to FE. I continued to monitor the birds' daily consumption of prey and state until they were either at their free-feeding mass (FFM [i.e., their mass at the beginning of the experimental phase]) or they were no longer consuming any defended larvae.

On each day of the mass manipulation phase, 18 larvae were presented to the bird as a sequence, six of each prey type (see Table 6.2 for the treatments of each prey type). In order to prevent the sequence of prey biasing behaviour for any one type of larvae, I ensured that two of each prey type was present in each third of the daily trial sequence. This meant that there were hundreds of different combinations of prey sequences available to be used for each individual of which only a small sample was.

All individuals had the same schedule on each day as they had during training (section 6.2.3) meaning that they were all weighed and fed between 0900-0930 hrs (BST). It was important to standardise the deprivation period in order to ensure that their energy levels were responsible for state-based effects. Therefore, all birds had access to food for at least 2 hr prior to the beginning of the food deprivation period. Only two birds could be run simultaneously so I staggered the times at which food was taken from their cages and thus the start times for each pair. Therefore, food was removed at 1100 hr from the first pair of birds, 1215 hr from the second pair, and 1330 hr from the last pair. Once the food was removed from birds' cages, the trial began 2 hr later. Food was put back into the cage at the end of the trial.

The presentation of prey was on a three minute cycle, which began with the placing of a new prey item at the bottom of the cage. I recorded if the bird attacked and consumed the prey, the latency to attack (time from the start of the presentation until a bird's bill touched the prey item), as well as other behaviours such as the rejection of prey, bill wiping (feeking), and head shaking. There were no occasions where trials had to be halted because of concerns for the bird's welfare.

6.2.5 Simultaneous choice trials

In order to test the relative preferences for each prey type, I conducted nine days of simultaneous choice trials at the end of the experimental phase. It was important to conduct these tests to ascertain if: (1) birds were basing their decisions on whether to consume prey on the colour cues provided and (2) that no cues other than colour were being used to make decisions. The trials followed the same basic procedure outlined above, except that instead of a single mealworm being presented, two prey types were presented simultaneously, and the birds were required to choose between them. Birds had a single session of 18 presentations on each of nine consecutive days. At each presentation, birds were allowed to attack one mealworm and the second mealworm was removed once the first was attacked. Over the nine days of the simultaneous choice trials, birds were given a choice between one of three prey dyads (undefended versus mildly defended, undefended versus moderately defended, or mildly defended versus moderately defended). On each day, the prey could be presented either, having both the colour cue present and the quinine injected (colour-quinine treatment), the colour cue present but no quinine (colour-only treatment), or the quinine injected but no associated colour cue (quinine-only treatment). Each treatment was presented on three of the nine days. The aim of the colour-quinine treatment was to get a baseline measure of preference, and was important to prevent birds learning that mealworms presented with colour were undefended during the colour-only treatment. The colour-only treatment allowed me to test whether or not birds were using the colours as the primary cue for decision-making purposes. Finally, the quinine only treatment allowed me to establish that there were no unintended visual or olfactory cues associated with the quinine. This is because quinine sulphate might have a visual cue associated with it that humans are unable to perceive (e.g., reflecting U.V. light). The order of the daily treatments was randomised to prevent the birds refusing to eat prey when they had no colour cues which might have occurred if the birds were given three consecutive days of the quinine-only treatment.

6.2.6 Statistical analysis

To test that birds had learned the discrimination by the end of the training trial, I used a Chi-squared test. For data where I was examining the effect for different prey types (e.g., the number of prey consumed in each prey trial), I utilised repeated-measures GLM models. I compared differences between fixed factors using the least significant difference (LSD) method to calculate the levels of significance between the estimated marginal means that had been corrected for multiple comparisons (Bonferroni method, α/n). These methods allowed for the comparisons of main fixed effects in the model.

I used regression analysis to test for relationships between morning mass readings and the visual fat scores of the birds taken before weighing. An analysis was completed for each individual to confirm this relationship.

In order to analyse the preferences of birds in relation to mass changes, I calculated standardised masses for each bird by dividing the daily mass by the free-feeding mass that I calculated at the start of the experiment. I then calculated the daily proportions of each prey type that individual birds consumed and entered these into bins based on 0.01 graduations of standardised mass. The mass bins ranged from 1.05 to 0.9 and it was these values I used to construct separate linear mixed models (LMM) for the mass loss and mass gain phases of the experimental trial. I used Akaike Information Criterion (AIC) in order to choose the best model. The lower the AIC score, the better the model is a general rule of thumb. For analyses on proportionate data, I used arcsine square-rooted to restore normality and homoscedasticity and allowed me to performed parametric tests. I used LMMs extensively in this chapter because I believe that they have a number of advantages over general linear models (GLMs). For example, the data that I gained from the mass manipulation phase of the experiment was only able to be analysed using LMMs, because there were many gaps in the data. Indeed, not one subject had standardised masses that encompassed the entire range of the readings which would precluded the use of GLMs. I used a variance components covariance matrix in these models. In cases, where all factors are fixed, LMMs can be considered analogous to GLMs. I compared differences between means using the least significant difference (LSD) method that had been corrected for multiple comparisons (Bonferroni method). All tests were two-tailed and had critical values of 0.05. I used the Mixed command in SPSS for PCs (versions 12.0, 14.0, and 15.0) and Mackintosh computers (version 11.1) to calculate all LMM statistics.

6.3 Results

6.3.1 Prey preference

By the end of the training period (the final three daily trials), birds learned to discriminate between the undefended and defended mealworms ($\overline{X}$ ± SE: undefended=15.2 ± 0.65, mild=7.8 ± 1.05, moderate=3 ± 0.26; all $\chi^2>7.462$, all $P<0.05$, df=2). In order to see if birds maintained this learned preference throughout the mass manipulation phase of the experiment I conducted two crude analyses on the data that I generated. First, I compared the number of each prey type consumed on each day of the mass manipulation phase. Second, I compared the number of days that the birds included all of a particular prey type throughout the mass manipulation.

For the first analysis, I expected the birds to maintain the preference they learned during training throughout the mass manipulation phase. Therefore, I calculated the mean number of each prey type consumed per daily trial throughout the entire mass manipulation phase as a conservative test of whether birds maintained their learned preferences. The mean number of prey consumed on each day throughout the experiment corresponded with the order of preference displayed in the last three days of training ($\bar{X}$ ± SE: undefended=5.81 ± 0.16, mildly defended=5.16 ± 0.18, moderately defended=4.33 ± 0.21; repeated-measures ANOVA: $F_{2,10}$=158.5, P<0.001, η^2=0.982, Fig. 5.2a). Comparisons between contrasts revealed that the daily consumption of all three prey types were significantly different from one another (LSD: undefended – mildly defended, P=0.001, undefended – moderately defended, P<0.001, mildly defended – moderately defended, P=0.001).

For the second analysis, I calculated the number of days where each bird consumed all of a particular prey type. If birds included mildly defended prey more readily than moderately defended prey, it is expected that the number of days where all moderately defended prey were consumed would be fewer than the number of days where all mildly defended prey were consumed. When birds consumed all of a particular prey type, I called this a day of full prey inclusion (or full inclusion for short) of that particular prey type. The analysis yielded a very significant result indicating that despite this conservative statistical method, the order of preference was highly robust ($\bar{X}$ ± SE: undefended=26.7 ± 2.32 days, mildly defended=18.2 ± 1.82 days, moderately defended=12 ± 1.46 days; $F_{2,10}$=75.152, P<0.001, η^2=0.938, Fig. 6.2b). *Post-hoc* comparisons between variables indicated that the birds' preferences for the three prey

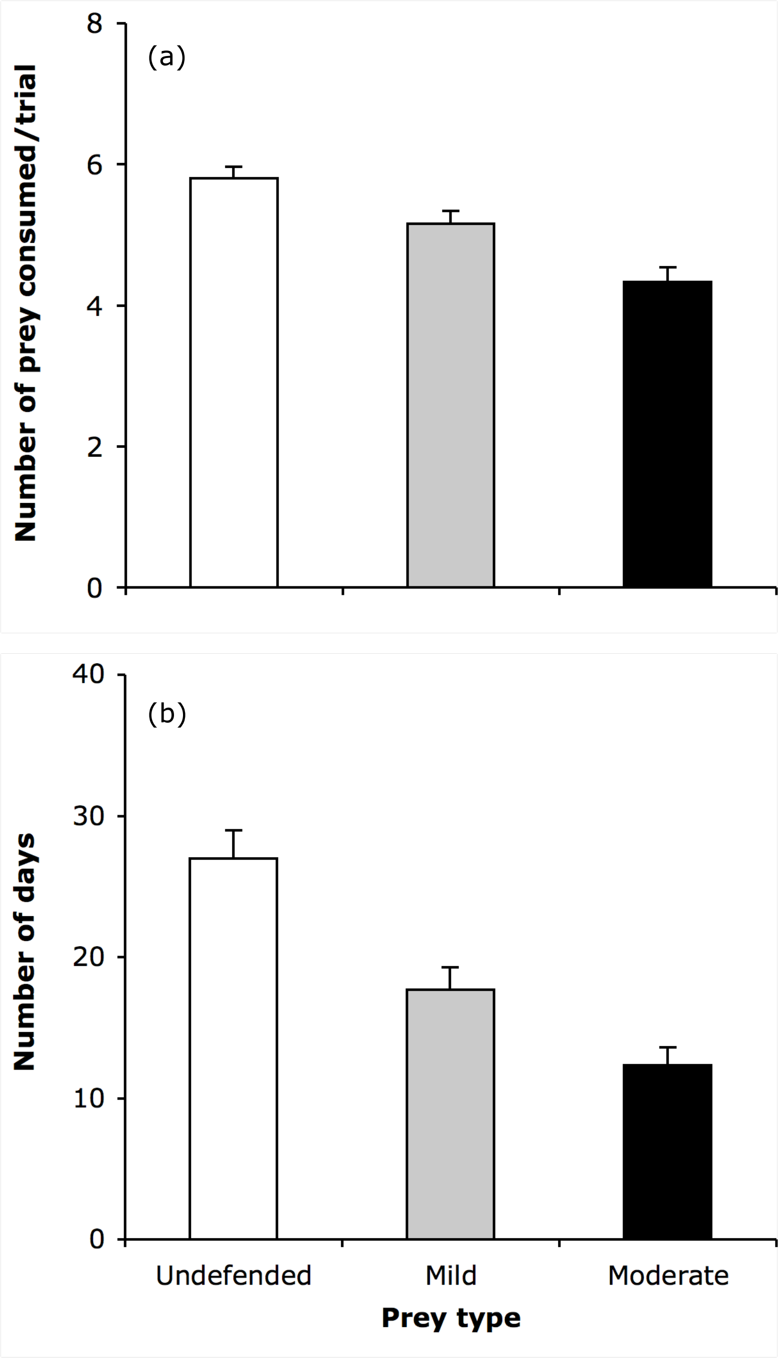

Figure 6.2. The measures of preference for each prey type measured by: (a) the mean number (+ SE) of each prey type consumed daily throughout the experiment and (b) the mean number of days (+ SE) when every prey of a given prey type was consumed. These results indicate that the prey were favoured in accordance with their toxin loads and hence, their profitability.

types were significantly different from one another (LSD: undefended – mildly defended, *P*=0.001, undefended – moderately defended, *P*=0.001, mildly defended – moderately defended, *P*=0.006). Taken together, these data confirm that birds were able to discriminate between the three prey types and that the order of learned preference corresponded with the level of chemical defences of the prey.

6.3.2 Experimental phase: mass manipulation and prey choice

In order to examine the relationship between body mass and furcular fat scores, I conducted regression analyses on each individuals daily masses and fat scores. There was a significant relationship between body mass and the furcular fat scores of birds. I performed a regression on the masses and furcular fat scores of individual birds during the experimental phase (linear regression: 19M, $F_{1,31}$=7.82, P=0.009, r^2=0.201; 20M, $F_{1,28}$=13.128, P=0.001, r^2=0.319; 21M; $F_{1,31}$=8.357, P=0.007, r^2=0.212; 39M, $F_{1,20}$=25.588, P<0.001, r^2=0.561; 48M, $F_{1,27}$=22.410, P<0.001, r^2=0.454). These data indicate that the mass changes during the restricted phases of the experiment, were due in large part to a changes in body fat reserves. Therefore, I use mass as a surrogate for energetic state for the rest of the chapter because the mass has been shown to be significantly related to fat reserves.

In order to test if attacked prey were then always consumed, I calculated the proportion of attacked prey that was subsequently consumed. I found that there were no differences in the proportions of attacked prey that were then consumed with almost all prey that were attacked being eaten irrespective of the prey type ($\bar{X}$ ± SE: undefended=1 ± 0; mild=1 ± 0; moderate=0.999 ± 0.001, repeated-measures ANOVA: $F_{2,10}$=1, P=0.402). Therefore, the rates of consumption can be taken as a good indicator of the rates of attack for the rest of this chapter.

The main aim of this chapter was to test the preferences of birds in relation to the levels of chemical defences of the prey and how the birds' preferences responded to changes in their energetic state. In order to test this, I constructed a LMM which included prey type and mass as fixed factors and an interaction term between mass and prey type for both the mass loss and mass gain phases of the experiment. Subjects were included in the analysis as a random subject variable. I defined the mass loss phase of the experiment as being all of the days where birds were losing mass until they consumed all prey offered to them in three consecutive days. If the bird consumed all of the prey during a daily session, I attempted to maintain the bird's mass at the level it was on the morning of the day when it consumed all prey offered.

In the mass loss phase of the experiment, birds increased the proportion of prey consumed with increasing mass loss (LMM: $F_{12,72}$=5.531, P<0.001, Fig. 6.3a). There were many significant differences in the contrasts when the masses were compared against other masses (Table 6.3). There were also significant differences in the numbers of each prey type consumed throughout the mass loss phase of the experiment ($F_{2,72}$=32.082, *P*<0.001, Fig. 6.3a). Pair-wise comparisons of contrasts for the proportion of each prey type consumed revealed significant differences between all three prey types (LSD: undefended – mild=15.557, *P*<0.001; undefended – moderate=30.221, *P*<0.001; mild – moderate=14.664, *P*=0.001). I was also interested to see if between-individual variation was a significant factor in the proportion of prey consumed. I found that variance between individuals accounted for only 17.8 % of the total variance of about 234 which was not statistically significant (Wald *Z* test: *Z*=1.145, *P*=0.252). The other 82.2 % of variation was accounted for by within-individual variation and error.

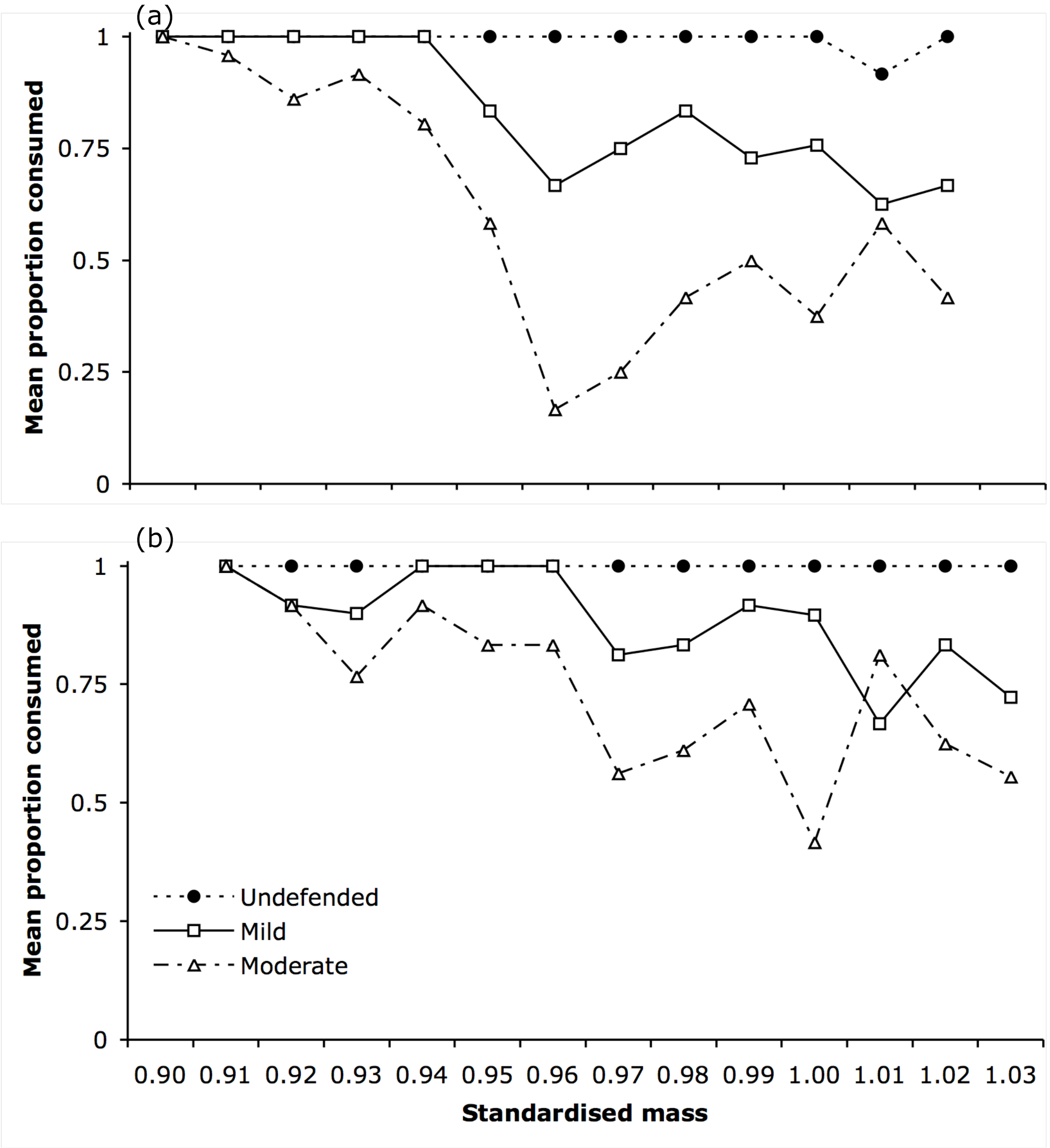

Figure 6.3. The mean proportion of larvae consumed by birds as they (a) lost mass and (b) gained mass. Standardised mass is the birds' mass as a proportion of their free-feeding mass that was calculated at the beginning of the experiment.

Once the birds had consumed all of the prey items offered for three consecutive days, I began incrementally feeding the bird more food in order to allow them to begin gaining mass. I defined the start of the mass gain phase of the experiment as first of the three consecutive days where birds did not consume at least one prey offered. Throughout the mass gain period, birds to slowly gained mass until they had reached their free-feeding masses. As with the mass loss data, I constructed a LMM, which included the factors mass and prey type, with individual birds nested in the analysis as a random subject variable. The results were similar to those from the mass loss phase of the experiment (compare Fig. 6.3a and Fig. 6.3b). The proportion of prey consumed was dependent on the mass of the birds as they gained mass (LMM: $F_{12,36}$=2.858, P=0.007, Fig. 6.3b). Unlike the mass loss phase of the experiment, none of the differences between the means for masses were significantly different from one another. This is most likely due to the alpha level of the test statistic being corrected using the Bonferroni method (i.e., α/n). There were also significant differences in the numbers of each prey type consumed throughout the mass gain phase of the experiment ($F_{2,36}$=34.631, P<0.001, Fig. 6.3b). Pair wise comparisons of the means for the proportion of each prey type consumed revealed significant differences between all three prey types in the mass gain phase of the experiment (undefended – mild=15.731, P<0.001; undefended – moderate=25.478, P<0.001; mild – moderate=9.747, P=0.010). Individual variation was not a significant factor in relation to the fixed variables. I found that variance between

Table 6.3. Significant pair-wise differences in the proportion of prey eaten at different standardised masses calculated using the LSD method as birds lost mass. All other pair-wise differences are non-significant.

Order (i)	Order (j)	Mean Difference (i-j)	Standard Error	df	P
0.90	0.97	36.0	9.12	72	0.014
	0.99	39.5	9.39	72	0.006
	1.00	37.8	9.64	73	0.015
0.91	0.97	30.9	6.80	71	0.002
	0.98	28.0	7.08	73	0.014
	0.99	34.4	7.33	73	0.001
	1.00	32.7	7.33	73	0.002
	1.01	32.5	8.64	74	0.027
	1.02	38.3	10.65	74	0.046
0.93	0.97	25.2	5.84	72	0.004
	0.98	22.2	6.11	72	0.040
	0.99	28.7	6.51	73	0.003
	1.00	27.0	6.51	73	0.007
0.94	0.97	27.1	6.45	71	0.006
	0.98	24.2	6.73	72	0.046
	0.99	30.7	6.68	71	0.002
	1.00	29.0	7.02	73	0.008

individuals accounted for only 4.6 % of the total variance of about 64.5 which was not statistically significant (Wald Z test: $Z=0.342$, $P=0.732$). The other 95.4 % of the variation was accounted for by within individual variation and error.

When the birds began gaining mass, I noticed some resistance for the birds to begin excluding the moderately defended prey. I had expected that the birds would start to exclude defended prey from the diet at about the same mass as when they had fully included them when they were losing mass. Therefore, I decided to compare the masses of birds FFI and FE for both the moderately and mildly defended prey in the mass loss phase and in the mass gain phase of the experiment. I found that the mass at FFI for moderately defended prey was significantly higher compared with the mass at FE ($\bar{X} \pm$ SE: standardised mass at FFI=0.949 ± 0.014, standardised mass at FE=0.977 ± 0.019, paired t-test: $t=-3.366$, $P=0.02$, df=5, Fig. 6.4a). However, this was not the case for the masses when birds fully included and began to exclude the mildly defended prey ($\bar{X} \pm$ SE: standardised mass at FFI=0.965 ± 0.012; standardised mass at FE=0.973 ± 0.013; paired t-test: $t=-2.319$, $P=0.068$, df=5 Fig. 6.4b). I decided to repeat this using the amount of food that they were receiving in order to see if the birds were also obtaining more energy at FE compared with FFI for the moderately defended prey. This pattern was confirmed when I compared the amounts of food being consumed in the preceding 24 hours at FFI and FE of moderately defended prey ($\bar{X} \pm$ SE: food eaten at FFI=13.8 ± 0.69 g, food eaten at FE=16.5 ± 0.66 g, paired t-test: $t=-7.291$, $P=0.001$, df=5, Fig. 6.4c). Again, for the mildly defended prey, there was no significant difference in the amount of food consumed on the first day of FFI compared with the amount eaten on the day of the FE ($\bar{X} \pm$ SE food eaten at FFI=14.1 ± 0.62 g; food eaten at FE=14.3 ± 0.64 g, paired t-test: $t=-0.672$, $P=0.530$, df=5, Fig. 6.4d). While the birds had their lowest levels of bodily energy reserves, the birds invested much less in singing behaviour, which is also likely to generally correlate with other non-maintenance behaviours (personal observation). It is possible that a prolonged period of reduced food availability will have meant that the birds were less reluctant to give up the nutritional advantages that these moderately defended prey offered to them. The other possibility is that birds built up a physiological tolerance to quinine. However, this would not explain why this trend was only evident in the moderately defended prey and not the mildly defended prey.

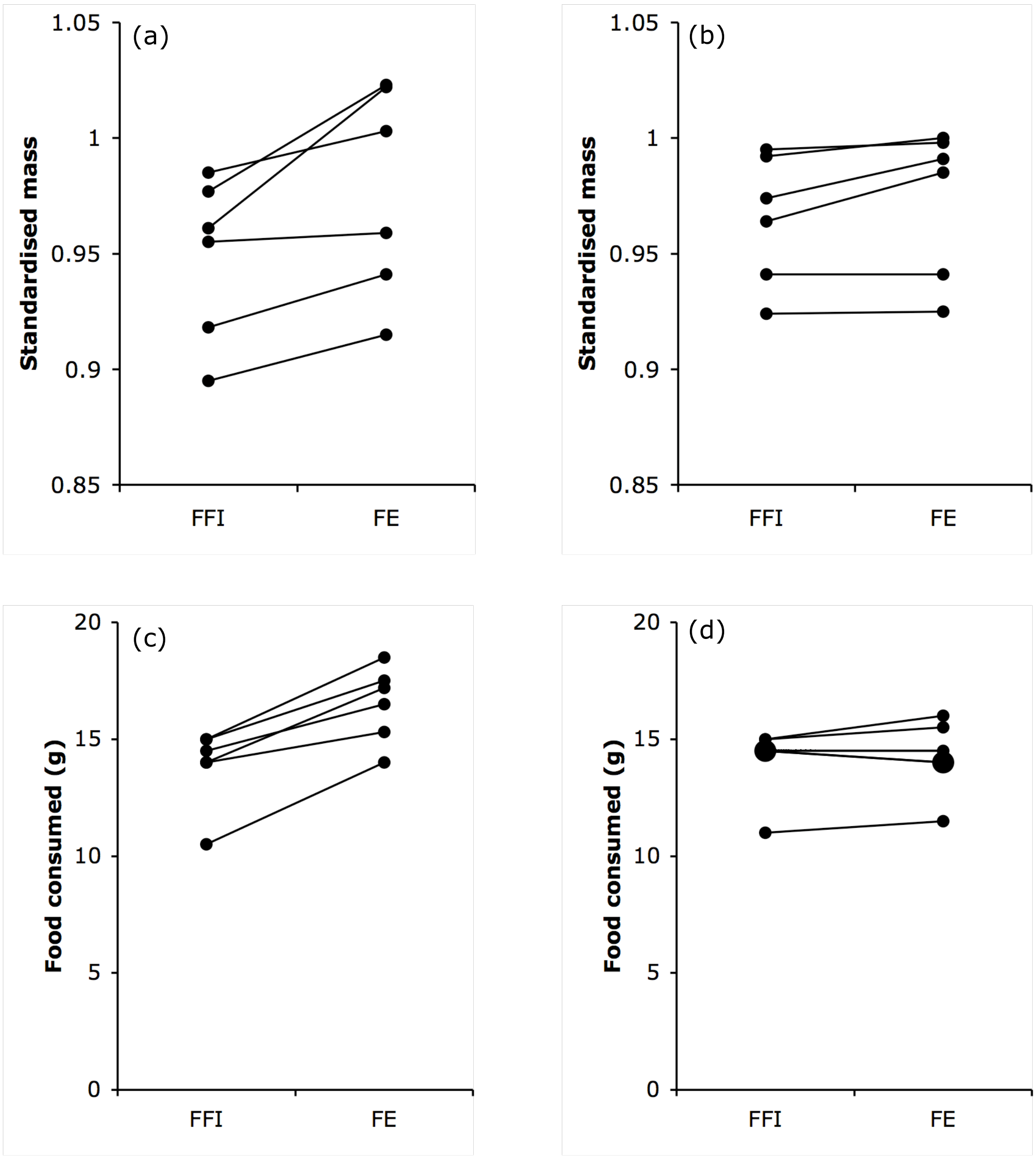

Figure 6.4. A comparison of the mean mass of individual birds when they either first fully included (FFI) each prey type as they lost mass and first excluded (FE) a prey when they were gaining mass for moderately defended (a) or weakly defended (b) prey. A similar pattern was evident in the amounts of food being consumed when the birds were considering either moderately defended prey (c) or weakly defended prey (d).

6.3.3 Within trial effects

I noticed that birds seemed to be consuming fewer defended prey as each daily trial progressed which might suggest that birds were strategically managing their levels of toxins ingested. In order to ascertain if there was a within-trial decrease in the number of defended prey consumed, I compared the numbers of each prey consumed in the first half of the daily trial with those consumed in the second half of the trial. Included data from throughout the entire mass manipulation phase of the experiment making it a conservative as it includes data from days where all prey were consumed. I found, as in the previous chapter, there was a significant decline of both defended prey types in the second half of the trials when compared with the numbers consumed in the first half of the experiment ($\bar{X}$ ± SE [mild], first half=0.943 ± 0.014, second half=0.823 ± 0.023, *t*=15.321, *P*<0.001, df=5; $\bar{X}$ ± SE [moderate], first half=0.843 ± 0.027, second half=0.623 ± 0.42, *t*=13.032, *P*<0.001, df=5; Fig. 6.5). However, the number of undefended prey consumed remained constant throughout the daily trials ($\bar{X}$ ± SE: first half=0.991 ± 0.004, second half=0.982 ± 0.012, paired *t*-test: *t*=0.434, *P*=0.682, df=5; Fig. 6.5), which might suggest that the birds were not decreasing the numbers due to satiation.

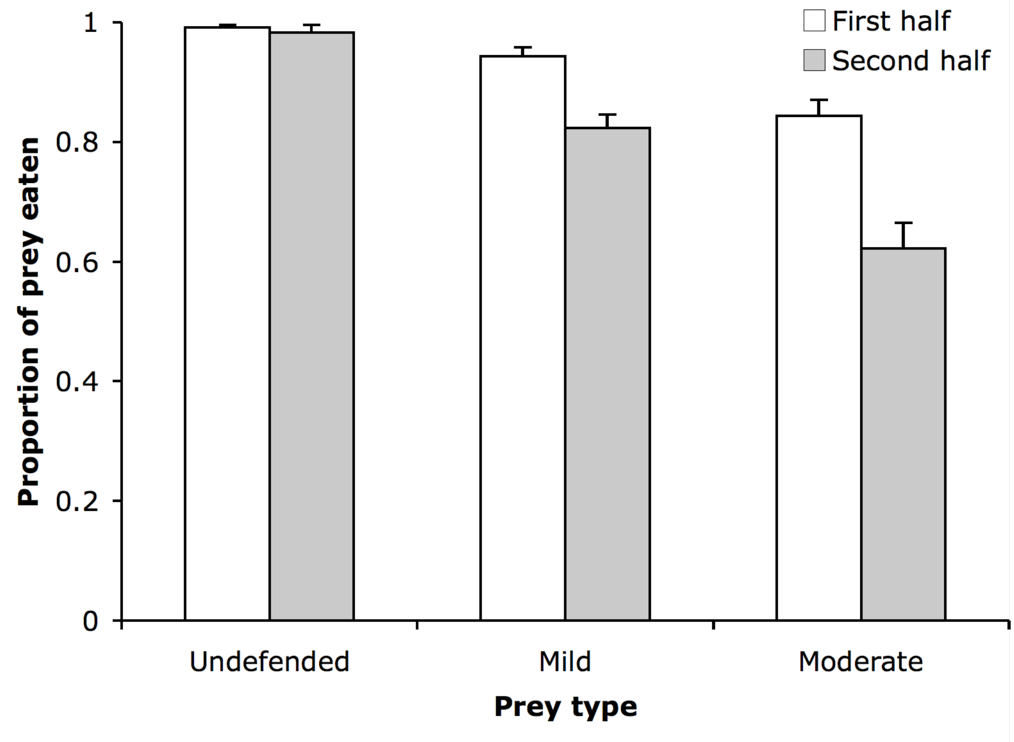

Figure 6.5. The number of each prey type consumed in the first half of a trial versus the second half of a trial. The birds showed a reduced tendency to consume the chemically defended prey in the second half of the experiment but the rates of consumption were similar in both halves for undefended prey.

6.3.4 Comparison of the ingestion of quinine levels between Chapter 5 and Chapter 6

One of the predictions of the SDP models of state-based consumption of chemically defended prey is that birds should have a critical level of toxins above which it is prohibitive for the birds to continue consuming chemically defended prey. Therefore, if we compare the differences in the levels of quinine ingested in the two experiments, there should be no significant difference between the levels of quinine ingested standardised for mass. I compared the average amount of quinine ingested corrected for body mass during the three days of training that were used to show that the birds had learned the prey differences in both experiments. There was no significant difference in the amounts of quinine ingested by birds in the two experiment ($\overline{X}$ ± SE: Chapter 5=11.5 ± 2.98 μg/g of body mass, Chapter 6=14.3 ± 0.64 μg/g of body mass; *t*-test: *t*=-0.963, *P*=0.358, df=10; Fig. 6.6).

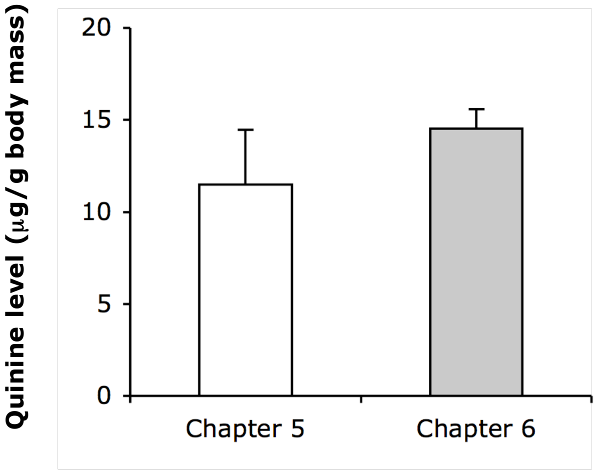

Figure 6.6. The mean (+SE) levels of quinine per gram of body mass that birds ingested while at comparable states in Chapter 5 and Chapter 6.

6.3.5 Differences in latency in accordance with prey type

I examined the birds' latencies to attack the different prey types in order to test if these were affected by quinine level and to see if the reductions in energetic state increased attack speeds. Differences in the latency data by prey type would indicate that the birds are educated about each prey type and that they may be more reluctant to attack chemically defended prey. Additionally, when birds lose mass, they might be quicker to attack prey in order to maximise the probability of capturing a prey item. I conducted two analyses of the attack latency data: (1) to test latencies according to prey type and (2) to compare differences in latencies in relation to changes in state.

The results of the first analysis indicated that the birds were quickest to attack undefended prey, followed by mildly defended prey, and the moderately defended prey having the longest latencies ($\overline{X}$ ± SE: undefended=3.4 ± 0.72 sec, mild=4.5 ± 0.87 sec, moderate=5.5 ± 1.17 sec, repeated-measures ANOVA: $F_{2,10}$=15.301, *P*=0.001, η^2=0.788, Fig. 6.7). Birds were significantly quicker to attack undefended prey compared with mildly defended prey (LSD: undefended – mildly defended, *P*=0.028) and moderately defended prey (undefended – moderately defended, *P*=0.027). However, there was no significant difference between mildly defended and moderately defended prey (mildly defended – moderately defended, *P*=0.080).

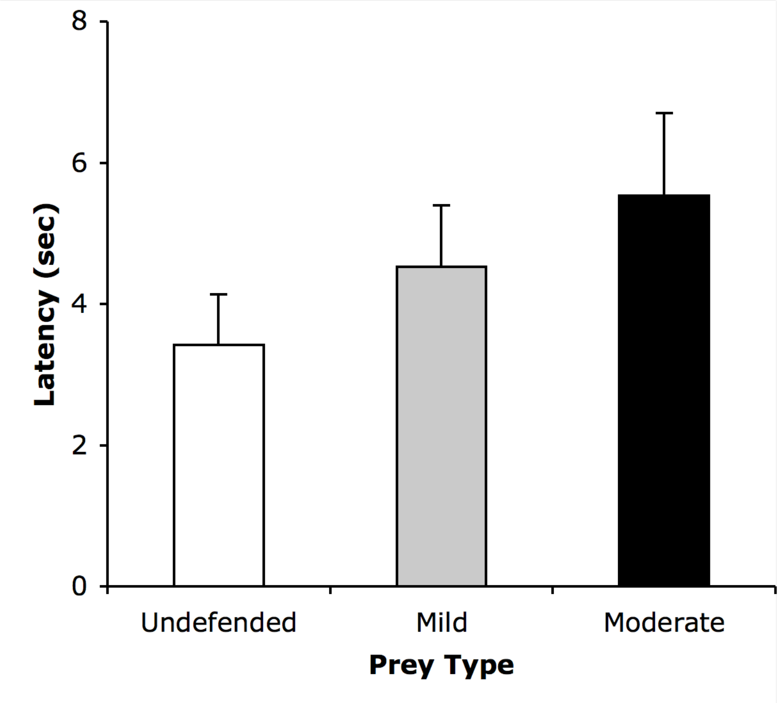

Figure 6.7. The mean (+SE) latencies to attack for each prey type for the experimental period.

In the second analysis, I was interested in how the latency to attack changed in response to changes in state. Therefore, I compared the latencies of birds for each prey type at two times within the experiment: (1) the three-day period used to demonstrate birds had been trained and (2) the day of FFI and the two days after this day. There were significant differences in the latencies between different times for undefended prey and moderately defended prey ($\bar{X}$ ± SE: undefended, early=4.1 ± 0.86 sec, full inclusion=2.2 ± 0.30 sec, paired *t*-test: t=3.097, P=0.027, df=5; moderate, early=7.3 ± 1.45, full inclusion=3.4 ± 0.33 sec, t=2.73, P=0.041, df=5; Fig. 6.8). However, there was no significant difference for the mildly defended prey despite the difference in the means (early=5.4 ± 1.15 sec, full inclusion=3.2 ± 0.33, t=2.158, P=0.083, df=5; Fig. 6.8).

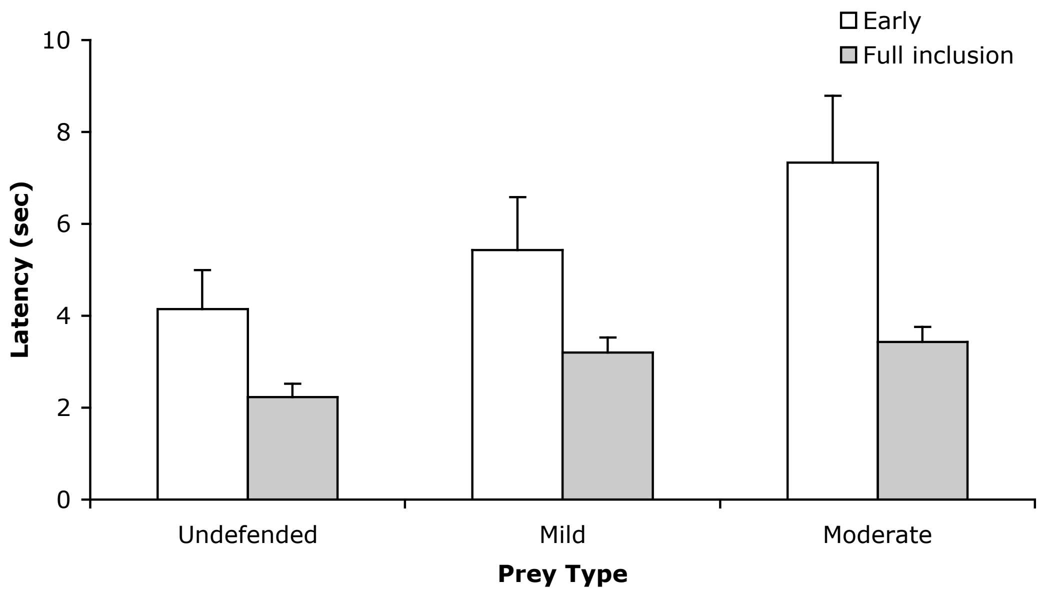

Figure 6.8. The mean (+SE) latencies to attacks of birds at the beginning of the experiment compared with the latencies at FFI for moderately defended prey. Generally, the latencies are significantly greater at the beginning of the experiment compared with the middle of the experiment.

6.3.6 Simultaneous choice trials

Simultaneous choice trials were conducted for two reasons: (1) to ensure that birds were making their choices of which prey to consume based on the learned colour cues and (2) to eliminate to possibility that birds were using other cues derived from the prey to make decisions. I totalled the number of prey consumed for each prey dyad and treatment and found that generally the birds favoured undefended over the two defended prey types and the mildly defended over the moderately defended prey in both the colour-quinine (Fig. 6.9a, Table 6.4) and the colour only treatments (Fig. 6.11b, Table 6.4). When the colour cues were not present, the birds did not discriminate between the defended and undefended prey (Fig. 6.9c, Table 6.4). Therefore, birds were making their attack decisions based upon the colour cues of the paper discs as opposed to any other cues that were associated with the defended mealworms.

Table 6.4. The results of paired *t*-tests for comparisons of prey types presented in simultaneous choice trials.

	Undef v. Mild		Undef v. Mod		Mild v. Mod	
	t-score	*P*-value	*t*-score	*P*-value	*t*-score	*P*-value
Colour-Quinine	6.779	0.001	7.68	0.001	3.53	0.017
Colour only	8.318	<0.001	7.787	0.001	1.714	0.147
Quinine only	0.653	0.542	0.696	0.518	-2.39	0.062

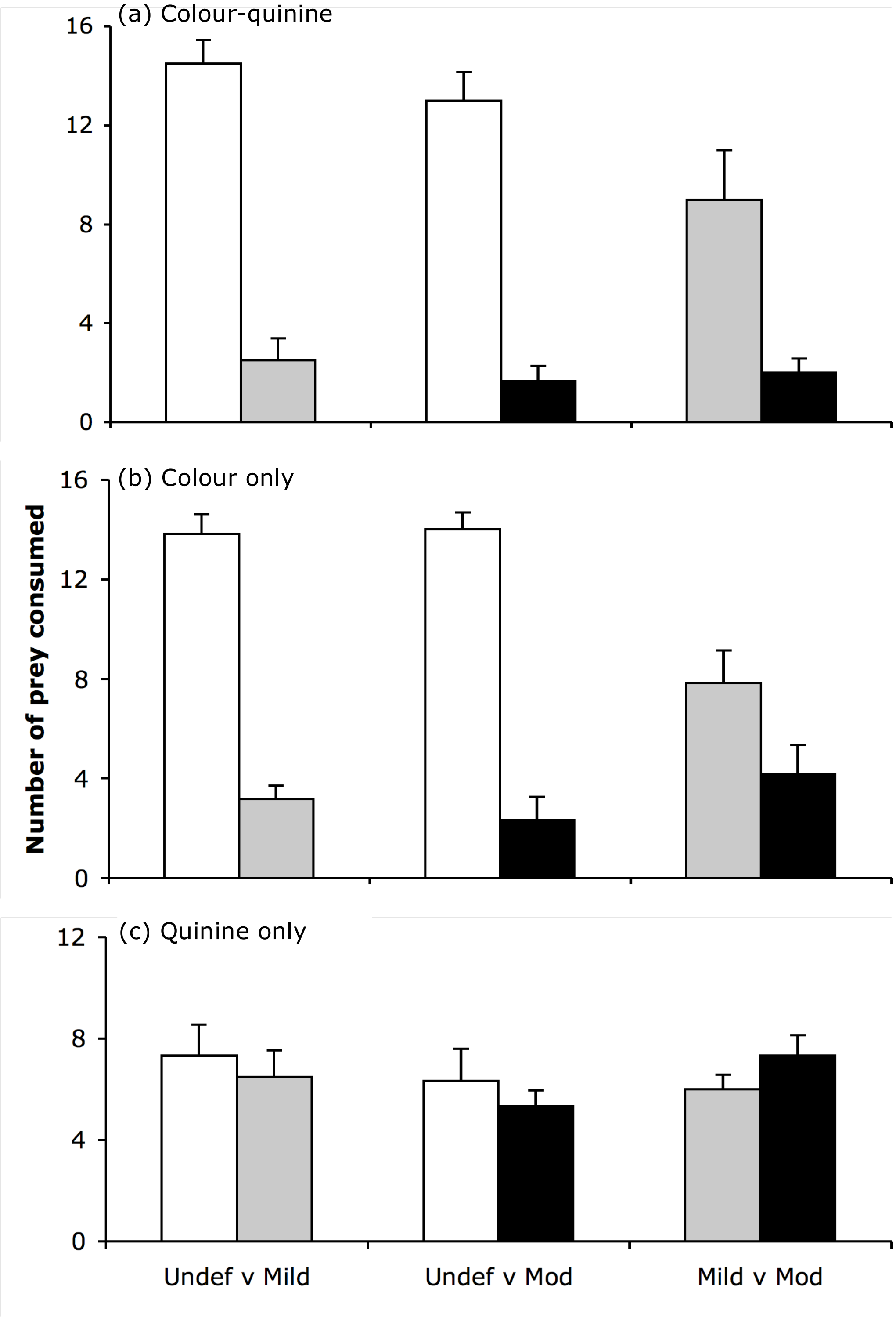

Figure 6.9. The mean numbers (+SE) of prey consumed over the simultaneous presentation trials. These results indicate that the generally chose prey in accordance with their expected quinine contents (a & b), but could not discriminate when the colour cues were removed (c).

6.4 Discussion

Together, these data support my two predictions: (1) birds were able to discriminate among three prey types and consumed them based on their levels of chemical defences and (2) as birds energetic reserves changed, they included defended prey in accordance with their levels of defence. This is one of the first studies that have shown that birds can form stable preferences over long time periods based on the levels of defence of their prey. Although there have been many studies that have fed numerous prey to predators and they have shown that these preferences may be related to levels of chemical defence (e.g., Jones 1932, 1934; Brower *et al.* 1963; Sargent 1995, Pinheiro 1996) and on their levels of energy reserves (e.g., Chai 1986), these studies have been hampered by a number of factors. First, as I have already mentioned, few of these studies have measured the energetic states of predators or demonstrated the effect of a supposed manipulation on the state of predators. Second, although the birds were being tested with actual aposematic prey, the levels of the preys' defences were often not validated meaning that there is no way of knowing if the predators were using the levels of prey defence to form their preferences. Finally, the prey in these other studies were aposematic and so it is unknown if the colouration was having an effect on the preferences that were being observed. My study effectively controlled for all three effects by careful measurement of energetic state of birds, careful control of the amount of quinine contained in each prey, and by offering the birds prey were non-aposematic. Therefore, the preferences of the birds were most likely the result of differences in the levels of chemical defences the prey contained and the bird's energetic state.

Another interesting result was that birds decreased the number of chemically defended prey in the second-half of the daily trials compared with the first-half. This suggests that the birds might have a critical level of toxins and they are able to strategically regulate their levels of toxins. These results are not due to satiation as birds are likely to have been losing mass during the course of the daily experimental session (further discussion in section 7.4). Therefore, these results support the SDP model of Sherratt *et al.* (2004) where they predict that birds may be able to strategically manage their intakes of toxins which they are able to ingest before the defended prey become prohibitively costly to consume. These results also add to the growing evidence that avian predators may be able to strategically manage their intakes of body toxins as has been shown in plant browsers (Belovsky 1982, Alm *et al.* 2002, Bergvall & Leimar 2005, Bergvall *et al.* 2006; Marsh *et al.* 2006).

These data support the idea that predators were making informed decisions for two reasons. First, the latency data suggest that the birds were quicker to attack the undefended prey compared with the other prey types. However, this trend decreased with decreasing energetic reserves although the overall trend remained significant. The latency data suggest that the birds were more hesitant to attack chemically defended prey and this may work in the favour of aposematic species as this fact may give them more time to escape compared with non-aposemes. Second, the results from the simultaneous presentations also suggest that birds were using only colour signals as a cue for their decision-making and that there were no other cues the birds used to make their decisions.

The fact that the birds' had attained greater energy reserves when they began excluding moderately defended prey in the mass gain period (FE) compared with their masses at FFI is an interesting finding. This is because it indicates that birds might have been using different mass regulation or behavioural strategies in the mass loss and mass gain periods of the mass manipulation phase (Godfrey & Bryant 2000). This is because in addition to being significantly heavier at the first exclusion for moderately defended prey, they were also consuming a greater amount of food. Although it is possible that birds could have built up a physiological tolerance to quinine, this would not explain why this trend also was not seen for the mildly defended prey. It is more probable that birds were utilising different mass regulation strategies. It has been demonstrated that birds are able to manipulate their mass strategically in accordance with SDP models (Thomas 2000; Barnett, unpublished data). Moreover, after periods of low energetic state, birds may gain greater amounts of mass as insurance against similar future events and so might continue to eat moderately defended prey until they attain a greater mass (Lima 1986; McNamara & Houston 1990; Houston & McNamara 1993; McNamara *et al.* 1994).

Birds have been shown to have unlearned aversions to aposematic prey (see Ruxton *et al.* Pp. 90-94). However, they can also learn to reject prey on sight, after capture and tasting (e.g., Järvi *et al.* 1981, Wiklund & Järvi 1982), or after the consumption and post-ingestive consequences such as emesis (e.g., Brower *et al.* 1967). These results indicate that birds learned the differences among the three prey types by associating the positive (energetic reward) and negative (quinine) stimuli with the appropriate colour cues through a feedback mechanism. The rate of learning of a food's aversive qualities is thought to be related to the lag between ingestion and the onset of the negative post-ingestive consequences and the size of the negative effect (Leimar *et al.* 1986; Yearsley *et al.* 2001, 2006). Little is known of the post-ingestive feedback mechanism that causes rejection of quinine-injected prey in birds. Birds have relatively few taste buds, in the order of 100, whereas humans have about 9000 (Kare & Mason

1986). This has led to some authors (e.g., Kassarov 1999, 2003; but see Skelhorn & Rowe 2005) to speculate that chemoreception via gustatory nerves is likely to be much less important in birds than in mammals. However, in subsequent experiments (Chapters 6 and 7), birds were seen to gag and cast defended mealworms almost immediately after ingesting them (i.e., < 5 seconds), which indicated that birds' reactions may have been mediated by gustatory or trigeminal pathways (Werner & Clark 2003). Matson *et al.* (2004) found that cockatiels (*Nymphicus hollandicus*) were able to perceive quinine at levels that were similar to those of humans. However, this experiment compared the differences in rates of consumption of water laced with different substances and so were not testing tasting ability *per se*. Therefore, all that can be stated with any certainty is that while quinine sulphate was a negative stimulus for the birds, its effects were probably not perceived immediately and could have been accompanied by a 5-20 second lag until a gustatory or general trigeminal response was registered (personal observation, Chapter 7, Chapter 8). Hence, although the negative stimuli were not immediate, their lags were experienced quickly enough for birds to learn the association between the stimulus, the prey, and the coloured paper disk. However, caution is advisable in interpreting the chemoreceptive abilities of birds, as it is likely that animals are adapted to taste compounds that are ecologically relevant to them (Werner & Clark 2003).

Interestingly, when ruminants that were injected with PSMs every 60 minutes in accordance with their foraging behaviour, they were unable to learn the association between unpalatable food sources and their post-ingestive consequences because the lag was too great (Duncan & Young 2002). Therefore, these results would suggest that if the lag between ingestion and stimulus infusion were decreased, the animal may have more chance of learning the correct association and post-ingestive consequence. This is despite the fact that herbivorous animals are known to balance the intake of dietary intakes of PSMs in mammals (e.g., cattle, *Bos taurus* [Pfister *et al.* 1997], common brushtail possums, *Trichosorus vulpecula* [Stapley *et al.* 2000], red deer, *Cervus elephus* [Alm 2002; Bergvall-Alm 2005; 2006] and moose, *Alces alces* [Belovsky 1982] and birds (e.g., bullfinches, *Pyrrhula pyrrhula* [Greig-Smith 1985] and ruffled grouse, *Bonasa umbellus* [Jakubus & Gullion 1990; Jakubus & Mason 1991]). This indicates that animals are capable of forming associations of food quality in relation to forage and that Duncan & Young's method might have been at fault (e.g., the experiment might not have been run for long enough).

The results of this chapter emphasise the role of energetic state in the decision-making processes of birds. Birds that remain in the same location throughout the year are known to fluctuate wildly in mass throughout the year and throughout the day. Small- to medium-sized passerines (≤ 50 g) are known to gain as much as ten percent of their morning body mass by the end of the day (Clark 1978, Haftorn 1989, 1992, Blem 1990, Meijer *et al.* 1994, Barnett & Briskie 2007). This mass gain is rarely constant throughout the day with the peak times of mass gain confined to a few hours after sunrise and a few hours before sunset (Thomas 2000, Barnett, unpublished data). The seasonal and diurnal effects of mass change are rarely considered in studies of aposematic prey choice. These results indicate that the energetic states of birds need to be considered in the design and interpretation of experiments on aposematic prey selection. Indeed, observations of insect behaviour indicate that aposematic species will become less behaviourally conspicuous during times of the annual cycle with low food availability (Hassell 1978; Majerus & Kearnes 1989).

These results demonstrate that as the birds lose energetic reserves, they increase the numbers of chemically defended prey in their diets which is consistent with previous research (Swynnerton, 1915; Sexton *et al.*, 1966; Gelparin, 1968; Chai, 1986; Hileman et al., 1995; Gillette et al., 2000). However, they also suggest that the birds do this in a strategic manner by increasing the numbers of mildly defended prey before increasing the numbers of moderately defended prey. This pattern is also repeated as birds gain mass when they continue to include greater numbers of mildly defended prey over moderately defended prey. Previous studies have exposed birds to prey of differing levels of chemical defence. Wiklund and Järvi (1982) fed young birds various aposematic prey and found that the rates of attack were very low and once attacked, the prey had a higher chance of survival compared with undefended prey. However, birds were presented with only one example of a particular aposematic species and so the data do not provide any further information as to the long-term dietary choices of educated predators Also, both altricial and precocial bird species were used and they may have had different developmental rates meaning results should not have been compared between species. Bowers and Farley (1990) fed grey jays seven different butterfly species and found that a group of four birds tended to prefer the more palatable butterflies. Birds were fed between three and six of each butterfly type. Therefore, although these studies provide evidence that birds are able to discriminate between prey, they provide no information of the long-term foraging behaviour of birds.

Moreover, there was no validation of the levels of toxins that prey contained, and the mechanisms that the birds were utilising to form their preferences was unknown.

The only study that has attempted to manipulate the energetic state of birds while feeding bird multiple aposematic prey was that of Chai (1986). He fed two rufous tailed jacamers various different species of butterflies. The first bird was fed 218 butterflies of 62 morphs and the second was fed 938 butterflies of 103 morphs. Generally, he found that birds were selective in taking only butterflies that were known to be palatable. However, there were differences in the numbers of prey taken which he also attributed to differences in hunger. Chai's study also provides a valuable insight into the discrimination abilities of birds and their powers of memory. However, the birds' energetic state was not measured and the number of subjects was too small for meaningful interpretations of the state-based consumption of defended prey to be derived. Other studies have also shown that birds are able to discriminate among different aposematic prey (Jones 1932, 1934; Brower *et al.* 1963; Sargeant 1995, Pinheiro 1996). However, one problem with these studies is that they are all confounded by the fact that butterfly imagos were used and so the effect of their warning signal cannot be disassociated from the decision to not consume the prey. Moreover, birds were fed insects at irregular intervals in many of these studies and so intake of food per unit of time was not standardised. This study controlled for both factors by carefully integrating energetic state into the design and by untilising non-aposematic signals to advertise the prey properties.

One problem of any study that negatively manipulates the energetic state of an animal is that the animal's cognitive abilities may become progressively impaired as its state deteriorates. There is some evidence to counter this argument both from this experiment and from the results of Chapter 5. First, the latencies to attack are related to the amount of quinine the mealworm contains meaning that generally as the quinine content increases, so does the latency to attack. Although the differences in latency between prey types decreased when the birds had lower fat reserves, they did not disappear meaning that birds were still making informed decisions. Second, if cognitive abilities were impaired at lower states, it would have been improbable that they would have chosen to exclude and include the two defended prey in accordance with their quinine contents. Moreover, In Chapter 5, I demonstrated that birds displayed a preference for the undefended prey type when they were presented simultaneously while on a restricted dietary intake that was designed to maintain their masses at 95 percent of their free-fed mass.

One common aspect of experiments that have fed birds large numbers of prey over long periods of time is that birds do not completely exclude the defended prey at free-fed masses (see also Chapters 4, 6, 7; Skelhorn & Rowe 2006b). Previous authors have interpreted these inclusions as being inconsistent with previous accounts of predator learning of aposematic prey and have come up with various models to explain inclusions of defended prey. For example, it has been suggested that partial preferences can be maintained as a result of the predators forgetting the defensive properties of the defended prey (e.g., Turner *et al.* 1984; Guildford 1990; Servedio 2000). However, in this experiment, birds were unlikely to have forgotten prey association as individuals were subjected to multiple prey presentations over many days (see also Skelhorn & Rowe 2006b). The implications of this research go beyond the boundaries of pure aposematism research. They also have implications for the recent debate regarding quasi-Batesian mimicry. If predators do strategically consume chemically defended prey this suggests that the preferences of prey may be maleable and so question traditional approaches to the aposematism and Müllerian mimicry which state that the continued consumption of chemically defended prey extinguish the future tendency to attack (e.g., Turner *et al.* 1984; Guildford 1990; Servedio 2000). However, these models ignore the benefits that accrue to predators by attacking defended prey at times of energetic need. Therefore, models that consider the costs and the benefits of consuming chemically defended prey are likely to produce more realistic predictions (e.g., Sherratt 2003; Sherratt *et al.* 2004)

This experiment emphasises the importance of incorporating energetic state as a factor in both mimicry and aposematism studies. Although most researchers acknowledge that energetic state is an important factor in determining a predator's decision to attack chemically defended prey, energetic state has become a forgotten factor in empirical research of mimicry and aposematism. For example, it now a standard procedure to food deprive birds before starting experiments. However, this is done with little consideration of how this treatment affects a bird's energetic state other than to increase the subject's 'motivation' to attack (motivation is generally a catch-all term that has become amorphous through loose application and poor definition). Therefore, by better incorporating and controlling for possible state based effects in experiments and between experiments might significantly aid researchers in generating less ambiguous data.

I have investigated the rates of attack upon three prey types of varying levels of chemical defence in response to changes in daily energetic state. This is the first time that it has been demonstrated in an integrated manner that birds choose whether to eat chemically defended prey is based on both their energy reserves and the levels of toxins that the prey contain. This study also shows that birds were able to discriminate between three prey types and rank them according to their quinine contents. These results reinforce the assumptions that have been made by recent SDP models on the consumption of chemically defended prey and evolution of mimicry (Sherratt 2003; Sherratt *et al.* 2004). There also needs to be a wider appreciation of the effects that energetic state can have on the predation of aposematic animals both in experiments and in nature.

6.4.1 Conclusions

In this study, I have shown unequivocally, that birds can also form preferences for prey based on their levels of chemical defence without bright warning colours. The problem with many of the previous experiments that have fed birds multiple types of chemically defended prey is that the reason for the birds' preferences have remained unknown. For example, in Chai's (1986) study, birds were fed many different types of butterfly although, the actual chemical composition of these prey were unknown. This meant it was unknown which cues the birds used to make basing their decisions. Darst *et al.* (2006) have demonstrated that colour is also an important factor in determining predator choices with brighter colours also acting to deter predators despite no correlation between brightness and level of defence (although see Bezzerides *et al.* 2007). Moreover, I have demonstrated that these preferences are consistent as birds lose or gain mass. One possible criticism with the mass manipulation in Chapter 5 was that the mass manipulation of the birds might have ecologically unrealistic. Therefore, demonstrating that even small changes in mass can cause changes in the number of chemically defended prey consumed indicates that energy reserves are an important consideration in determining predators' probability of attack. These are also important findings because they support the assumptions of the SDP models that birds can choose prey based on the levels of chemical defences that the prey contain and their own levels of energetic reserves. The interactions between energetic state, prey defence, and predator toxin burden are complex. Therefore, the results of this study need to be replicated both in European starlings in different physiological states and in different species with different mass regulation strategies and physiological capacities for toxins. Only then, will we be able to gain a comprehensive understanding of how these factors all interact in real predator-prey systems.

Chapter 7

THE RELATIONSHIP BETWEEN ENERGETIC STATE, SIGNAL RELIABILITY, AND PREY CHOICE IN A BATESIAN MIMICRY SYSTEM

7.1 Introduction

7.1.1 Signals and their reliability

The bright warning colouration of chemically defended animals such as butterflies are examples of visual signals. Otte (1974, p. 385) defined "signals" as "behavioural, physiological, or morphological characteristics fashioned or maintained by natural selection because they convey information to other organisms". Therefore, signals are traits that have evolved specifically to alter the behaviour of recievers. Cues on the other hand, are features of an organism that are not related to communication *per se,* but are used by receiving organism as information to guide future decisions (Maynard Smith & Harper 2003, pp. 3-6). This definition of a cue differs from that of Hauser (1996) who wrote that cues and signals both evolved but differ in the fact that cues are continuously expressed whereas signals are expressed discontinuously. I prefer the distinction of Maynard Smith and Harper (2003) in using a functional explanation to distinguish between cues and signals. Hence, my differentiation between cues and signals focuses on the intention of the signal. If the intention of the signal is to alter the behaviour of the receiver, then the conveyed information is a signal. Alternatively, if the information conveyed is incidental or a latent property of the signaller, then it is a cue.

Generally, signals are thought to be costly to produce. This is because of the assumption that, for a signal to convey reliable information, it ought to incur some form of cost to the signaller (Guilford & Dawkins 1993b; Hauser 1996; Maynard Smith & Harper 2003). If signals incur no cost, then the communication system will break down because cheats can corrupt and devalue the meaning of the signal. This is because receivers should respond to a signal only when it benefits the receiver to act on the information conveyed by the signaller. If a signal can be used by too many cheats; it will no longer convey the information that was intended, and receivers should no longer respond to the signal. The costs of signals and their reliability are still contentious issues within the field of animal signalling. For example, bird song is thought to function as a signal for males to attract mates, but the costs of singing are still debated keenly with many researchers arguing that song is no more costly than other behaviours (e.g., Horn *et al.* 1995, Ward *et al.* 2003a,b), whilst others have argued that the costs of singing may be significant (e.g., Thomas 2002; Barnett & Briskie 2007). Therefore, signal honesty and reliability are major issues in the study of animal communication.

If signals are reliable, then the receiver can have confidence in the veracity or truthfulness of the signal. Searcy and Nowicki (2005 p.3) formalised a definition of a reliable signal where a signal was reliable if: (1) some characteristic of the signal … is consistently correlated with some attribute of the signaller or environment; and (2) receivers benefit from having information about this attribute. Although there are problems with this definition (e.g., how to define the term “consistently correlated”), it is a useful starting point within a biological context.

Theoretically, aposematism may be an example of an honest signal as the possessor of such a signal has a quality that makes it unprofitable to attack (e.g., Sherratt 2002; Sherratt & Beatty 2003; Speed 2003; Broom *et al.* 2006; Speed & Ruxton *et al.* 2007). Aposematic signals are conspicuous and may increase the detectability of individuals and initially evolve as honest signals of prey unprofitability. However, this view is complicated by the fact that even within an aposematic species, the levels of defences can vary between individuals (Brower & Moffitt 1974; Brower *et al.* 1975; Brower *et al.* 1984; Cohen 1985; McLain & Shure 1985; Malcolm & Brower 1989; Eggenberger & Rowell-Rahier 1991; Bowers & Williams 1995; Pasteels *et al.* 1995; Moranz & Brower 1998; Ritland 1994; Tullberg *et al.* 2000). Automimicry is a form of intra-specific mimicry where an aposematic species has variation in the level of defences that individuals contain. Automimicry is similar in principle to Batesian mimicry because in both forms of mimicry, the mimic gains the protective advantage of the model’s defences, but does not pay the costs of sequestering of producing defensive compounds (see Sherratt *et al.* 2004, pp. 55-64 for a discussion of the costs of chemical defences). The model (the defended species) gains nothing from the relationship meaning that it is a parasitic relationship.

7.1.2 Experiment introduction

In Chapters 5 and 6, I presented some data that suggest that predators may be able to strategically manage their body levels of a specific toxin that is contained within chemically defended prey. If this is the case, then the reliability of an aposematic signal's association with its chemical defence could be an important factor in determining a predator's decision on whether or not to attack a defended prey item. This is because, as a predator ingests greater quantities of a toxin during a foraging bout, its body burden of toxins may approach some point where further ingestion of defended prey items becomes prohibitively costly. Sherratt *et al.* (2004) called this point the 'critical toxin burden'. This means that if a predator is strategically managing its toxin burden, the reliability of an aposematic signal may become more important the closer it gets to the critical toxin burden. In a situation where a predator could encounter two prey types each with the same average level of defence, but one with a reliable signal and the other with an unreliable signal, it might be expected that predators prefer the prey with reliable signals as the predator began to approach its critical burden of toxins. In this way, a predator can manage its toxin burden more precisely. Therefore, the variation in defence may confer an advantage to a mimicry complex by reducing attacks (all other things being equal).

Aposematic signals are made up of two or more sensory components and it is possible for each component of the signal to vary. For example, visual signals may vary as in the case of imperfect Müllerian mimicry and chemical defences may vary both within a species (automimicry) or between species (Batesian and Müllerian mimicry) (Ruxton *et al.* 2004). Recent studies have investigated how differences in visual signals and chemical defences can interact with one another (Lindström *et al.* 2006, Ihalainen *et al.* 2007). However, these studies have not been concerned with how signal reliability may influence predators' prey choices.

I aimed to investigate how signal reliability might affect the prey choices of birds in relation to mass loss by offering birds sequential presentations of three prey types that had distinct visual signals: (1) an undefended prey type; (2) a "constant" prey that contained a constant amount of quinine per prey (i.e., it had reliable colour signal of underlying quinine content); and (3) a "model-mimic" prey that had variable defences, but the same average level of defences as the constant prey (i.e., it had unreliable colour signals of underlying quinine content). The two defended prey had the same average levels of quinine, but they differed in the variance in the amount of quinine around the mean. By comparing the rates of prey consumption between these two defended prey types, I was able to examine the effects that signal reliability might have on the foraging choices of European starlings.

Recent SDP models on the state-based consumption of chemically defended prey have predicted that predators should consume chemically defended prey when they become energetically stressed (Sherratt 2003; Sherratt *et al.* 2004). Sherratt *et al.* (2004) also predict that predators should regulate their intake of chemically defended prey so that they do not exceed their intake above a critical burden. Therefore, if average toxic effect of the two prey types are equal then, then it would be predicted that birds should display no difference in preference for either of the defended prey types. However, from a signal reliability perspective, the birds ought to favour the signal that is more reliable (i.e., the constant prey). This is because as birds approach their critical body burdens of toxins, they should pick an option that allows them to be able manage their levels of toxins with more certainty. The constant prey has the more certain outcome and so should be favoured despite the birds knowledge of each prey type being the same. This is the first study that has presented this sort of foraging problem to predators and examined the strategies that they develop when they are exposed to these prey over long periods of time and hundreds of prey presentations. This experiment aimed to examine additional benefits to prey with variable defences based on the uncertainty that predators might experience when attacking these prey types compared to non-mimetic defended prey.

7.2 Methods

7.2.1 Study species and housing

The subjects were seven wild caught male European starlings (*Sturnus vulgarus*) caught under licence from English Nature (Licence Nos. 20040489). The birds were individually identifiable throughout the experiment through use of numbered white celluloid split leg-bands. The birds were taken from the free-flight aviary and housed individually in wire mesh cages measuring 450 x 750 x 450 mm (h x w x d) with a metal drawer at the bottom, which could be removed for cleaning. The birds were subjected to a 14:10 hour light/dark cycle (lights on at 0900 hr; lights off at 2300). During the experiment, birds were fed pheasant breeder pellets in accordance to their previous days mass change (see section 6.2.2 for details). During training, birds were fed *ad libitum* pheasant breeder pellets and were given a piece of fruit daily. Water was available to birds continuously at all times. When food was changed each day, the remaining food was weighed so I could calculate the amount of food consumed per 24 hr. At the end of the experiment, birds' masses and fat scores were monitored until they had stabilised, when they were returned to the free-flight aviary.

7.2.2 Prey-preparation and training

The objective of the experiment was to examine the responses of birds to three prey types: undefended prey, model-mimics (with unreliable colour signals), and constant prey (with reliable colour signals) (Table 7.1). Prey were *Tenebrio* sp. larvae (henceforth, mealworms) of approximately 20 mm in length. Prey of this length was used in order to standardise the nutritional value of mealworms. The masses of mealworms and their nutritional value would be similar to those used in other chapters (see section 5.2.3 for details). Mealworms were injected intra-orally into the body cavity with quinine or water. This method allowed liquids to be introduced into the mealworm while maintaining its body shape and minimising the seepage of bodily fluids from the exoskeleton.

Table 7.1. The treatments of the four prey types used in this experiment.

Prey Type	Treatment
Undefended	0.2 mL water
Constant prey	0.2 mL 2 % quinine sulphate suspension
Mimics	0.2 mL water
Models	0.2 mL 4 % quinine sulphate suspension

This experiment differed from those in Chapters 5 and 6 in that birds were trained to flip lids that had been placed over a Petri dish containing a mealworm. The mealworms in this experiment were covered by a coloured cardboard lid meaning that the birds were unable to see the mealworms directly until they flipped the lid off the dish. The lid flipping technique is preferable because means that only the colour cues are available to birds before deciding whether to attack the prey or not.

Birds were trained to flip the lids (42 mm diameter) that were initially uncoloured (white) off the tops of Petri dishes (38 mm diameter). All birds learned this within three days of training. I initially food deprived birds for two hours prior to starting the training, but quickly realised that the birds would participate in training and experimental sessions without pre-trial food deprivation. Therefore, I eschewed food deprivation for the rest of the experiment. Birds were not moved in their cages or hidden behind curtains for this first part of training meaning birds could see one another. I habituated birds initially to eat undefended mealworms that had not been injected with any liquid substance from uncovered Petri dishes that had been left in the centre of its cage for one minute after which I removed the dish. There was then an interval of two minutes before the next mealworm was presented. Once a bird had consumed a mealworm five times in succession, I placed a circle of white card next to the Petri dish with the next mealworm. Again, the birds were required to consume five of the mealworms before the disk was propped against one side of the Petri dish. With each presentation, I continued to leave the Petri dish containing a mealworm in the bird's cage for one minute before it was removed and another two minutes allowed to elapse before presenting the next mealworm. If the bird attacked a mealworm on five occasions within 10 seconds of each pressentation, I moved the disk to cover a slightly greater part of the Petri dish. This was done in quarters whereby at first the disk covered a quarter of the dish. Once a bird had consumed the mealworm five times within the first 10 seconds of a presentation, the disk was moved to cover a half of the Petri dish. This process was repeated until the cardboard disk completely covered the Petri dish and the bird had completed five successful lid flips and consumed the mealworms in under 10 seconds. At this point, I began using coloured lids in the training of birds and conducting the trials in visual isolation.

Five minutes prior to the start of the trial, birds were moved in their cages to a new position within the room and left to habituate. They were positioned behind a curtain in order to ensure that the other birds in the room could not see the subject and *vice versa*. The bottom of the curtain was level with the bottom of the cage. In order to observe the bird, I used a video camera connected to a television monitor.

Birds were first habituated to the coloured paper lids (pink, blue, green, and yellow), which were balanced between the subjects as much as possible to minimise the possible effects of colour biases. Birds were presented with 18 undefended mealworms in small Petri dishes concealed under coloured card disks. The order in which I presented the different coloured lids was randomised within a daily training trial, but with the rule that two of each colour were presented in each third of the daily training trial. For each prey presentation, the birds had one minute in which they could attack and consume the prey. If a bird consumed the mealworm, the dish was removed immediately and the bird was left until three minutes had elapsed before another prey was presented. If the bird failed to consume the prey after one minute, the Petri dish was removed and another two minutes elapsed before the next prey was presented.

Once the birds consumed all 18 larvae in a trial using undefended prey, the birds could be trained to learn the colour associations between colour and prey type. A different colour was used exclusively to indicate each prey type and there were six presentations of each prey type in each daily trial. Prior to the first training trial, I presented five undefended prey covered with the coloured paper lids so that birds would not stop eating mealworms as they might if they consumed defended larvae during initial presentations. I then presented a further 18 larvae, with two of each prey type presented in each third of the session. I assumed that birds had learned the differences in prey properties when the total number of prey eaten in the previous three days of the trials were significantly different using a chi-square test from what would have been expected if the birds were feeding without preference (see section 7.3.1 for results). Generally, the mean number of trials it took birds to learn the colour associations was 12.7 trials (SE: ± 1.85 sessions; range=8-20 sessions).

On two training days, I weighed each bird (to the nearest 0.1 g) at the beginning and end of the daily training trial. I chose the day I weighed each bird at random within the period where birds were learning the colour prey associations, but I allowed at least four days between the first and second day's readings. I weighed birds so I could ascertain the mass change of birds throughout an experimental trial. I did this during training, to avoid any additional handling of birds interfering with the data collection during the experimental trials. Although it is possible that this procedure could have interfered with the birds' learning of the colour/prey associations (as they grow agitated during handling), I did not notice any elevated level of restlessness or reluctance to participate in training on days when I weighed birds (personal observation). For example, they settled down in their cage quickly after being placed back in there and they did not seem reluctant to attack mealworms when they were presented (i.e., their latencies did not seem longer and they approached mealworms in a manner similar to how they did on other days).

On each day during the training period, I weighed and visually assessed fat levels as set out in the next section. I calculated the birds free-feeding masses from this data using the five days data up to and including the day where the birds had been shown to have learned differences among the three prey type. I also collected the food remaining at the bottom of the bird's cage in order to calculate the amount of food consumed within a 24-hour period. I compared the mean amount eaten on the five days that were used to calculate the bird's average food consumption. Although this did not take account of the food spilled from the cage, it positively correlated with the masses of the individual birds with heavier individuals consuming more food in a 24-hour period than lighter individuals ($F_{1,6}$=6.632, P=0.050, r^2 = 0.5701, Fig. 7.1).

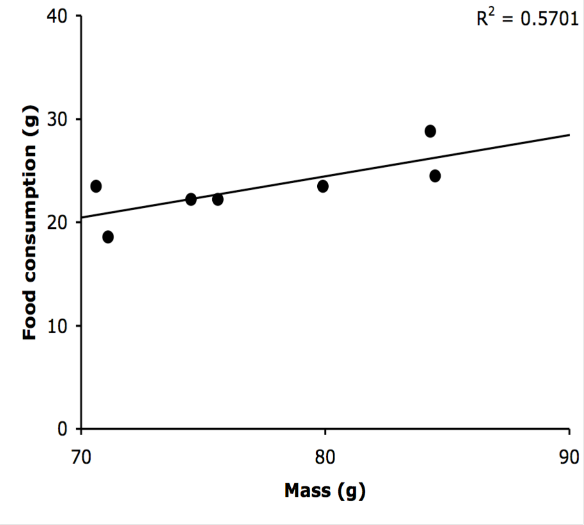

Figure 7.1. The mean amount of food consumed by individuals in a 24-hour period correlated with their mean body masses during training.

7.2.3 Experimental trials: mass manipulation and prey choice

As in Chapter 6, I made visual inspections of the furcular fat levels and weighed the birds each day (see section 6.2.2 for details). I also manipulated the birds' energetic state through food restriction using the reactive adjustment method (see section 6.2.2 for details) and calculated the bird's free-feeding mass (FFM). I measured the circumference of the birds around their chest, wings, and back at the point where the sternum was deepest at the beginning of the experiment and on the day after the bird consumed all of the prey for the first time (FFI). I did this in order to measure if the birds lost muscle throughout the mass manipulation. If the birds were severely energetically stressed, then the birds would be expected to metabolise muscle as well as fat tissue, which would cause muscle atrophy.

The mass manipulation phase of the experiment had two periods: a mass loss and a mass gain period. The birds were fed after weighing (between 0915-930 GMT) either 50 g of food during training or a restricted amount that was calculated according to the reactive adjustment method (outlined in section 6.2.2). This method allowed birds to gradually gain and lose mass the rate of about half a gram or less of mass each day ($\bar{X}$ ± SE: mass loss phase=-0.33 ± 0.058 g, mass gain phase=0.50 ± 0.079 g). During the mass loss period, the birds' levels of consumption of the different prey types were recorded until all of the prey were being consumed. At the point where a bird consumed all of a particular prey type, I noted this and called it the day of first full inclusion (FFI) for that particular prey type. Eventually, the birds' energetic state declined to the point where they consumed all of the prey that were offered to them. On FFI, I fed the bird its previous days food minus the mass change in order to stop its mass from changing. Once all the prey were consumed for three consecutive days, I allowed the birds to begin gaining mass. I included the criterion to avoid the possibility that the day on which all prey were consumed was not an isolated occasion. All birds reached this point whilst above 90 % of their FFM. I continued the daily trials as I switched birds from the mass loss period of the experiment to the mass gain period. The prey types that were consumed were recorded until a bird either reached its 100 % free feeding masses or when it was no longer consuming any defended prey.

In order to make mass comparisons within the same individual, I first calculated an average mass of the individual birds whilst under experimental conditions. I calculated the mean mass of the birds on the last five days of training phase of the experiment which was then used to calculate a standardised mass that could be compared among individuals. The masses did not need to be standardised using tarsus or wing length as each bird was acting as its own standard for making comparisons.

Individual birds followed the same schedule on each day. However, I could run only two experimental trials simultaneously, which meant that I had to stagger the start and begin a new pair of birds every 90 minutes. The first pair of birds was started at 1300 hr and it took about six hours to fully complete trials for all seven birds. Prey presentation was identical to that during training. On each day of the mass manipulation phase, 18 larvae were presented to birds in a sequence which constituted a daily trial. I recorded if the bird flipped the lid and whether the prey was attacked and consumed. I also recorded the latency to attack (from when the prey was placed in the cage until the bird's bill touches the prey item) and the birds' behaviour.

7.2.4 Behaviour while attacking and consuming prey

During the course of the experiment, it became evident that birds' behaviour towards the different prey types was changing. Initially, birds did not handle prey and quickly consumed the prey after flipping the lids off the Petri dishes. As the experiment progressed, I noticed that the birds' prey handling times began to increase and that they were increasing the types of behaviours displayed. I identified a number of different behaviours that the birds displayed before attacking the prey, while handling the prey, or after eating or rejecting the prey (see Table 7.2 for descriptions). I also hand-timed the handling times all prey presentations.

Table 7.2. Classification of different behaviours observed during the daily experimental trials.

Behaviour	Description
Pre-attack	
Jumping Jack	Bird approaches prey and then jumps back quickly and flaps wings.
Bill wiping	Wiping bill on cage, perch, or floor.
Bill gaping	Bill open for an extended period of time.
Feather ruffling	Feathers ruffled quickly or for protracted period.
Casting	A food item brought up from crop.
Handling	
Jumping Jack	Bird approaches prey and then jumps back quickly and flaps wings.
Head biting	Biting the cephalic region of mealworm
Body crushing	Biting the body of mealworm up and down its length
Wiping	Wiping the mealworm in the floor of the cage
Whacking	Hitting the mealworm against cage floor
Post-ingestive	
Bill wiping	Wiping bill on cage, perch, or floor.
Bill gaping	Bill open for an extended period of time.
Feather ruffling	Feathers ruffled quickly or for protracted period.
Casting	A food item brought up from crop.
Head shaking	Head shaken vigorously for short time (≈ 1 sec).

7.2.5 Statistical procedures

On each day of the mass manipulation phase of the experiment, I calculated the birds' standardised masses. Throughout the mass manipulation experiment, the bird's standardised masses ranged between 0.9 and 1.04 (although no individual bird's masses encompassed the entire range of variation). Therefore, I constructed a scale of 0.01 graduations of standardised masses. This allowed the data to be pooled for each individual bird during the mass loss and mass gain periods of the experiment. This allowed me to compare the relationships between changes in birds' masses and their changes in prey preference. This also allowed me to remove time as a factor, as often the birds' mass changes were not consistent in their downward or upward trends. This allowed me to remove these differences and concentrate on the relationship between energetic state and consumption of the different prey types.

I used the data obtained from the above method to construct separate linear mixed models (LMMs) for the mass loss and mass gain phases of the experiment. I used Akaike Information Criterion (AIC) in order to choose the best model. The lower the AIC, the better the model is a general rule of thumb. All analyses that were on proportionate data, I arcsine square-rooted the data and performed parametric tests on the resultant transformed value. I compared differences between means using the least significant difference (LSD) method that had been corrected for multiple comparisons (Bonferroni method).

The analyses in section 7.3.2 were calculated by hand and checked three times for accuracy. I used Tukey's HSD (honestly significant difference) test, which is a *post-hoc* method of comparing differences between means. This method is one of several methods that ensure that the chance of finding a significant difference in any comparison is maintained at the alpha level of the test. Therefore, the alpha-level does not need to be corrected.

I also conducted an analysis on the number of behaviours that were expressed in relation to prey type and time throughout the mass manipulation phase of the experiment in order to examine if handling behaviour increased as the experiment progressed. In order to do this, I standardised the length of the experiment between individuals since the number of experimental days experienced by each bird varied between individuals (Range=43-56 days). Therefore, I converted the day number into a proportion by dividing it by the total number of days that bird was subjected to experimental trials. I then put each time into one of twenty time bins which were split into 0.05 graduations beginning at 0 and ranging to 1. I transformed the resulting value using the square root transformation ($\sqrt{[x + 0.5]}$) and analysed the resulting values using a LMM.

I conducted all statistical analyses using SPSS for PCs (Versions 14.0, 15.0) and for Macintosh computers (Version 11.1). I checked all data for normality and transformed the data with appropriate transformations if they were not normal.

7.3 Results

7.3.1 Training

I considered the birds to be trained to the defence-colour association when a chi-square test was significant for the totals of the previous three days' consumptions of each prey type. The number of trials that it took for birds to learn the prey associations ranged from 8 to 20. The birds favoured undefended prey over constant prey, which were in turn favoured over model-mimics ($\bar{X}$ ± SE; undefended=17.7 ± 0.18, constant=10.1 ± 0.94, model-mimics=6.9 ± 0.46). I conducted a chi-squared test of the each bird's total number of each prey type consumed over the three trials of training against the assumption that the number of prey consumed would be randomly distributed between the three prey types. I found that the number of prey consumed differed significantly from the expectation of random prey preference (all $\chi^2>7.462$, all $P<0.05$, df=2).

In order to ascertain the change in mass during an experimental trial, I had measured the average mass change between the mass at the beginning and at the end of the two daily training trials. Using the mean mass change during these two trials, I found that the birds lost mass during the trials and that they were significantly lighter at the weighings at the end of the trials ($\bar{X}$ ± SE: start mass – end mass=0.6 ± 0.19 g, paired *t*-test: $t=3.175$, df=6, $P<0.02$). This suggests that the birds lost mass during a training trial, although it is possible that some of this mass loss was due to defecation (since they were not food deprived). Moreover, the method of weighing was likely to have been stressful for birds meaning that a corticosterone response could have been triggered, which would increase the metabolic rates (and thus, mass loss) of birds. Therefore, it would have been better to have remotely weighed the birds rather than handle them twice within an hour. However, the birds seemed behaviourally unaffected by the weighing by the start of the trial and participated without in the training trial without obvious differences to days when they were not weighed.

7.3.2 Rates of attack

In the last two chapters, the rates of attack have matched the rates of consumption. In this chapter, I changed the method of prey presentation. Therefore, I conducted two crude analyses to investigate how the rates of attack corresponded with the consumption of prey. First, I calculated the rate of attack for each individual bird and prey type throughout the mass manipulation phase of the experiment. I pooled the average proportion of prey attacked by individual and prey type and conducted a repeated-measures ANOVA on the arcsine square root transformed data. I found that the proportion of prey attacked varied significantly between prey types (repeated-measures ANOVA: $F_{2,12}=84.97$, $P<0.001$, Fig. 7.2a). Post-hoc tests of the differences between the means revealed the undefended prey were attacked at significantly higher rates than both the constant prey and undefended prey (Tukey's HSD: undefended –

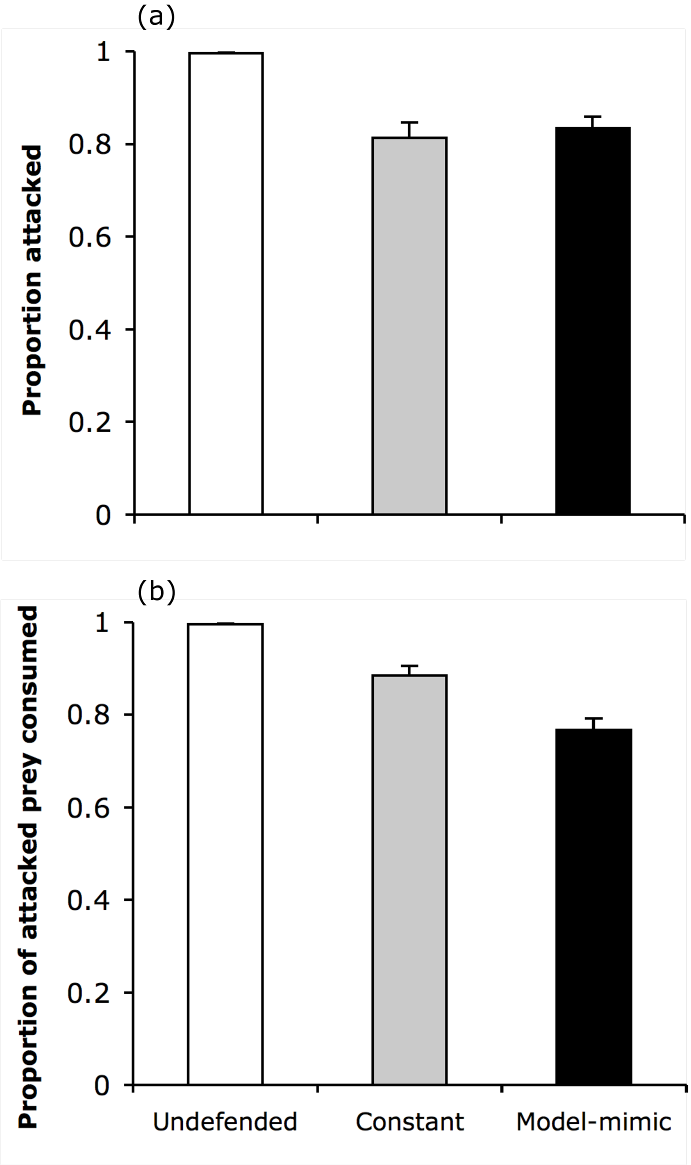

Figure 7.2. The mean (+SE) proportions of prey attacked in relation to prey type (a) and the mean proportion of attacked prey then consumed (b).

constant=22.17, *P*<0.01) and model-mimics (undefended – model-mimics=20.67, *P*<0.01). However, this was not the case for the difference between constant prey and model-mimics (constant–model-mimics=-1.50, *P*>0.05).

The second analysis I completed was and examination of the proportion of prey that were attacked which were subsequently consumed. This is not analogous to the rate of prey consumption (see Fig. 7.3) because the prey consumption is the product of these two factors. I analysed the data collected on the rates of attacked prey that were then consumed that had been pooled by prey type and individual. I found that the proportion of attacked prey that were subsequently consumed differed significantly between prey types (repeated measures ANOVA: $F_{2,12}$=50.32, *P*<0.001, Fig. 7.2b). There were significant differences between the mean proportions of attacked prey that were consumed by prey type (Tukey's HSD: undefended–constant=17.29, $P<0.01$; undefended–model-mimics=24.60, $P<0.01$; constant–model-mimics=7.31, $P<0.05$; Fig. 7.2b).

7.3.3 Changes in energetic state during the mass manipulation phase

In order to examine the relationship between body mass and furcular fat scores, I conducted regression analyses on each individual's daily masses and fat scores. These analyses revealed a significant relationship between bird mass and their levels of furcular fat scores (linear regression: 51M: $F_{1,85} = 122.61$, $P < 0.001$, $r^2 = 0.591$; 54M: $F_{1,83} = 108.63$, $P < 0.001$, $r^2 = 0.562$, 61M: $F_{1,85} = 44.32$, $P < 0.001$, $r^2 = 0.335$; 63M: $F_{1,84} = 62.79$, $P < 0.001$, $r^2 = 0.428$; 64M: $F_{1,85} = 46.30$, $P < 0.001$ $r^2 = 0.353$; 78M: $F_{1,85} = 113.98$, $P < 0.001$, $r^2 = 0.573$, 81M: $F_{1,85} = 227.94$, $P < 0.001$, $r^2 = 0.728$). These data indicate that the mass changes during the mass manipulation phase of the experiment, were due in large part to a reduction in body fat reserves. Therefore, I use mass as a surrogate for energetic state for the rest of the chapter because the mass has been shown to be significantly related to fat reserves.

It is also possible that there was also muscle atrophy during the mass manipulation phase of the experiment which I tested by measuring the pectoral circumference of birds at the beginning of the experiment and at FFI when the birds should have been theoretically most energetically stressed. I found that the diameter of the birds around the deepest part of their sternum was significantly smaller on FFI ($\bar{X}$ ± SE: diameter at beginning – diameter at FFI=-8.4 ± 2.00 mm, paired *t*-test: $t=-4.218$, $P<0.01$, df=6). This is likely to be due to atrophy of the pectoral muscle since there is little subcutaneous fat around any part of the bird at this point. Moreover, the pectoral muscles are the largest muscle group on a flighted bird's body and so may indicate an overall reduction of body muscle.

7.3.4 Gross indicators of prey preference

In order to see if birds maintained this learned preference throughout the mass manipulation phase of the experiment I conducted two crude analyses on the data that I generated. First, I compared the number of each prey type consumed on each day of the mass manipulation phase of the experiment. Second, I compared the number of days that the birds included all of a particular prey type throughout the mass manipulation phase of the experiment.

For the first analysis, I calculated the mean number of each prey type consumed per daily trial throughout the entire mass manipulation phase of the experiment. I found that the daily consumption of prey during the mass manipulation phase of the experiment differed by prey type (repeated-measures ANOVA: $F_{2,12}$=40.134, P<0.001, η^2=0.870, Fig. 7.3). Comparisons between contrasts revealed that the daily consumption of the undefended prey was significantly higher than the other two prey types (LSD: undefended – constant=1.1, P=0.002, undefended – model-mimic=1.47, P=0.001). The numbers of constant prey consumed tended to be higher compared to the model-mimics, although, the trend was not quite significant (constant – model-mimic=0.37, P=0.072). This result indicates that the bird's preferences that they formed in training were partly maintained throughout the mass manipulation phase of the experiment.

When I compared the difference in the number of models and mimics consumed throughout the mass manipulation phase, I found that mimics were consumed at significantly higher rates than models ($\overline{X}$ ± SE: models=97.4 ± 3.99, mimics=99.9 ± 4.03; paired t-test: t=-6.58, df=6, P<0.001). This difference over the mass manipulation phase meant that approximately 2.4 extra mimics were consumed over the entire experimental phase of the experiment (range=43-56 days). Therefore, this result is not biologically meaningful because it is the result of a small standard error of the differences between the two means which generated a high t statistic.

For the second analysis, I calculated the number of days where each bird consumed all of a particular prey type. Although, this was a crude measure of preference, it yielded a very significant result indicating that despite this conservative

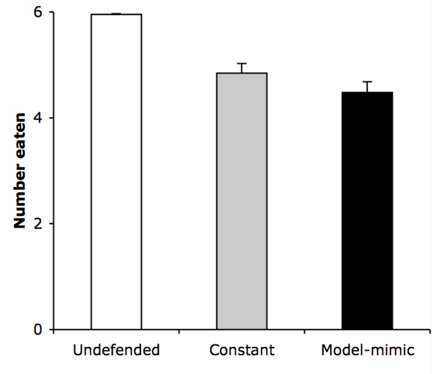

Figure 7.3. The mean number of each prey type (+SE) consumed on each day of the mass manipulation phase of the experiment.

statistical method, the order of preference was highly robust ($\bar{X}$ ± SE: undefended=50.57 ± 2.319, constant=18.17 ± 1.815, model-mimic=12 ± 1.461; repeated-measures ANOVA: $F_{2,12}$=55.622, P<0.001, η^2=0.903, Fig. 7.4). Pair wise comparisons by prey type indicated that all undefended prey presented in a daily trial were consumed on a significantly greater number of days when compared with constant prey and model-mimics (LSD: undefended – constant, P=0.001; undefended – model-mimic, P=0.001). However, there was no significant difference between the number of days where all mealworms were consumed for the two defended prey types (constant – model-mimics, P=0.176).

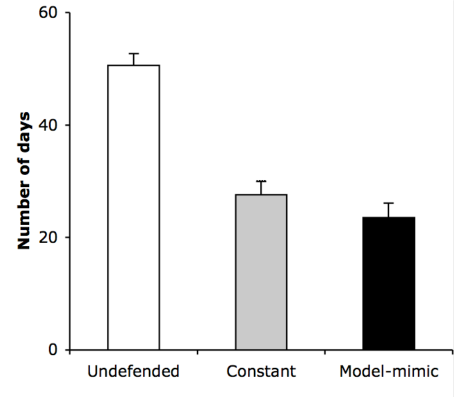

Figure 7.4. The mean number of days (+SE) on which birds consumed all of a particular prey type.

7.3.5 Preferences of birds with changes in mass

As with the previous experiment (Chapter 6), one of the aims of this experiment was to ascertain the effects that short term changes in energetic state have on the consumption of chemically defended prey. I predicted that if signal reliability and defence variability are is important for birds attempting to strategically manage bodily toxin burdens, birds may prefer prey with colour cues that reliably indicate their level of chemical defence (constant prey) over prey with cues that are not good predictors their levels of defence (model-mimics).

I constructed a linear mixed model (LMM) of the proportion of each prey type consumed in association with the birds standardised mass (split into 0.01 graduations) during the mass loss and mass gain periods of the experiment. The mass loss phase of the experiment was defined as being all of the days where birds were losing mass until they started eating all the prey offered to them (FFI). I also included the next two days after FFI in order to ensure that the consumption of all the prey on the first day was not a chance event. This also aided in standardising the endpoint of each bird's mass loss phase. I included standardised mass and prey type as main factors and individual birds included as a random subject variable. The arcsine square root transformed mean proportion of prey consumed was the dependent variable. The model for the mass loss phase of the experiment shows that as birds lost mass, they increased the number of chemically defended prey in their diets (LMM: $F_{14,135}$=4.534, *P*<0.001, Fig. 7.5a). There were many significant differences in the pair-wise comparisons between different masses, which have been included in Table 7.3. There was also a significant difference in the numbers of prey consumed by prey type ($F_{2,135}$=74.654, *P*<0.001, Fig 7.5a). Pair wise comparisons between the means revealed that there were significant differences in the proportions of prey consumed among all three prey (LSD: undefended – model-mimics=32.9, *P*<0.001; undefended – constant=23.6, *P*<0.001; model-mimics – constant=-9.2, *P*<0.001). The interaction term for mass by prey type was not significant ($F_{28,135}$=0.913, *P*=0.596). The estimated total variance of the model was approximately 212 of which 42.5 (20.11%) was attributable to variation between individuals. This meant that between subjects variance was not a significant source of variation for the model as a whole (Wald *Z*: *Z*=1.467, *P*=0.142).

Once the birds had consumed all of the prey items offered for three consecutive days, I began incrementally feeding the bird more food in order to allow them to begin gaining mass. The first day when a subject did not eat all of the prey offered was the day of first exclusion (FE). I continued to allow birds to slowly gain mass until the birds had reached the free-feeding masses that had been calculated for them at the beginning of the experiment. From the first day, that the bird did not include all of the prey items I included the three days prior to first exclusion in the LMM. This was to ensure all the birds' foraging choices were being started at the same point. As with the mass loss data, I constructed a LMM, with standardised mass and prey type as main factors and individual birds as a random subject variable. The arcsine square root transformed proportion of prey consumed was the dependent variable. The proportion of prey consumed was dependent on the standardised mass with fewer defended mealworms being eaten as the birds gained mass (LMM: $F_{14,120}$=3.698, *P*<0.001, Fig. 7.5b). Comparisons of the means between different masses revealed that the differences were among 0.9 and six other mass classes (LSD: 0.9 – 0.96=21.1, *P*=0.019; 0.9 – 0.97=22.2, *P*=0.004; 0.9 – 0.99=21.3, *P*=0.017; 0.9 –1.00=23.1, *P*=0.046; 0.9 – 1.01=26.5,

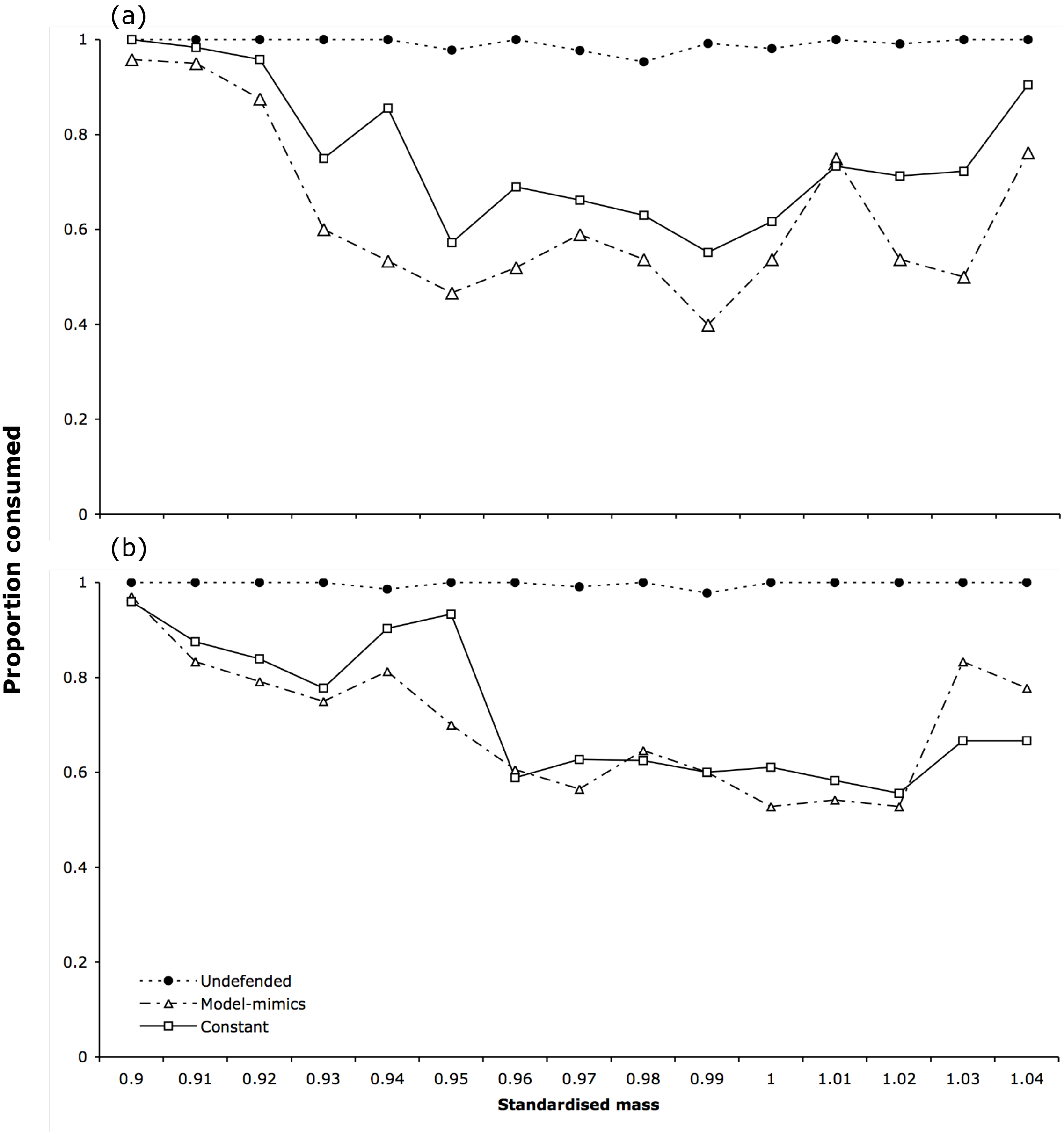

Figure 7.5. The mean proportions of each prey type consumed in each mass class as birds lost mass (a) and gained mass (b). Standard error bars omitted for clarity.

Table 7.3. Significant pair-wise differences in the proportion of prey eaten at different standardised masses calculated using the LSD method as birds lost mass. All other pair-wise differences are non-significant.

Mass (i)	Mass (j)	Mean Difference (i-j)	Standard Error	df	P
0.90	0.95	29.5	6.46	132	0.001
	0.96	24.1	6.46	132	0.031
	0.97	27.6	6.25	132	0.002
	0.98	27.6	6.46	132	0.004
	0.99	32.5	6.25	132	<0.001
	1.00	28.7	6.45	133	0.002
0.91	0.95	27.4	6.46	132	0.004
	0.97	25.6	6.25	132	0.008
	0.98	25.5	6.46	132	0.013
	0.99	30.5	6.25	132	<0.001
	1.00	26.7	6.45	133	0.007
0.92	0.95	24.5	6.46	132	0.024
	0.97	22.6	6.25	132	0.045
	0.99	27.5	6.25	132	0.002
	1.00	23.7	6.45	133	0.036

P=0.002; 0.9 – 1.02=31.8, P=0.009). All other comparisons among different masses were non-significant. The proportions of prey consumed also varied between prey types ($F_{2,120}$=56.075, P<0.001, Fig. 7.5b). The birds preferred to consume undefended prey as opposed to the other two prey defended prey types (LSD: undefended – constant=30.9, P<0.001; undefended – model-mimic=27.3, P<0.001; Fig. 7.5b), but there was no significant difference in the proportions of constant prey and model-mimics consumed (constant – model-mimics=3.6, P=0.776). The interaction between mass and prey was not significant as birds gained mass ($F_{28,120}$=0.964, P=0.524). The estimated total variance of the model was approximately 285 of which 67 (23.5%) was attributable to variation between individuals. This meant that between subjects variance was not a significant source of variation for the model as a whole (Wald Z: Z=1.011, P=0.312).

Taken together, these analyses of the two phases of mass loss and mass gain indicate: (1) that the proportion of chemically defended prey consumed was dependent on mass (Fig. 7.5), and (2) that as birds lost mass there was a preference for constant prey over the model-mimics which was not evident as birds began gaining mass again.

7.3.6 Behaviour of birds while attacking and consuming prey

Throughout the mass manipulation trials, I had been collecting the types of behaviours that birds had been displaying towards to prey while handling them. This allowed me to conduct three analyses on the data that I collected: (1) I compared the amount of handling time birds displayed towards each prey type; (2) I examined the number of behaviours that birds exhibited towards the different prey types as the mass manipulation phase of the experiment continued; and (3) I analysed the frequency each behavioural type was exhibited in relation to prey type.

I hand timed the latencies to attack for each prey presented throughout the mass manipulation phase of the experiment. These data allowed me to compare the amount of time that birds spent handling each prey type by pooling data for the three prey types used throughout the entire mass manipulation phase of the experiment. I found that the birds handled the different prey types for significantly different lengths of time (repeated measures ANOVA: $F_{2,12}$=17.206, P<0.001, η^2=0.741, Fig. 7.6). Pair-wise comparisons revealed that there were significant differences between the undefended prey compared with both the constant prey and model-mimics (LSD: undefended – constant=-5.7, *P* = 0.020, undefended – model-mimic=-5.3, *P*=0.014). However, there was no difference between constant prey and model-mimics (constant – model-mimic=0.4, *P*=0.819). When I compared at the models and mimics separately, I found that models were handled for significantly longer than for mimics ($\bar{X}$ ± SE: mimics=5.0 ± 0.92 sec, models=8.2 ± 1.65 sec, paired *t*-test: *t*=4.288, df=6, *P*=0.0052).

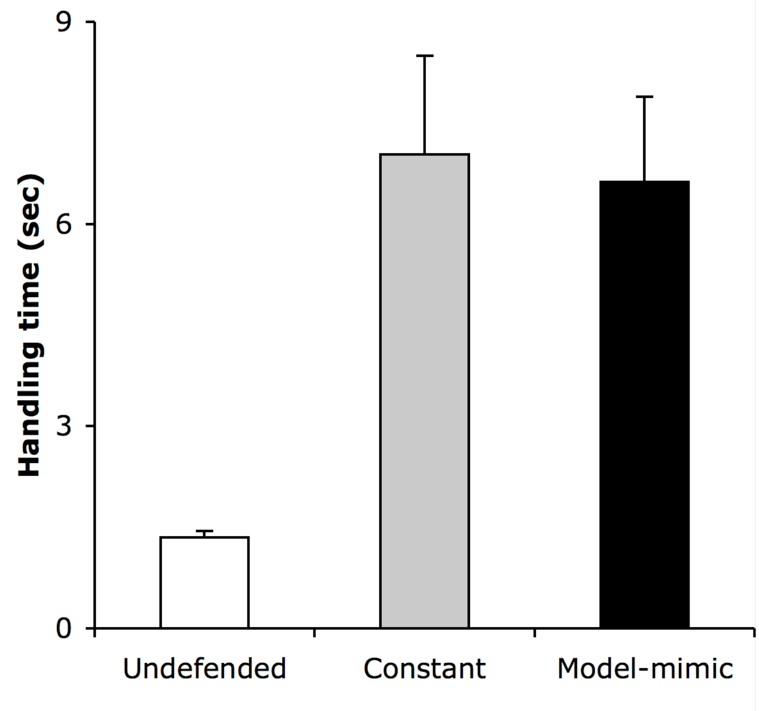

Figure 7.6. The mean handling times (+SE) of each prey type throughout the experimental phase.

I analysed the mean number of behaviours that birds expressed towards prey as the experiment progressed using a LMM on the square-root transformed data in which prey type and standardised time were factors. Subjects were included in the model as a random subject variable and the number of behaviours expressed per trial was the dependent variable. The model revealed that as the mass manipulation phase progressed birds exhibited greater numbers of behaviours towards all prey (LMM: $F_{19,354}$=4.368, P<0.001, Fig. 7.8). Pair-wise comparisons between means for the numbers of behaviours expressed indicated that there were many significant differences in the means (Table 7.3). There was also a significant effect of prey type ($F_{2,354}$=167.285, P<0.001, Fig. 7.7) with the birds expressing far fewer behaviours when handling undefended prey (LSD: undefended – constant=-0.477, P<0.001; undefended – model-mimics=-0.468, P<0.001). However, there was no significant difference in the number of behaviours expressed in each trial when constant prey were compared with model-mimics (constant – model-mimic=0.009, P=1). The interaction between time and prey type was not significant ($F_{38,354}$=1.008, P=0.461). The estimated total variance of the

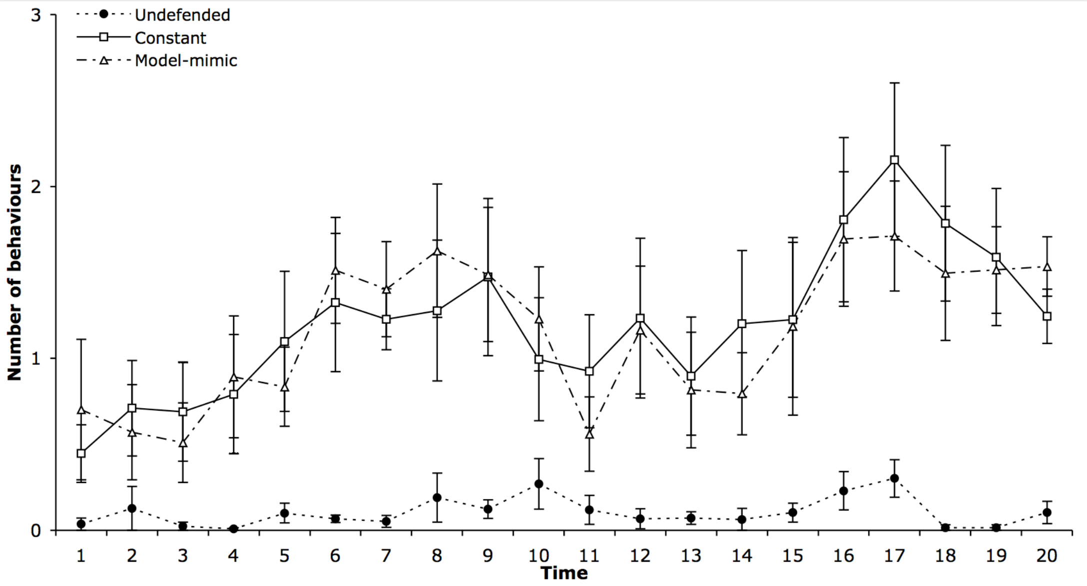

Figure 7.7. The mean number (±SE) of handling behaviours plotted against standardised time throughout the course of the mass manipulation phase of the experiment.

Table 7.3. Significant differences in the pair wise comparisons between the means in the number of behaviours displayed in a presentation at different times throughout the mass manipulation period of the experiment calculated using the LSD method.

Time (i)	Time (j)	Mean Difference (i-j)	Standard Error	df	P
1	16	-0.35	0.077	354	0.001
	17	-0.41	0.077	354	<0.001
	18	-0.29	0.077	354	0.047
2	16	-0.34	0.077	354	0.013
	17	-0.37	0.077	354	0.001
3	16	-0.34	0.077	354	0.003
	17	-0.40	0.077	354	<0.001
4	17	-0.34	0.077	354	0.003

model was approximately 0.0937 of which 0.0313 (33.4%) was attributable to variation between individuals. This meant that between subjects variance was not a significant source of variation for the model as a whole (Wald Z: Z=1.676, P=0.094). Interestingly, I also found that the birds expressed a greater number of handling behaviours when they attacked the models compared to the mimics ($\bar{X}$ ± SE: mimics=0.989 ± 0.229, models=1.383 ± 0.275, paired t-test: t=6.401, P<0.001, df=6).

In order to examine if there were differences in the way birds handled the three prey types, I examined the proportion of all trials where eight different behaviours that had been listed in Table 7.2 were exhibited. I also constructed a LMM for the arcsine square root transformed proportion of trials in which the behaviours were expressed. Prey type and type of handling behaviour were included as fixed variables and subjects were included as a random subject variable. The eight behaviours (biting, crushing, wiping, hitting/shaking, head shaking, pecking, gaping/casting, bill wiping) that were chosen were the eight most common as judged by the proportion of trials in which they were expressed. All other behaviours were negligible or very rare. The model indicated that there were significant differences in the proportion of trials in which any of these eight behaviours were expressed by prey type (LMM: $F_{2,144}$=34.875, P<0.001, Fig. 7.8). Pair wise comparisons of the birds' handling behaviour indicated there were differences between the undefended prey and the two defended prey types, but there was no significant difference between constant prey and model-mimics (LSD: undefended – constant=-14.505, P<0.001; undefended – model-mimic=-18.076, P<0.001; constant – model-mimics=-3.571, P=0.365; Fig. 7.8). There was no significant effect for the proportion of times that each behavioural type was exhibited ($F_{7,144}$=1.802, P=0.091). However, given that there was a lot of noise in the data due to increased prey handling as the experiment progressed (Fig. 7.8) and the P-value being less than 0.1, this might be indicative of a developing trend (see section 8.3.4 for further evidence of differences between treatment of models and mimics). There was also no significant difference in the interaction between prey type and behaviour ($F_{14,144}$=0.211, P=0.999).

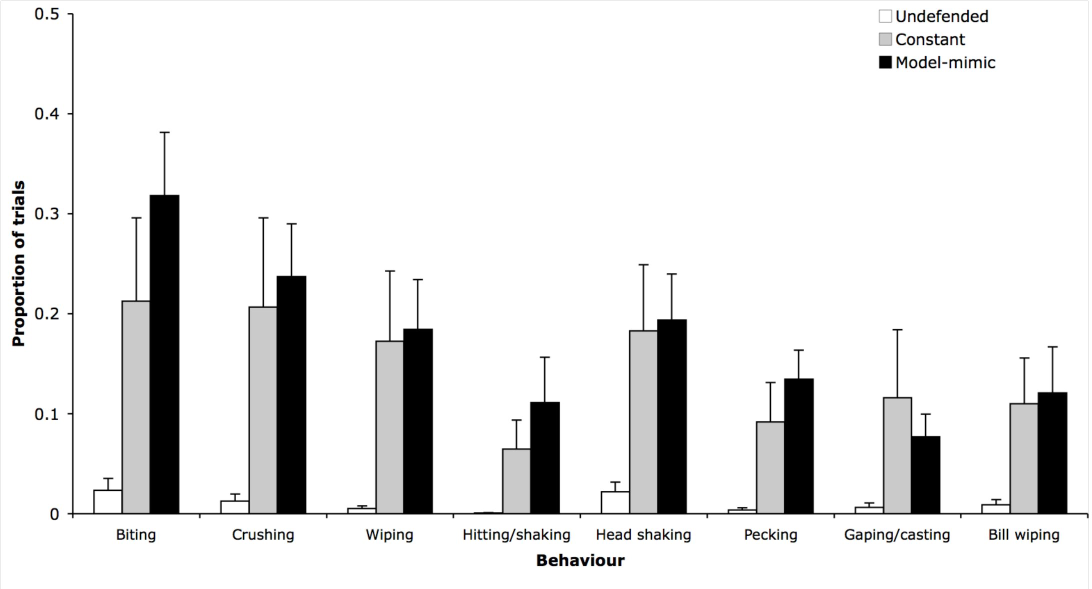

Figure 7.8. The mean proportion (+SE) of trials in which the most commonly expressed handling behaviours and post-ingestive reactions were expressed when attacking each prey type.

7.3.7 Attack latencies throughout the experiment

Attack latencies may be an indicator of energetic stress of the birds throughout the mass manipulation phase of the experiment. In order to examine this, I constructed a linear mixed model on the square-root transformed means of the latencies of the birds at three points during the data collection phase of the experiment: (1) the last three days of training where there were significant differences in the number of prey consumed among prey types (as reported in section 7.3.1); (2) the three days when the birds consumed all the prey in the mass manipulation phase; and (3) the last three days of the experiment. I included the prey type and time within experimental phase as factors and subjects were included as random subject variables. There was a significant effect of the experimental phase (LMM: $F_{2,48}$=4.634, P=0.014, Fig. 7.9). Pair-wise comparisons of the means indicate that the latencies at the beginning of the experiment were significantly longer than those recorded during the middle of the experiment (LSD: 0.245, P=0.017). Neither of the other pair-wise comparisons were significant (start – finish=0.052, P=1, mid-experiment – finish=-0.193, P=0.083). The model also indicated that there were significant differences between the attack latencies for each prey type in the experiment ($F_{2,48}$=6.705, P=0.003, Fig. 7.9), although the differences were between the undefended and the two defended prey types (LSD: undefended – constant=-0.296, P=0.003, undefended – model-mimics=-0.23, P=0.028). There was no significant difference between the constant prey and model-mimics (constant – model-mimics=0.67, *P*=1). The interaction between time and prey type was not significant ($F_{4,48}$=0.365, *P*=0.832). The estimated total variance of the model was approximately 0.226 of which 0.151 (66.57%) was attributable to variation between individuals. This amount seems high but the standard error in was also vary high relative to the mean which meant that between subjects variance was not a significant source of variation for the model as a whole (Wald *Z*: *Z*=1.64, *P*=0.101).

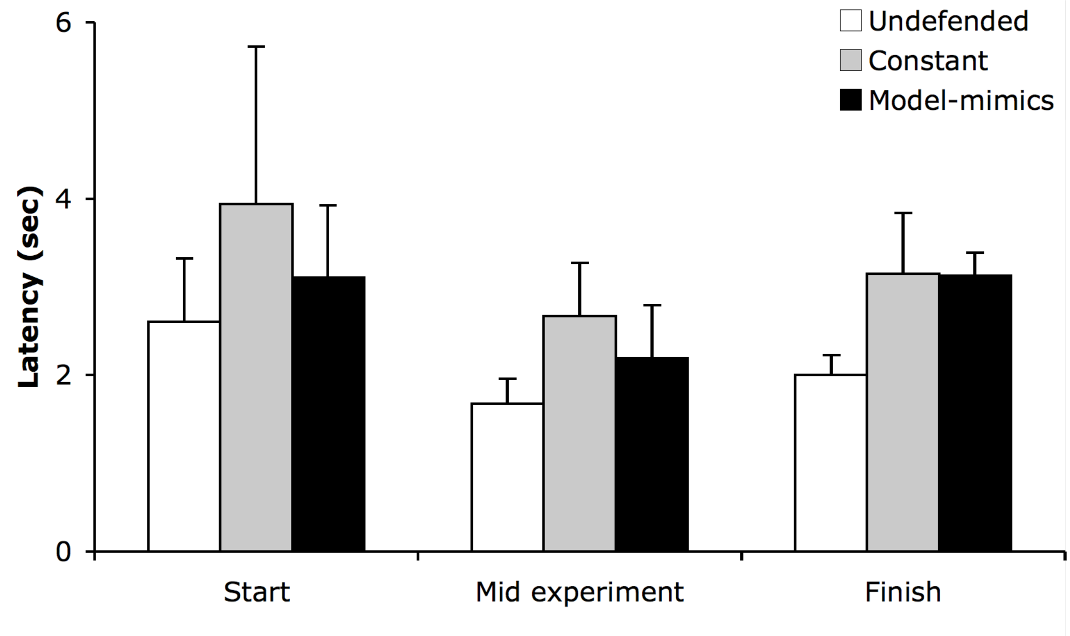

Figure 7.9. The mean latencies to attack (+SE) in relation to prey type and at different times throughout the experimental phase of the experiment.

7.4 Discussion

The aim of this experiment was to assess the effect of signal reliability and defence unpredictability on the foraging choices made by birds foraging on two types of chemically defended prey and how these choices were affected by changes in mass. The results indicate that the prey with the unreliable signals and variable defences (model-mimics) tended to have an advantage over the prey with reliable signals and constant defence (constant prey). As the mass manipulation phase of the experiment progressed, this advantage declined. This suggests that the reliability of signals and variability of chemical defences could also play a role in how educated predators choose among different types of chemically defended prey. This indicates that not only the level of defence is important, but also the reliability of the signal may be important in the decision making process. Signal reliability is an area of intense debate in other areas of animal signalling research. The debates centres around whether signals need to entail a cost in order to be reliable. The debate has been resolved to a degree as there is growing evidence that reliable signals can evolve that do not entail costs (Maynard Smith & Harper 2003).

Aposematic signals have recently been discussed as reliable indicators of prey defence because aposematic species can afford to pay the cost of being detected (Sherratt 2002; Sherratt & Beatty 2003; Speed 2003). However, these discussions focussed on explanations favouring the evolution of distasteful models away from less well-defended mimics in order to create easily identifiable signals to aid predator recognition. The problem presented in this chapter was different because birds were presented with two defended prey that differed in not in their average level of quinine, but in their level of variation around this mean. This meant that the colour cue was an unreliable signal of defence level in the model-mimics. To my knowledge this is the only study that has attempted to present this problem to predators feeding on chemically defended prey. However, there is another field in foraging theory that deals with different rewards with identical means but differing amounts of variance about the mean.

Risk sensitive foraging is an area which has studied similar problems (i.e., where prey types have different distributions of variance of nutrient reward [see Chapter 3]). Surprisingly, most studies of risk-sensitive foraging fail to acknowledge the importance of uncertainty as a possible factor promoting risk-aversion (but see Real & Caraco 1986; Real 1992). Risk sensitive foraging studies also show that animals are more likely to show risk aversion for amount of rewards (see Kacelnik & Bateson 1996; Bateson & Kacelnik 1998 for reviews), which could be partly explained by the birds being certain of the reward when selecting a constant food option. Bateson and Kacelnik (1998) discuss the possible explanations for risk-sensitive foraging. However, it is difficult to see how many of these explanations would apply to the problem as presented. For example, the energy budget rule cannot predict the results of this chapter as it only deals with variation in the amount in food. Although the different defended prey used in this experiment had variations in their levels of quinine that could have made caused variation in their net profitabilities there are sufficient differences in the design between this experiment and traditional risk-sensitive foraging approaches to suggest that the two approaches are not analogous. For example, most risk sensitive foraging experiments for amount present the prey options simultaneously. In this experiment, prey were presented sequentially meaning that the preferences for one prey over another could not be tested in the same method as in a risk-sensitive foraging experiment. Therefore, there is little that risk sensitive foraging can tell us about these results.

The results also indicate that birds were also able to perceive the difference in the levels of variance between the constant prey and the model-mimics which lead to the initial preference for constant prey over mimics. Therefore, it is likely that birds may have been able to perceive quinine via the gustatory or trigeminal nervous pathways (Werner & Clark 2003). However, the post-ingestive pathway that leads to predators learning the association between ingestion of a food type and its consequences are still poorly understood. Recently, Yearsley *et al.* (2006) published a model of learning based on ideas from information theory. Their general finding was that the rate of learning and memory retention will be dependent on a number of factors including the temporal disassociation between the ingestion of food and the onset of the post-ingestive consequences. This finding may lead to a preference for the constant prey as the reliability of information that the bird experiences with the model-mimics may lead to difficulties in forming associations between the ingestion of the prey and its post-ingestive consequence.

As I slowly reduced the amount of food to which birds had access, this reduced the levels of energetic reserves of the birds and caused the number of chemically defended prey (both constant and model-mimics) eaten during daily trials to increase. There was a significant difference between the two defended prey types consumed with the constant prey being preferred to the model-mimics. In the mass gain phase of the experiment, the difference in the proportion of constant prey and model-mimics was no longer significant. This meant that there was an initial benefit for the model-mimics, but this benefit declined as the experimental mass manipulation continued. The likely cause was increases in handling behaviour. This might have been due to birds learning to taste the model-mimics given that the visual cue provided for the model-mimics was unreliable. However, it is also possible that the birds were attempting to reduce the amount of quinine contained by the mealworms (see section 8.4 for a more detailed discussion of this matter).

There are three possible reasons for the early preference of the constant prey over the model-mimics. First, birds might have been utilising only visual cues associated with the mealworms early in the experiment. Therefore, the birds might have favoured the constant prey over model-mimics because of their higher signal reliability associated with the prey item. As the experiment proceeded, the birds learned that they could also taste the mealworms in order to distinguish between model-mimics. Second, a variable defence as in the model-mimics is predicted to slow the rate of learning (Yearsley *et al.* 2006). This is the opposite of what I found as birds excluded the variable prey in greater numbers earlier in the experiment. However, it is also possible that the birds preferred to eat prey about which they have learned more. Third, it is possible that the birds' perceptions of the levels of quinine in the models and constant prey were non-linear. For example, a model may have been perceived as being more than twice as distasteful as the constant prey. If this were the case, then this could lead to preference for the constant prey types over the model-mimics.

The experiment manipulated the energetic state of birds in a manner similar to Chapter 6. This was a further test that birds will increase their intake of chemically defended prey as their energy reserves decline. Again, the results of this chapter provide evidence of the energy/toxin trade-off that animals will consume greater numbers chemically defended prey at times of energetic stress, which is not a new idea (e.g., Poulton 1890; Swynnerton 1915; Brower *et al.* 1968; Speed 1993b). The saturation hypothesis (Mallet & Joron 1999; Mallet 2001) stipulated that predators might readily consume defended prey until their body levels of toxins reach a saturation point. More recently, this idea was formalised in a SDP model that examined the relationship between the consumption of chemically defended prey and physiological state (Sherratt *et al.* 2004). This model had two state variables, which were the levels of energy reserves and the level of body toxins. The model predicted that the birds should attack chemically defended prey depending on the amount of constant food available and the levels of energy reserves of the bird. The results from this study indicate that animals increase the number of chemically defended prey in their diet with decreasing energy reserves. Therefore, these results indicate that partial prey preferences (asymptotic rate of attack [Speed 1993a]) are dependent on the levels of energy reserves. However, the data also indicate that the birds accept higher body burdens of quinine as their energetic reserves decline. This indicates either that the critical value of quinine that birds accept changes with energetic state or that the birds are strategically trading-off the costs of ingesting toxins against the energetic benefits derived from the mealworms at a level below the upper limit of their possible quinine intake. It is unlikely that the increase in consumption of defended mealworms is wholly due to an increasing tolerance of quinine as the birds decreased the consumption of chemically defended prey when they began gaining mass again. Therefore, it is likely that the birds were increasing the number of chemically defended prey that was below the critical toxin burden, but were prohibitively costly to ingest at times of lower nutritive need. This indicates that the birds are able to facultatively manage their levels of quinine intake in accordance with their levels of energy reserves.

Birds have been shown to be able to strategically manage their levels of body mass in the field (Thomas 2000; Barnett, unpublished data). However, there is comparatively little previous evidence that animals are able to manage their levels of body toxins (Skelhorn & Rowe, unpublished data). Recently, Skelhorn and Rowe (unpublished data) conducted an experiment where European starlings were fed with three undefended mealworms or three mealworms containing quinine before the start of an experimental trial where birds were presented sequentially with eight defended (quinine injected) and eight undefended (water injected) prey. They found that when birds were pre-fed with mealworms containing quinine, birds consumed fewer defended mealworms during the experimental trials. In earlier chapters, it was shown that birds started excluding defended prey earlier in the daily trials. Chapter 5 also indicated that this trend was more extreme for the more heavily defended mealworms. In earlier chapters, it has been found that if the daily consumption data are plotted for the birds, that they consumed fewer defended prey later in daily trials (section 5.3.2, Fig. 5.5, and Fig. 5.6) and that this effect is more extreme for more heavily defended prey (section 6.3.3 and Fig. 6.4). Therefore, these data together indicate that birds are able to control the intake of quinine.

It is unknown how the birds might be able to strategically manage their levels quinine as the perceptual pathway that links the perception of the colour signal with the distasteful compound is still poorly understood. One possibility is that the birds continue to consume the prey containing quinine until they begin to experience physiological effects of quinine. However, the fact the birds continue to consume undefended prey indicates that the birds were not suffering from nausea or other side effects, which might be expected if quinine was making birds sick. How birds perceive quinine and other toxins and strategic regulate their body toxin loads is an interesting research area in need of further research. Despite our lack of knowledge regarding the probable mechanism, these data add further support for birds having the ability to strategically manage their body loads of toxins.

Another possibility is that birds might learn to taste the prey rather than rely on colour cues associated with the prey. Signal detection theory indicates that when the discriminability between two signals becomes low then other means should be used in order to differentiate between options (see Wiley 2006 for a review). This is especially the case in order to discriminate between two almost visually indistinguishable prey types as may be the case in perfect Batesian mimicry and automimicry (Oaten 1975; Getty 1985). For example, if the visual signal of a prey is unreliable as in the case of a Batesian mimic, then the predator should begin tasting the prey for chemical defences. An example of this has been observed in the black-backed oriole (*Icterus galbula abeillei*) which seems able to taste reject monarchs that contain higher levels of cardenolides (Fink & Brower 1981, Brower & Calvert 1985, Brower 1988). Moreover, birds select the parts of the butterfly that contain the least cardenolides (Brower 1988). This behaviour suggests that animals are able to mediate their levels of toxin through behavioural means in what Brower (1988) called an exapation (the modification of a pre-existing trait for a new function [Gould & Vbra 1982]) of foraging behaviour. However, it is unknown if the behaviour is a foraging innovation that occurred *de novo* or if the behaviour was an exapation.

Animals have been known to mediate the chemical defences of their prey for a long time. However, the majority of examples come from specialised predators. Perhaps the most famous example of handling behaviour is that of bee eaters (family Meropidae). Bee-eaters are known to consume a large proportion (20-96 % of all insects of all insects caught) of hymenoptera in their diets, which they catch on the wing by hawking (Fry *et al.* 1992). Before eating a hornet, the bee-eater kills the prey by striking it on the head in order to kill it. Then they apply pressure to the insects' abdomen forcing the venom and sting from it before consumption. This is highly specialised behaviour and extremely effective. Most studies of mimicry and aposematism have been primarily concerned with the rates of predation of prey or the rate of survival by prey. Moreover, most of these studies have only investigated birds' rates of attack rather than handling behaviour. For example, Marples *et al.* (1994) showed that birds displayed more head-shakes towards prey that were more potently defended compared with other prey which suggests that predators' handling behaviour does have the potential to indicate prey differences. However, no previous study has presented the development of prey handling behaviour in a generalist foraging species.

The results from the last three chapters build a compelling case for the strategic consumption of chemically defended prey in relation to energetic need. In all three state based experiments I have carried out, I have found a very strong state effect where birds increase the number of chemically defended prey they consume with decreasing state. They suggest that energetic state is an important factor in determining the level of predation on aposematic prey. The data also suggest that the birds may strategically manage their body burdens of toxins throughout the daily trials by reducing the number of defended prey that they consume. However, the data cannot be taken as conclusive experimental evidence for this occurrence. This chapter extends this work by indicating that increasing risk and signal unreliability can affect the preference over the short-term, but over the long term, animals adopt behavioural strategies to help mediate the fact that a visual signal may have little value for one prey. It would be interesting to attempt replicating this result without the mass manipulation. I predict that if this were done, the preference of the birds for the constant prey over model mimics would take much longer to extinguish or may even persist indefinitely. This is because my mass manipulation made birds much more likely to attack prey than they would otherwise have been. Through their increased rates of attacks and consumption, the birds learned that they may have been able to taste the differences in quinine between models and mimics. They may have also learned that they could mediate the levels of quinine through squeezing it out of the prey.

7.4.1 Conclusions

This study has tested the value of defence variation and signal reliability to birds in a long term feeding experiment that also manipulated the subject's energetic states. The results indicated that the model-mimics had an advantage over the constant prey in the mass gain period of the experiment, but this advantage declined as the experiment progressed. This early advantage for the model mimics over the constant prey was probably due to the ambiguity of the colour signal associated with the model-mimics. As the experiment progressed, the birds began exploiting other sensory cues associated with the prey (e.g., taste). The results of this experiment also suggest that the birds were able to manage their intakes of toxins strategically in line with predictions of the recent SDP model that indicated that birds may have a critical value for the levels of toxic defences ingested. Finally, the behavioural data indicated that the birds treated the three prey types differently and that the birds may have learned to reduce the levels of quinine contained in the mealworm through prey manipulation.

The results of this experiment are important as they suggest new factors that might be significant in promoting the evolution of mimicry. First, the uncertainty that may be associated with model-mimic systems has not been considered an important factor in the study of mimicry. The data show that prey belonging to mimicry systems may gain at least some initial protection from the uncertainty of prey type that would be encountered in addition to the protection from chemical defences. Signal uncertainty and defence variation has not often been thought to directly influence predator decisions of whether to consume chemically defended prey. For predators strategically managing their levels of chemical defences they may favour prey with reliable signals and predictable levels of chemical defences. These results also indicate the potentially important role that behaviour can have in regulating the intake of distasteful or toxic substances. Finally, these results add to the growing amount of evidence that indicates that the birds physiological state in the form of energy reserves and levels of toxins play an important and interrelated role in birds' decisions to consume chemically defended prey.

Chapter 8

THE EFFECT OF MIMIC ENCOUNTER RATE ON PREDATION AND SURVIVAL IN A BATESIAN MIMICRY SYSTEM

8.1 Introduction

Batesian mimicry is the resemblance between two warningly coloured species, one of which is defended (the model) and the other is not (mimic). Generally, Batesian mimics gain a protective advantage from resembling the model meaning the relationship between models and mimics is considered parasitic. Mimics reduce the effectiveness of the model's chemical defences at deterring predation, leaving models to pay the costs of producing and storing chemical defences. Many factors can affect the rates of predation on species in a Batesian mimicry system.

The rate of predation on aposematic models and undefended Batesian mimics is dependent on three main factors: (1) the proportion of models to mimics, (2) the potency of the models defences, and (3) the availability of alternative undefended prey (see section 4.2.1 for a review of each of these points). Bates (1862) stated that undefended mimetic species were likely to be rare in relation to models because the efficacy of a signal as a deterrent is thought to be directly related to the level of defence that is associated with the signal (Yearsley *et al.* 2006). As palatable mimic frequency increases the average level of defence experienced or the frequency a model encountered by the predator is diluted. Increases in the abundance of mimics within a Batesian mimicry system have a negative effect on the efficacy of aposematic signals. This has been confirmed in many empirical studies where experimental increase in the number of Batesian mimics increased the rates of predation (Brower 1960; Duncan & Shappard 1965; Lea & Turner 1972; Pilecki & O'Donald 1971, Goodale & Sneddon 1977, Huheey 1980; Avery 1985, Nonacs 1985, Lindström *et al.* 1997).

8.1.1 Experiment introduction

A recent SDP model (Sherratt *et al.* 2004) analysed the state-based consumption of chemically defended species and their mimics. In this model, the authors analysed how a predator's energetic state can play a role in determining the attack rates on the mimicry system when models and mimics are considered separately. I have tested various predictions of this and other related SDP models in earlier chapters, and have found that my data generally support the SDP models of the state-based consumption of moderately defended prey. However, these models also predict other behaviours that are not considered by the traditional models of aposematism and mimicry.

One counter-intuitive prediction of the Sherratt *et al.* (2004) model was that under some conditions, increasing the probability of encountering a mimic would decrease the probability of attack for both models and mimics (the SDP models talk only of attack although I equate this with predation). This prediction seems contrary to previous findings that have increased the frequency of Batesian mimics and have found that attack rates were higher with greater frequencies of mimics (Brower 1960; Duncan & Shappard 1965; Lea & Turner 1972; Pilecki & O'Donald 1971, Goodale & Sneddon 1977, Huheey 1980; Avery 1985, Nonacs 1985, Lindström *et al.* 1997). If increasing the frequency of mimics were to decrease the rate of attack or ingestion of models and mimics, this would be termed a quasi-Müllerian effect because a normally parasitic Batesian mimic would reduce predation in a manner similar to that of a defended Müllerian co-mimic. Consider a situation where a predator's foraging bout is split into a number of discrete foraging periods. If the predator's probability of searching and finding food per foraging period is x, then the probability of not finding food is $1 - x$. If the amount of food found per unit of time is increased, then we can affect the overall density of food in the system. If this increase in food availability comes from mimics that are indistinguishable from models, then rather than dilute the protection of the model, this may actually increase the overall level of avoidance of the mimicry complex. This is because at high mimic density there is more food available, but because there is still a risk associated with the mimicry system, it may allow the predator to be more conservative in its diet unless it becomes critically low in energy reserves (Sherratt *et al.* 2004).

In this experiment, I specifically tested this prediction. All birds received two treatments where they were sequentially offered prey of three types. Sequential trials were used because simultaneous presentation of prey has been criticised by some authors (e.g., Hetz & Slobodchikoff 1988; Lindström *et al.* 1997) because predators might rarely encounter prey simultaneously in the field. Moreover, simultaneous presentations generally restrict the predator to attacking one prey item. In reality, a predator encountering two prey items at once, might attempt attacking both prey upon encounter. Hence, simultaneous presentations of defended and undefended prey may overestimate the survival advantage of the species in the mimicry system. The three prey types used in this experiment were alternative (undefended) prey, models (injected with quinine sulphate), and perfect Batesian mimics (resembled the models but were undefended) (Table 8.1). The alternative prey types also had a different colour signal from the model-mimics making them visually distinctive from them. In addition to presentations of these three prey types, the birds also had unsuccessful searching periods where no prey were encountered. By altering the number of unsuccessful searches and mimics between treatments, it was possible to test this novel prediction from the SDP models that the increase in mimics would increase the effectiveness of the mimicry system. This study is the first study to attempt investigating if quasi-Müllerian effects occur in a Batesian mimicry system. If the birds display quasi-Müllerian behaviour to potentially defended prey, this will change the way that Batesian mimics are thought to affect the predation of prey in Batesian mimicry systems.

8.2 Methods

8.2.1 Birds and Housing

I used six European starlings that had been used in Chapter 7 because they had been trained to the colour prey associations for the different types of defended prey which were maintained in this experiment. One bird was not used because it was getting stressed by being in the cage and was returned to the free flight. There were no state manipulations in this experiment and so the birds were fed *ad libitum* water and pheasant breeder pellets. Fruit was given to the birds with their daily food ration (see sections 2.2.1 and 7.2.1 for further details of bird husbandry).

8.2.2 Lid flipping

The birds had already been taught to flip lids for the previous experiment (see section 7.2.3 for details). Therefore, there was no initial training.

8.2.3 Training and prey preparation

In this experiment, I had alternative prey signalled by one colour, and a model-mimic complex signalled by another colour. Since all birds had already experienced these types of prey in the previous experiment (see section 7.2.3), I used the same colours that I used for these prey in the previous chapter. The alternative prey in this chapter were the same as undefended prey in the last experiment. The model-mimics in this experiment were also identical to those in the last chapter. The colour associations that the birds learned in the last chapter were also maintained. All other methods of bird training and prey preparation were the same as in the previous chapter (see 6.2.4 for further details).

8.2.4 Experimental protocol and daily schedule

The experiment aimed to simulate a period of foraging by a bird. This period was split into discrete time units (in the case of this experiment 24 three minute time units), during which a bird would encounter a prey item or not (see Fig. 8.1). When the bird encountered a prey item, it had the option of choosing to flip, attack, and consume the mealworm. The prey were of three types: (1) alternative prey which had no chemical defences, (2) models that were moderately defended with quinine sulphate, and (3) mimics that contained no chemical defences but were visually identical to the models. In some of the foraging periods, no prey was offered to the birds, which was intended to simulate a period where searching was unsuccessful. The birds were given two treatments that differed in the number of mimics presented and the number of times that no prey were presented (Fig. 8.1). Both treatments had eight alternative prey and four defended models (Fig. 8.1). In the low mimic frequency treatment, four mimics were presented and there were eight occasions where no prey were presented. In the high mimic frequency treatment, the mimic frequency increased so that 10 mimics were presented to birds, but that there were only two occasions where birds received no food (Table 8.1, Fig. 8.1). All prey were all prepared in accordance with the methods that were described in Chapter 7 (see section 7.2.5 and Table 7.1 for details).

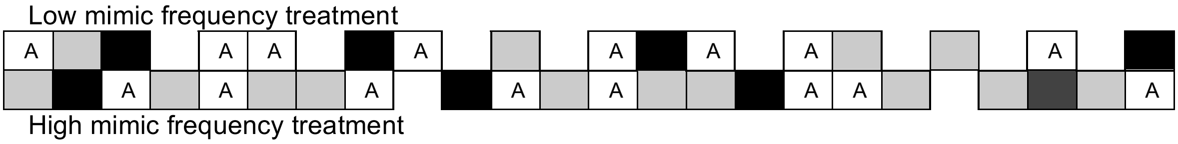

Figure 8.1. An example of the prey presentation sequence in the daily trials in the two experimental treatments. Each square represents a three minute time block in which birds were presented with a prey item. Squares with an A, indicate the presentation of an alternative prey; squares with grey shading indicate a mimic; and squares shaded black indicate models. Empty squares without full borders indicate an occasion where no prey was presented.

Table 8.1. The presentations on each daily trial for the two experimental treatments (proportion of all trials in treatment represented in brackets).

Search outcome	Low mimic frequency	High mimic frequency
Alternative	8 (0.333)	8 (0.333)
Models	4 (0.167)	4 (0.167)
Mimics	4 (0.167)	10 (0.417)
No prey	8 (0.333)	2 (0.083)

There were 24 three minute intervals in each daily trial, compared to 18 in the previous two chapters (see sections 5.2.5, 6.2.4, and 7.2.3 for details). This increase in the number presentations was required to ensure that I could increase the number of mimics and still have presentations containing no prey. The presentation order was different each day, and was random except for these following two conditions: (1) the first and last presentations had contain prey and (2) the proportions of each prey type encountered in the first and second halves of the daily trial were equal (Fig 8.1). Prior to the start of the experiment, I fed the birds 22 mealworms at three minute intervals in order to ensure that birds were able to consume all mealworms that might be offered during a daily session. Out of the six birds, five consumed all 22 mealworms and the other bird consumed 21. This bird's single rejection occurred because of noises emanating from other areas of the laboratory, and was unlikely to be due satiation or some other internal factor.

8.2.5 Analysis of prey survival

If the mealworm was attacked but then rejected, I retrieved it and examined it for signs of outward body damage that would indicate the bird inflicting a fatal injury. Such signs included removal of cephalic region, puncturing of the exoskeleton, absence of legs, and obvious bill marks. If such signs were found, I defined the injuries as fatal. Absence of outward signs of damage meant that I recorded the prey as having survived. However, this method obviously discounts possible fatal internal injuries which were not ascertainable. This is due to the insertion of the syringe needle during prey preparation which would have ruptured the alimentary canal and the coelomic cavity and caused the obliteration of many internal structures. Using this crude measure, I was able to calculate rates of survival for prey which allowed me to conduct a basic survival analysis for each prey type by treatment.

In cases where I was unsure of the damage, I scored the prey as killed. This happened on 5 occasions out of 160 recorded instances of attacked prey not being consumed. Overall, I believe the system I used was a fair reflection of the probability of survival of this species. Rates of mortality and survivorship have been used by other authors studying mimicry. However, although they differ in their definitions of how much damage was likely to cause mortality. For example, Lindström *et al.* (1997, 2001) considered the prey to be 'killed' if the bird ate, took bites from, tested, or were seen hammering the prey. However, the classifications in other studies have stated that prey were considered to have been survived if the legs remained intact and the cuticle was not ruptured (Järvi *et al.* 1981; Sillén-Tullberg *et al.* 1982; Wiklund & Järvi 1982). My classification was closer to those of the studies of Järvi and colleagues (Järvi *et al.* 1981; Sillén-Tullberg *et al.* 1982; Wiklund & Järvi 1982).

8.2.6 Handling times and behaviours

I collected of behavioural data in order to determine if the birds were behaving in a different manner towards the different prey types. The behaviours that I recorded are described in Table 7.2. I also hand-timed the latencies of the birds and the total handling time of birds for each prey type using a hand-held stop watch to the nearest 0.1 sec.

8.2.7 Statistical procedures

In most cases, I used linear mixed models (LMM), which are a versatile set of statistical methods to calculate the statistics. On occasions where LMMs where not used, the statistical method is stated in the results. In order to complete *post-hoc* comparisons of contrasts, I used the least significant difference (LSD) method after the alpha values had been corrected using the Bonferroni method (i.e., α/N). I used the Mixed procedure in SPSS to fit linear mixed models to my data. This procedure is more general than the general linear model (GLM) procedure. For example, it is possible to complete an analysis of unbalanced repeated measures data. This is something that is not possible using GLMs.

The experiment was a repeated-measures design, which is preferable for a number of reasons. First, fewer animals are used in the experiment. Second, differences between individuals are eliminated as a source of variance meaning that any effects are much more likely to be a result of the factor which is being manipulated. Finally, SDP models often specify optimal behavioural strategies for individuals meaning that the best way to test these models are through repeated measures of the same individuals under the same experimental conditions.

I converted data to proportions and ran statistical tests including the variables treatment, day, and prey type. Overall, the day of the experiment was not a significant factor and was excluded unless otherwise stated. I arcsine square root transformed proportions in order to restore normality and homoscedasticity and analysed the resulting data using parametric methods. The data on the number of handling behaviours exhibited towards the prey was square root transformed using the formula:

$$X' = \sqrt{(X + 0.5)}.$$

This treatment of the data resulted in the lowest score for the Akaike's information criterion. Akaike's information criterion (AIC) was used to choose the best model when models had different sets of parameters. AIC was calculated for each model as:

$$-2(\text{log-likelihood}) + 2 \times p$$

where p is the number of parameters estimated in the model. AIC therefore represents a measure of the explanatory power of the model discounted by the number of parameters that have gone into its construction; a lower value indicates the 'better' model.

I completed all statistical analyses using SPSS for PCs (version 15.0) and Mackintosh computers (version 11.1).

8.3 Results

8.3.1 The rates of attack and the relationship between attack and consumption

I defined an attack as when a bird's bill was seen to touch a prey after the bird had flipped the lid off the Petri dish. I ran a LMM on the proportion of prey attacked with prey type and treatment as fixed factors. Subjects were included in the models as a random subject variable and the proportion of prey attacked was the dependent variable. The model indicated that there were significant differences in the proportions of mealworms attacked by prey type (LMM: $F_{2,25}$=34.28, P<0.001, Fig. 8.2). Comparisons of the differences in the estimated marginal means revealed that the alternative mealworms were attacked significantly more frequently than both the mimics (LSD: difference=19.34, P<0.001) and models (difference=14.50, P<0.001). However, there was no significant difference in the difference between the proportions of mimics and models attacked (difference=-4.84, P=0.172) which indicated that birds were not able to discriminate between them prior to attacking.

My prediction was that by increasing the density of mimics, at the expense of unsuccessful searches, the attack rate on the mimicry complex as a whole would decrease. Despite the trend of the data indicating that the levels of attack might have declined with increased mimic abundance (Fig. 8.2), the model revealed that the effect was not significant ($F_{1,25}$=3.036, P=0.094). The interaction between treatment and prey type was also not significant ($F_{2,25}$=0.604, P=0.554). The estimated total variance of the model was approximately 46.14 of which 10.66 (23.12%) was attributable to variation between individuals. This meant that between subjects variance was not a significant source of variation for the model as a whole (Wald Z: Z=1.005, P=0.315).

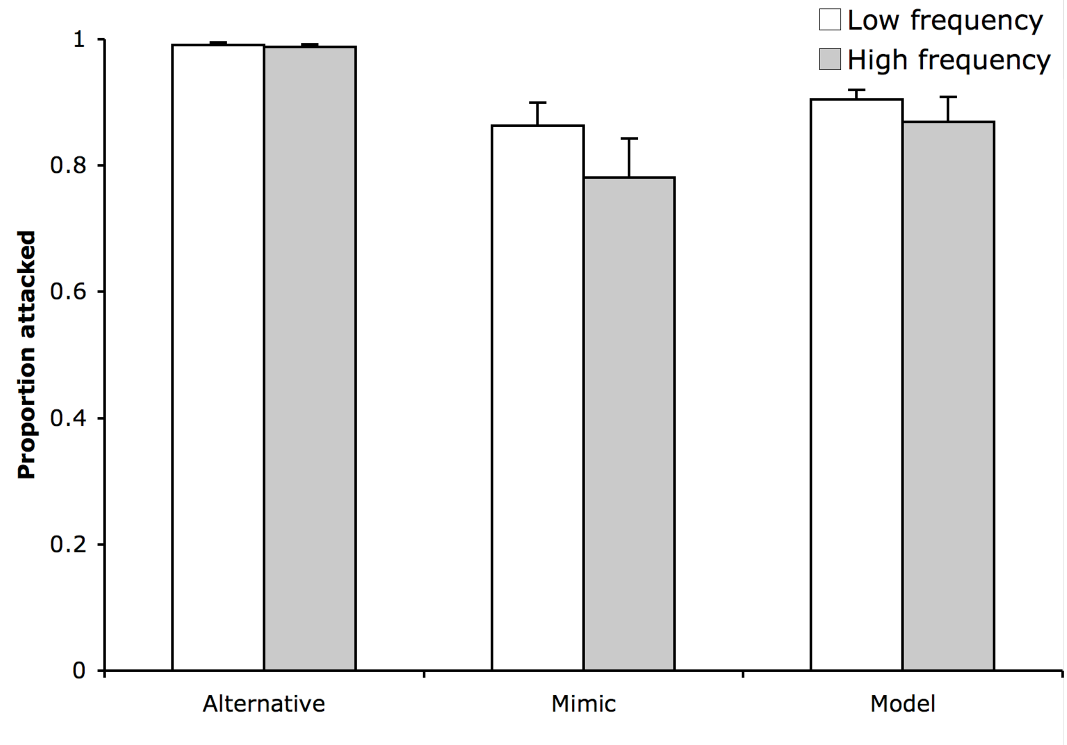

Figure 8.2. The mean proportions of prey (+ SE) that were attacked.

I also constructed a LMM to test if there were differences in the proportions of prey attacked that were subsequently consumed. The proportion of attacked prey that were subsequently consumed is not an analogue to the proportion of prey consumed. The rate of prey consumption is the product of the rate of attack and the proportion of attacked prey consumed. I included treatment and prey type as factors and subject as a random subject variable. I found there was a significant effect of prey type (LMM: $F_{2,30}$=66.148, P<0.001, Fig. 8.3), with the proportion of alternative prey attacked and then consumed being significantly greater than the proportions of consumption for both the mimics and the models (LSD: alternative - mimic=14.04, $P<0.001$; alternative – models=35.273, $P<0.001$). Notably, the proportion of attacked mimics that were consumed was also significantly greater than that of the models (mimic – model=21.234, $P<0.001$). There were no other significant main effects or interactions (treatment: $F_{1,30}=0.341$, $P=0.564$; treatment*prey: $F_{2,30}=1.06$, $P=0.359$). The estimated total variance of the model was approximately 8.27×10^{-3} of which 8.72×10^{-4} (10.54 %) was attributable to variation between individuals. This meant that between subjects variance was not a significant source of variation for the model as a whole (Wald *Z*: $Z=0.634$, $P=0.526$).

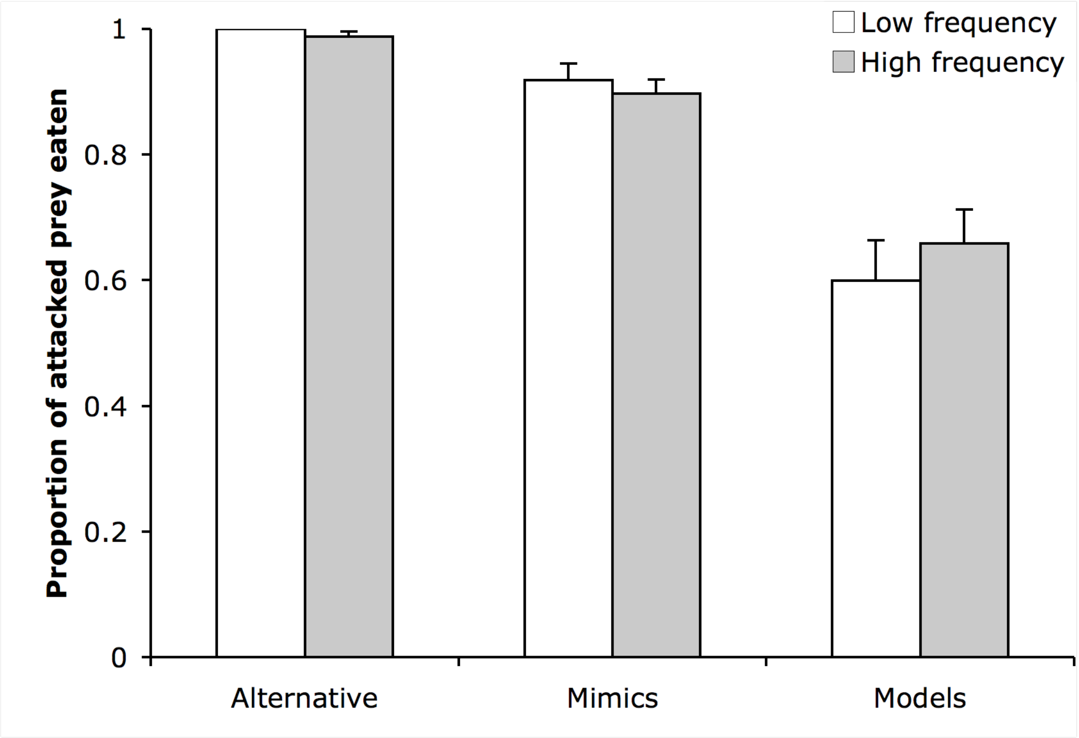

Figure 8.3. The mean proportions (+SE) of the attacked prey that were subsequently eaten.

8.3.2 The prey preferences for the different prey

Although the attack rates of were not significantly different between treatments, the proportion of prey consumption might be another factor for the survival of prey. Figure 8.4 illustrates the proportion of prey consumed from the total number of prey offered on each day during the experiment. The data for the mimicry complex is combined. It shows that the rate of consumption was lower for model-mimics in the high mimic frequency treatment compared with low mimic frequency treatment. I illustrated the data with the model-mimics combined in order to give an impression of the pattern of prey consumption from the birds' perspective or based on the visual distinctiveness of the prey

Although there were differences in attack rate that were not significant between treatments, there might have been post-attack differences in birds behaviour that could cause differences in survival of different prey types. For example, there may have been differences in the rates of prey consumption between the three prey types. In order to test for differences in the proportions of prey consumed between treatments, I set up a full factorial LMM that included treatment, day within the treatment, and prey type (alternative, models, and mimics) as fixed factors. Individual subjects were included as a random subject variable and the proportion of prey consumed was the dependent variable. The model indicated that there were significant differences in the proportion of prey consumed between the two treatments (LMM: $F_{1,205}$=13.023, P<0.001). The difference in the estimated marginal mean indicated that the proportion of prey consumed was lower in the high mimic frequency treatment when compared with the low frequency treatment (LSD: treatment 1 – treatment 2=6.964, P<0.001, Fig. 8.5). There were also significant differences among the proportions of each of the three prey types that were consumed ($F_{2,205}$=157.387, P<0.001, Fig. 8.5). *Post-hoc* comparisons of contrasts revealed that there were significant differences in the proportion of prey consumed between the different prey types (alternative – mimics=24.366, P<0.001; alternative – model=41.734, P<0.001; mimic – model=17.369, P<0.001). Over the seven days of each experimental treatment, there were no differences in the proportion of prey consumed between days within a treatment ($F_{6,205}$=1.815, P=0.098). There was a significant interaction between treatment and prey type ($F_{2,205}$=7.959, P<0.001). This was because the slopes for the proportion of prey broken down by prey type and treatment type had significantly different slopes from one another. There were no other significant two-way interactions (treatment*day: $F_{6,205}$=1.037, P=0.402, day*prey: $F_{12,205}$=1.103, P=0.360) or three-way interactions (treatment*day*prey: $F_{12,205}$=1.520, P=0.119). The estimated total variance of the model was approximately 267 of which 32.5 (12.18%) was attributable to variation between individuals. This meant that between subjects variance was not a significant source of variation for the model as a whole (Wald Z: Z=1.349, P=0.177).

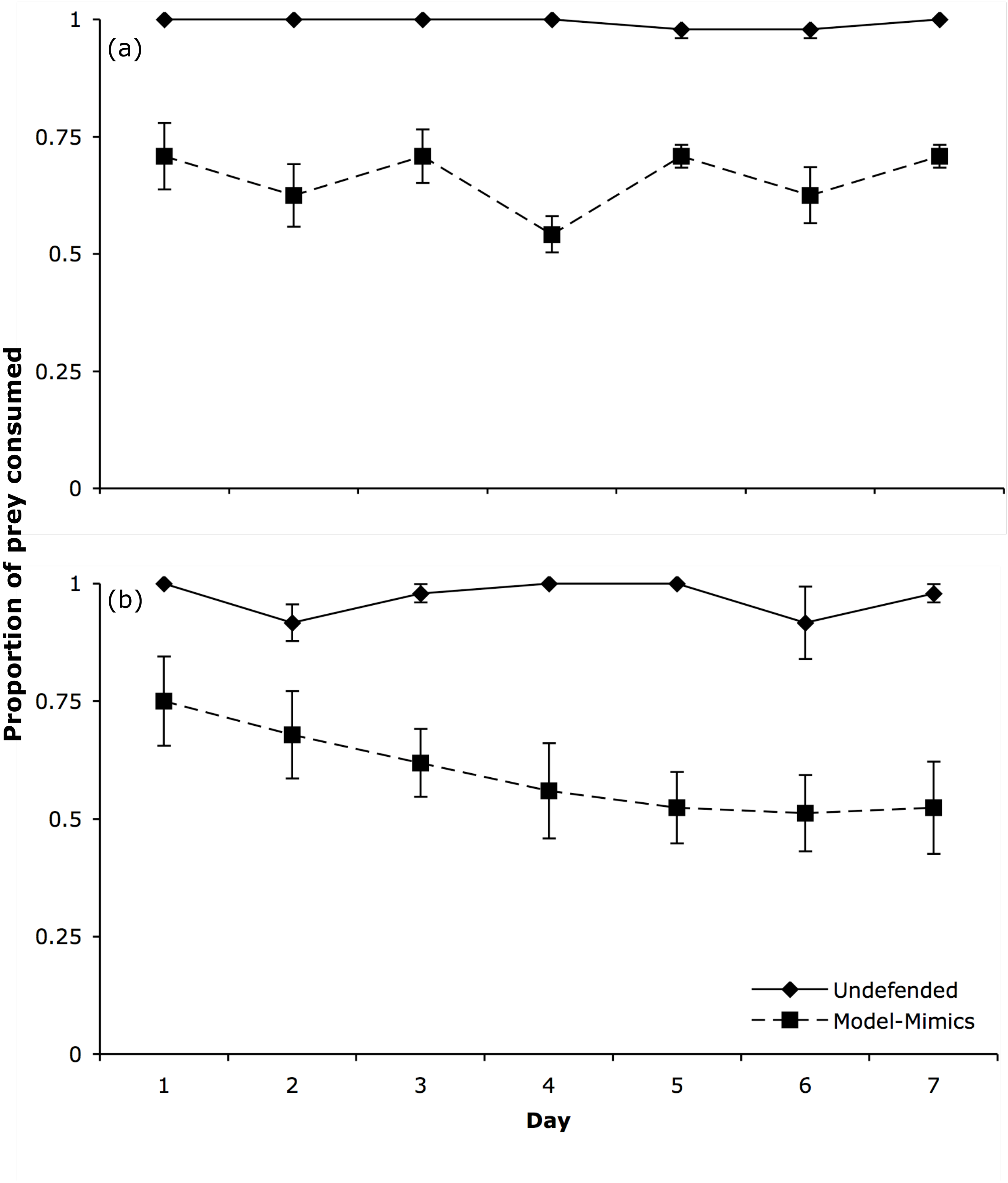

Figure 8.4. The daily mean proportions (± SE) of the alternative and model-mimics consumed in (a) the low mimic frequency treatment and in (b) the high mimic frequency treatment.

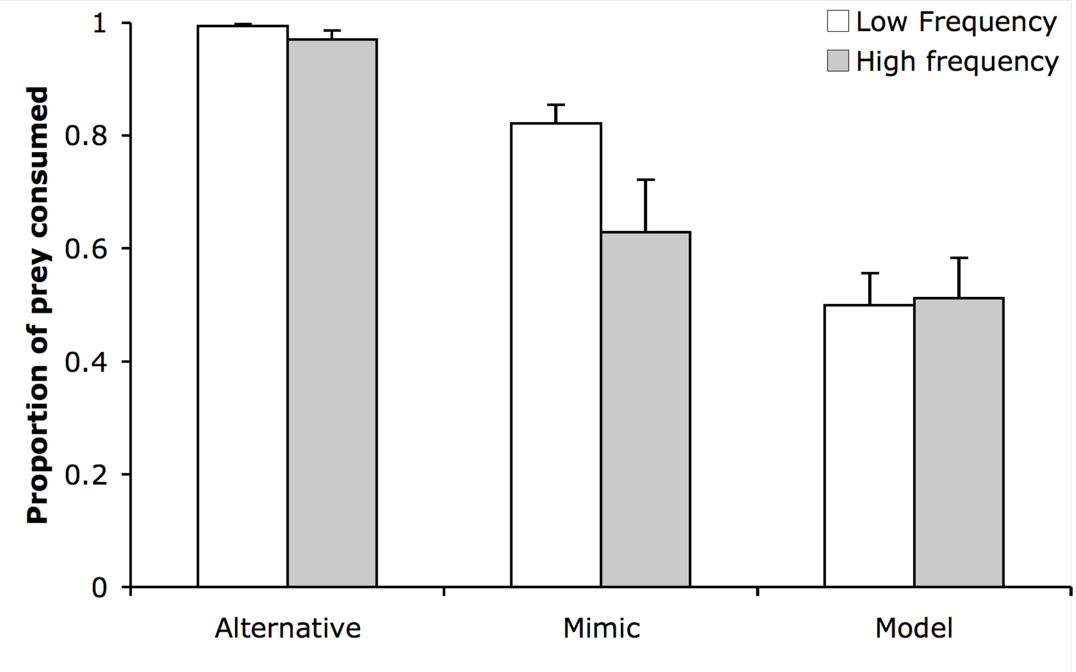

Figure 8.5. The total mean (+SE) proportion of each prey type consumed in each treatment.

Finally, I compared the number of prey consumed in each treatment in order to ascertain if an increase in food availability meant that this would increase the number of prey consumed. The addition of the mimics in high mimic frequency treatment meant that the overall number of prey in a trial was greater compared to the low mimic treatment. This might have meant that the birds were able to consume greater numbers of prey in the second treatment. This was indeed the case when I compared the differences in the mean number of prey consumed per day for each treatment (paired *t*-test: *t*= 2.583, *P*=0.0493, df=5; Fig. 8.6).

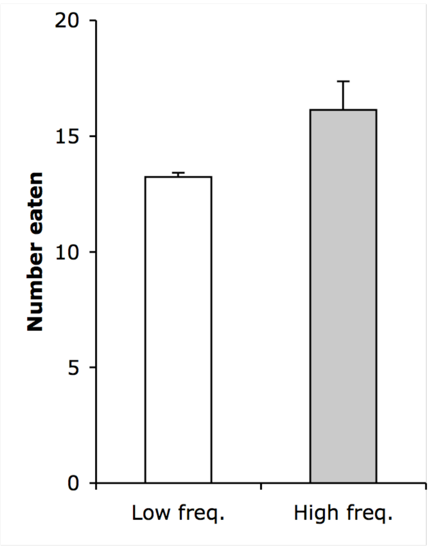

Figure 8.6. The mean number (+ SE) of prey eaten per day for each of the treatments.

8.3.3 A simple analysis of prey survival

Since I had estimated the probability of survival for mealworms from damage they incurred during attacks, I used this in an additional analysis of survival. I added the number of prey that had not been attacked with the number of individuals that I deemed to have survived attack and divided this sum by the total number of prey offered for that prey type in order to calculate a proportion of individuals that survived for each trial. I analysed the results in a LMM, which include prey type and treatment as fixed factors. Subjects were included in the model as random subject variables and the dependent variable was the transformed proportion of prey surviving attacks. I found that there was a significant difference in the proportion of prey surviving between the treatments of different mimics frequencies (LMM: $F_{1,25}$=5.901, P=0.023, Fig. 8.7). Comparisons of the contrasts in the estimated marginal means revealed that prey survivorship was significantly lower in the low mimic frequency treatment when compared with the other treatment (LSD: difference=-6.14, P=0.023, Fig. 8.7). The proportion of prey surviving also differed according to prey type ($F_{2,25}$=42.892, P<0.001, Fig. 8.7) which was because the alternative prey had lower levels of survivorship compared with mimics and models (alternative – mimics=–23.063, P<0.001; alternative – models=–26.307, P<0.001; Fig. 8.7). However, there was no significant difference between the proportion of mimics or models surviving (mimics – models=–3.243, P=0.915; Fig. 8.7). The interaction

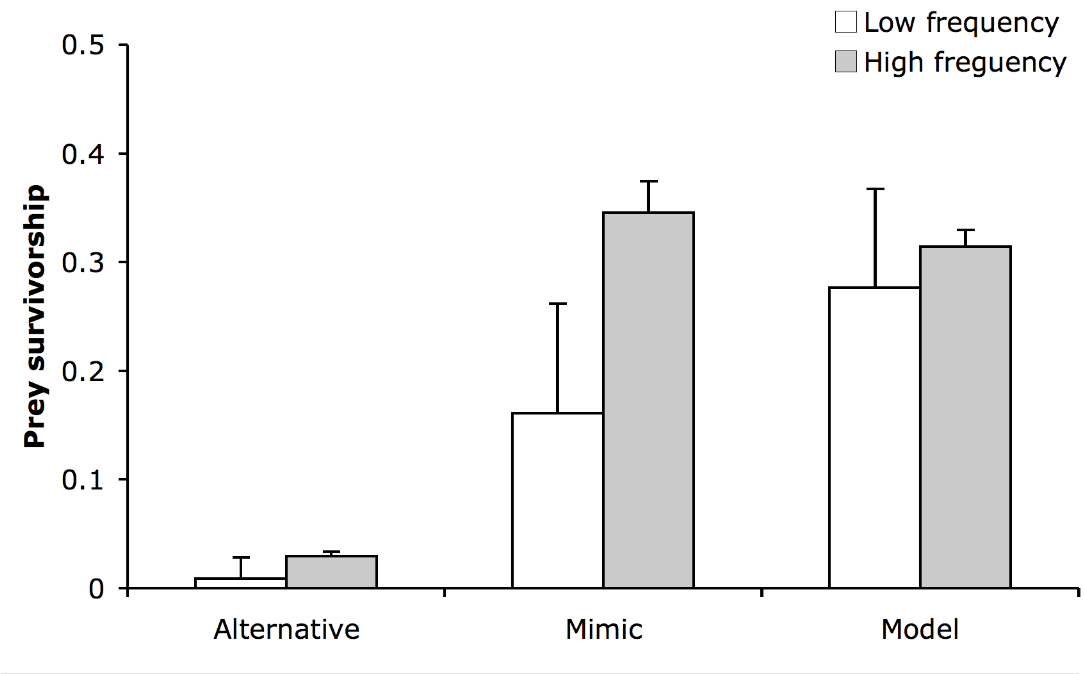

Figure 8.7. The mean proportion (+SE) of prey types surviving in both treatments.

between treatment and prey was not significant ($F_{2,25}$=1.493, P=0.244). The estimated total variance of the model was approximately 87 of which about 28 (33.67 %) was due to between subject differences. This level of variance was not significant meaning that between subject variance was not a significant factor in the overall model (Wald Z: Z=1.183, P=0.237).

I examined the handling times of the prey that sustained external injuries that I considered would have been fatal and it became clear that there was some relationship between the handling time and the probability of subsequent mortality. Those larvae that sustained fatal injuries were handled for significantly longer periods than survivors ($\bar{X}$ ± SE: survivors=7.21 ± 1.13 sec, non-survivors=28.96 ± 2.72 sec; paired t-test: $t_{(2),5}$=-7.922, P=0.001).

8.3.4 Handling times and behaviours

The SDP models predictions are based on predators using the visual signals of prey in order to make decisions. However, the birds might learn to tell the difference between prey using taste, olfactory or tactile cues of the prey. In order to examine this possibility, I conducted an analysis of the post attack handling behaviour of birds. I conducted three analyses to examine this possibility. First, I examined the handling times of birds in relation to treatment and prey type. Second, I examined the proportion of trials each of the most eight frequently expressed post-attack behaviours were expressed in relation to prey type. Finally, I examined the number of different behaviours that birds expressed per trial in relation to treatment and prey type.

Data for handling times were $\log_{10}$ transformed in order to restore normality and homoscedasticity thus permitting the use of parametric statistical methods. I constructed an LMM and incorporating the fixed factors of treatment and prey type. Subjects were included in the model as random subject variables. The model revealed that there was a significant effect of prey type (LMM: $F_{2,25}$=54.79, P<0.001, Fig. 8.8) with alternative prey having significantly shorter handling times than both the models and mimics and the mimics having shorter handling times than models (LSD: alternative – mimics=-0.434, P<0.001; alternative – model=-0.868, P<0.001; mimics – model=-0.435, P<0.001). There were no other significant main effects or interactions (treatment: $F_{1,25}$=1.954, P=0.174; prey*treatment: $F_{2,25}$=0.229, P=0.797). The estimated total variance of the model was approximately 0.066 of which about 0.025 (31.81 %) was caused by between subject differences. This level of variance between subjects was not significant meaning that between subject variance was not a significant factor in the overall model (Wald *Z*: Z=1.233, P=0.217).

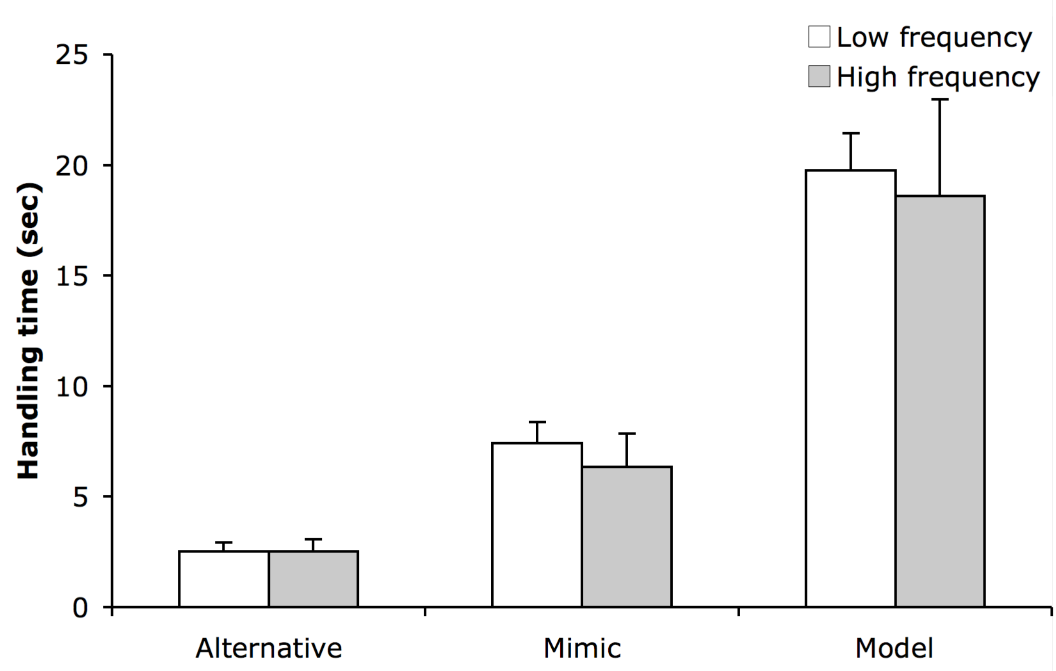

Figure 8.8. The mean handling times (+SE) of the three prey types in each treatment.

In the second analysis, I examined the handling behaviour of the birds in more detail. It was clear that the birds treated the three prey types differently from one another (Figure 8.9; Table 8.2). In order to analyse the prey handling behaviour, I used a LMM, which included the eight most common behaviours that I recorded during the trials (see Table 7.2. for operational definitions). I included behaviour type and prey type as fixed factors and included bird ID as a random subject variable. The transformed proportion of trials in which a behaviour was recorded was the dependent variable. The model indicated that the birds expressed some behaviours more frequently than other behaviours during prey presentations (LMM: $F_{7,115}$=108.356, P<0.001). This means that the behaviours were not expressed indiscriminately or randomly between trials and were related to the type of prey being handled (Fig. 8.9). Another observation that is evident from Figure 8.9 is that there appeared to be three clusters of behaviour which were expressed at different rates between clusters but similar rates within the cluster. These were based on the differences between the pair wise comparisons of the contrasts (Table 8.2). The first cluster of behaviour consisted of biting which was clearly the most commonly expressed behaviour and expressed significance more often than other behaviours (Fig. 8.9). The second cluster of behaviours consisted of crushing, wiping, and hitting behaviours which were expressed in about a third of all trials. The remaining cluster of behaviours consisted of the remaining four behaviours (head shaking, pecking, gaping/casting, and bill wiping) and were expressed in less than 10 % of trials (Fig. 8.9; Table 8.2).

Table 8.2. Comparison of the differences in the estimated marginal means of the proportion of times that each behaviour was expressed during the trials. * Significant at α=0.05 (Bonferroni corrected).

Behaviour (i)	Behaviour (j)	Mean difference (i-j)	Standard error	df	*P*
Biting	Crushing	16.679*	2.050	115	<0.001
	Wiping	19.087*	2.050	115	<0.001
	Hitting/shaking	23.473*	2.050	115	<0.001
	Head shaking	40.499*	2.050	115	<0.001
	Pecking	39.863*	2.050	115	<0.001
	Gaping/casting	41.505*	2.050	115	<0.001
	Bill wiping	38.235*	2.050	115	<0.001
Crushing	Wiping	2.408	2.050	115	1
	Hitting/shaking	6.794*	2.050	115	0.034
	Head shaking	23.820*	2.050	115	<0.001
	Pecking	23.184*	2.050	115	<0.001
	Gaping/casting	24.826*	2.050	115	<0.001
	Bill wiping	21.556*	2.050	115	<0.001
Wiping	Hitting/shaking	4.385	2.050	115	0.966
	Head shaking	21.412*	2.050	115	<0.001
	Pecking	20.775*	2.050	115	<0.001
	Gaping/casting	22.418*	2.050	115	<0.001
	Bill wiping	19.148*	2.050	115	<0.001
Hitting/shaking	Head shaking	17.026*	2.050	115	<0.001
	Pecking	16.390*	2.050	115	<0.001
	Gaping/casting	18.033*	2.050	115	<0.001
	Bill wiping	14.763*	2.050	115	<0.001
Head shaking	Pecking	-0.636	2.050	115	1
	Gaping/casting	1.006	2.050	115	1
	Bill wiping	-2.263	2.050	115	1
Pecking	Gaping/casting	1.643	2.050	115	1
	Bill wiping	-1.627	2.050	115	1
Gaping/casting	Bill wiping	-3.270	2.050	115	1

There were significant differences in the proportion of times that behaviours were expressed depending on prey type ($F_{2,115}$=218.248, P<0.001, Fig. 8.10). The differences between all the prey types were significant (LSD: alternative – mimic=-9.691, P<0.001; alternative – model=-25.950, P<0.001; mimic – model=-16.258, P<0.001). This clearly demonstrates that the birds were in some way able to taste the prey. However, there is also likely to be some reliance on the colour of the lid when the signal is expected to be reliable (i.e., for the alternative prey). There was a significant behaviour by prey interaction meaning that the trends of behaviour expression differed significantly between prey types and the type of behaviour that was being expressed ($F_{14,115}$=7.199, P<0.001). The estimated total variance of the model was approximately 55 of which about 18 (31.81 %) was caused by between subject differences. This level of variance between subjects was not significant meaning that between subject variance was not a significant factor in the overall model (Wald Z: Z=1.452, P=0.147).

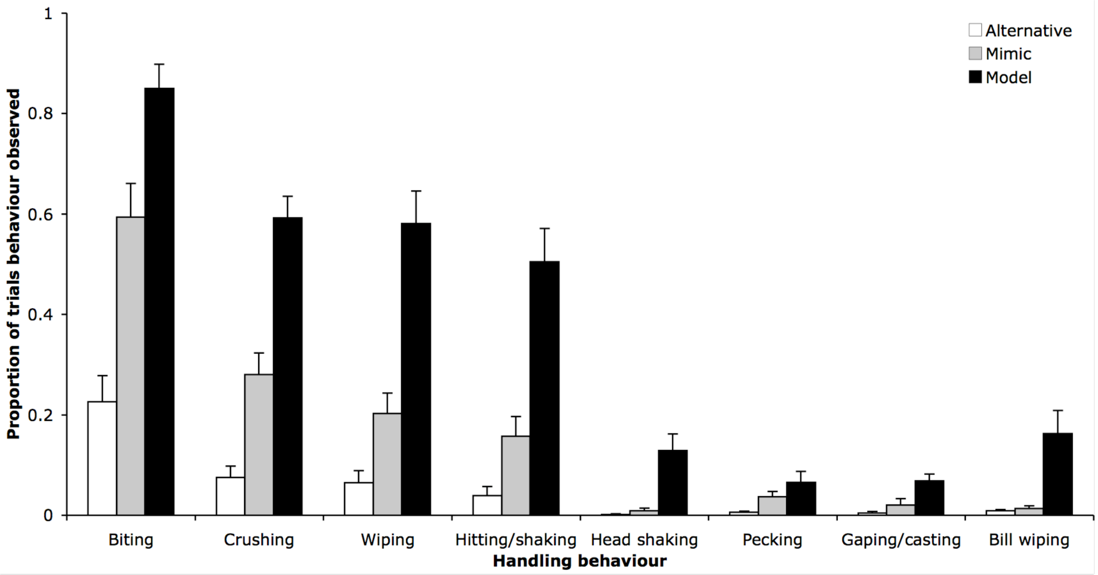

Figure 8.9 The mean proportion (+SE) of trials in which each behaviour was exhibited by birds. All behaviours increased in frequency of expression from the undefended alternative prey to the moderately defended models.

For the final behaviour analysis, in addition to the proportion of trials where certain behaviours were observed I was able to conduct a LMM analysis on the number of behaviours birds exhibited in each trial. The AIC indicated that the square-root transformed model was better as it had the lowest AIC number (untransformed=597.908, transformed=159.814). I constructed a LMM on the transformed number of behaviours expressed per prey presentation. I included prey type and treatment as fixed factors. Subjects were included as random subject variables and the number of behaviours per presentation was the dependent variable. The model revealed that the numbers of behaviours exhibited varied significantly by prey type (LMM: $F_{2,205}$=187.257, P<0.001, Fig. 8.10). Pair wise comparisons of the differences between the means revealed there were significant differences in the number of behaviors birds exhibited between all prey types (LSD: alternative – mimic:-0.367, P<0.001; alternative – model=-0.844, P<0.001; mimic – model:-0.477, P<0.001). No other factors or interactions were significant (treatment: $F_{1,205}$=1.914, P=0.168; day: $F_{6,205}$=1.953, P=0.074; treatment*prey: $F_{2,205}$=0.402, P=0.670; treatment*day: $F_{6,205}$=0.617, P=0.717, prey*day: $F_{12,205}$=0.955, *P*=0.493; treatment*prey*day: $F_{12,205}$=1.467, *P*=0.139). The estimated total variance of the model was approximately 0.11 of which approximately 0.03 (27.26%) was caused by between subject differences. This level of variance between subjects was not significant meaning that it was not a significant factor in the overall model (Wald *Z*: *Z*=1.487, *P*=0.137).

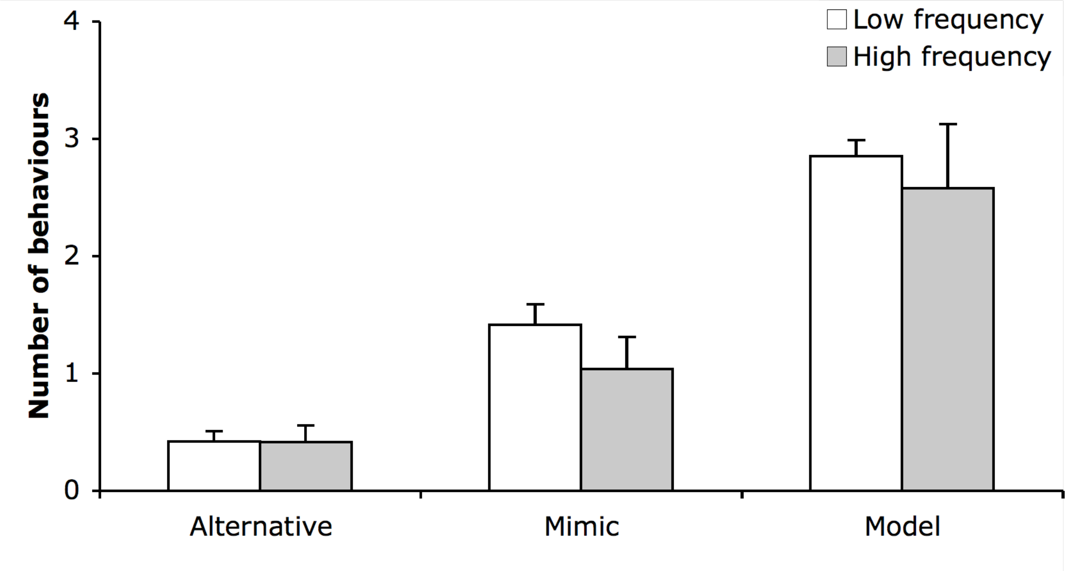

Fig 8.10. The mean number of behaviours (+SE) expressed per trial in relation to prey type and treatment.

8.3.5 Latencies to attack

Latencies to attack can indicate the birds motivation to attack the prey and declined with energetic state in the previous chapters (e.g., Chapters 5, 6, and 7). I $\log_{10}$ transformed the raw data to restore normality and homoscedasticity which allowed me to conduct parametric statistics on the data. I constructed a fully factorial LMM, which included prey type and treatment as fixed factors and subjects included as random subject variables. The model was not significant for any of the factors (LMM: treatment $F_{1,25}$=1.893, *P*=0.181; prey type $F_{2,25}$=1.702, *P*=0.203; treatment*prey $F_{2,25}$=0.237, *P*=0.791). The estimated total variance of the model was approximately 0.0487 of which approximately 0.0374 (76.81%) was caused by between subject differences. However, this level of variance between subjects was not significant meaning that between subject variance was not a significant factor in the overall model (Wald *Z*: *Z*=1.505, *P*=0.132). This analysis shows that the latencies provide any evidence that there were differences in the birds' motivation to attack prey in either of the experimental treatments.

8.4. Discussion

In this experiment, I tested a prediction arising from the SDP model on the state-based consumption of chemically defended prey by Sherratt *et al.* (2004). This model predicted that the increased frequency of Batesian mimics may decrease the rate of attack on all prey types in a mimicry system as the increase in the number of mimics will make the predators more conservative in their consumption of the species in the mimicry system. The data from this chapter show unequivocally that increases in mimic frequency, increased the effectiveness of the mimicry system through decreasing consumption and increasing survivorship of both models and mimics in the mimicry complex. Moreover, birds developed sophisticated behavioural methods of mediating the levels of chemical defences in the models. Something that had been associated with more specialised predators (e.g., Fry *et al.* 1993).

The survivorship of models and mimics increased in the high mimic frequency treatment, with the increase disproportionately favouring the mimics. The reason for this is unknown although it could be further evidence that birds may be in some way managing their body burdens of toxins so that once they have ingested a certain number of prey they stop further ingestion of potentially defended prey. The SDP models of the state-based consumption of chemically defended prey predict that birds ought to manage their intakes of secondary metabolites and perhaps birds stopped consuming prey once their body levels of quinine had reached a critical point.

These results contradict the classical conditioning models of learning, which have dominated in the literature of Batesian mimicry (Huheey 1976; Owen & Owen 1984; Speed 1993a; 1999, 2001; Turner & Speed 1996, Speed & Turner 1999 although see Darst 2006). These models generally assume that experience with brightly coloured chemically defended prey will lower the probability of future attacks, whereas consumption of an undefended mimic will increase the probability of a future attack. This was found in earlier empirical studies that investigated the attack probability on a Batesian mimicry complex when the frequency of mimics increased (Brower 1960; Duncan & Shappard 1965; Lea & Turner 1972; Pilecki & O'Donald 1971, Goodale & Sneddon 1977, Huheey 1980; Avery 1985, Nonacs 1985, Lindström *et al.* 1997). However, these studies considered the relative model-mimic frequency in isolation from the rest of the system (with the exception of Nonacs [1985]).

The high frequency mimic treatment in this experiment provided more food meaning that the mimicry complex potentially gained protection from being in a system that had more prey. This protection came from the perceived risk that the additional food could possibly be models, meaning the birds could become more cautious in their consumption of the mimicry complex. This increase in food availability paralleled the result of other studies that have increased the amount of alternative prey in the system. These studies suggest that predators decrease their rate of predation on the model-mimics even when mimicry is imperfect (Nonacs 1985; Lindström *et al.* 2004). Nonacs (1985) found that long-eared chipmunks (*Eutamais quadrimaculatas*) decreased predation on model-mimics when alternative undefended food sources were more abundant. Lindström *et al.* (2004) also found that increases in alternative food abundance decreased the rate of relative predation on the models and the mimics (albeit using imperfect mimics). The results from these two studies are likely to be related the predator's preference for alternative prey above prey from the mimicry system. The two experiments were run for a set period of time within which, predators were allowed to forage freely until they had attacked a certain number of prey (Lindström *et al.* 2004) or a set time had elapsed (Nonacs 1985).

If Nonacs (1985) had allowed his subjects to forage for longer, they would have become more likely to attack defended prey from the mimicry system. My experiment was differed from that of Nonacs (1985) and Lindström *et al.* (2004) insofar that the birds never encountered the prey simultaneously and the maximum rate of food delivery was controlled within the daily trials. Presenting birds with food simultaneously may induce attacks on prey and may be unrealistic in that birds might rarely be faced with situations where they are required to make multiple decisions (Hetz & Slobodchikoff 1988; Lindström *et al.* 1997). Moreover, when state is a factor of interest, detailed knowledge of the food intake per unit time is an important factor that could influence rates of attack on chemically defended prey.

The results of this chapter are important and have far reaching implications for how we view Batesian mimics in a mimicry system. Until now, there has been massive theoretical and empirical evidence gathered which indicates that Batesian mimics have a parasitic effect on their models (see Ruxton *et al.* 2004, pp. 139-163 for a review). This is the first study that has shown that under some circumstances, increasing frequencies of Batesian mimics may actually enhance the effectiveness of the Batesian mimicry system. One possible mechanism for this effect might be that more frequent reinforcement of the warning signal may force the birds' to remember the unpleasant defence associated with the model-mimics more often (Guilford 1991). The increased rate at which the association between the warning signal and its post-ingestive consequence are encountered in the high mimic frequency treatment may cause the bird to remember these negative post-ingestive consequences of the prey more readily.

In this experiment, the probability of encountering a mimic increased in the high mimic frequency treatment along with the probability of prey encounter. This led to the birds consuming more mealworms in the high frequency treatment than in the other treatment (Fig. 8.6). Although the number of mealworms eaten in the high frequency treatment was significantly greater than in the low mimic frequency treatment. The extra mealworms probably would not have significantly affected a bird's energetic state. This difference equated to 2.9 more mealworms being eaten each day in the high frequency treatment compared with the low frequency treatment, providing birds with an extra 2.4 kJ in energy in the high frequency treatment. This would account for about one percent of their daily energetic needs (≈270 kJ [Nagy *et al.* 1999]). The absolute increases in consumption of prey did not translate into significant increase in mortality on a per capita basis in the high mimic frequency treatment. This might have been expected had the mimics diluted the perception of chemical defences in the system. This also demonstrates that the birds were more cautious towards the model-mimics in the high mimic frequency treatment than they would have been if the extra food had come from alternative prey.

The risk of ingesting quinine may have provided a significant deterrent to birds fully exploiting the extra food available in the high mimic frequency treatment. I calculated that given that birds daily energy requirements, they needed to gain about 0.33 kJ of energy every minute throughout the foraging day (i.e., 14 hours) to provide their 24 hr energy requirements. In this experiment, the most they could gain in the low mimic frequency treatment was 0.19 kJ per minute whereas the high frequency treatment provided the birds with a maximum of 0.26 kJ per minute. These calculations are supported by the fact that in Chapter 7, birds lost mass over a 54-minute trial period where birds had access to up to 0.28 kJ per minute. Therefore, even if birds were maximally consuming prey, they would have still been consuming an insufficient amount of food to provide their energetic needs even during the experiment. The birds in this experiment were not food deprived prior to starting this experiment meaning that they were probably more cautious than in earlier chapters (e.g., Chapter 2, 5, and 6). However, this potentially makes the results more realistic as free-living birds are unlikely to have extended periods without food access throughout the day except at dawn.

The rates of prey survival show a survival advantage for the models and mimics in the high mimic frequency treatment. This survival advantage came from the lower number of prey being consumed in this treatment and a propensity for the birds to abandon attacks in greater numbers in these trials. However, I believe that the experimental method may have encouraged attacks by birds as the birds seemed to often flip the lid and then make an attack decision. This may have been an artefact from the last experiment or may indicate that the birds were basing their decisions on a conditioned response. This is because when birds became energetically stressed, they tended to consume all prey and so may have learned to flip the lids very quickly and before the bird had made its decision about attack and handling. Interestingly, the rates of consumption after attacks upon prey in this chapter and in Chapter 7 were lower than the rate of consumption after attack in Chapters 5 and 6. This may have been a result of differences in the method, or due to differences between individuals used in this experiment. This was not the only behaviour of the birds that was interesting. I collected data on the post attack handling and subjugation behaviour of the birds and analysed the results closely.

Generally, the predatory sequence of animals can be split into three phases: the recognition phase, the pursuit/attack phase, and the subjugation/handling phase (Caro 2005). In this experiment, the recognition and pursuit/attack phases could not be differentiated from one another and so must be considered together. There was little evidence to suggest that the birds were able to discriminate between models and mimics prior to prey handling. Either this is because there were no differences in the latencies to attack, or the proportion of prey attacked between these two prey types. The alternative prey type were attacked significantly more frequently than the model-mimics although the differences in the latencies were not significantly different. This indicates that the colour cue was important in discouraging attacks on the model-mimics compared with the alternative prey.

During the handling phase of the predatory sequence, the handling times increased for the model-mimics. It is likely, that the first five to ten seconds of the handling period the birds were manipulating the prey in order to taste for quinine. Birds may have been able to taste quinine as sometimes prey would have residual quinine around the cephalic region from the intra-oral injection, although sometimes the head was removed in order to sample the viscera. It is clear that for the models, the birds handled prey for longer and displayed a greater number of behaviours per handling period. Therefore, it is likely that birds were able differentiate between models and mimics through a gustatory or general trigeminal nervous response. The majority of mimics that were rejected post attack were not handled for long periods and sometimes came after the consumption of a model. This raises the possibility that these rejections of mimics could have been caused through confusion of prey type due to residual taste effects of the quinine into subsequent trials following model ingestion. When I tasted quinine, the flavour was evident for a period longer than three minutes although it cannot be assumed avian taste perception is in any way similar to human taste perception (despite the claims of Royama 1970, p. 339). Once the bird had identified a model, the bird either abandoned the attack (≈ 35 % of the time) or continued to handle the prey item (≈ 65 % of the time). On a few occasions individuals continued to handle the models until the end of the 60 seconds, when the prey was removed. Therefore, it is likely that the rates of consumption and handling time were underestimated. However, the prey had in all cases sustained what would have been fatal injuries meaning that the survivorship levels were not influenced by this occurrence.

The starlings may have been attempting to mediate the levels of quinine that the mealworm contained through prey manipulation. For example, a common method (five out of the six birds demonstrated this behaviour) employed by birds was to remove the head and then hold the mealworm from the posterior end and hit it against the bottom of the cage. After 5-10 seconds of hitting, the bird would then retaste the mealworm and consume if satisfactory or continue hitting and tasting. This method could allow the birds to reduce the mealworms level of quinine to an acceptable level for consumption. It would have been useful to keep some of the rejected mealworms and compared their levels of quinine with those of non-handled mealworms that had been injected with quinine. However, time was not available for these analyses.

The behavioural sequence that I described above is very sophisticated behaviour for a species that is considered a generalist predator. The majority of studies that have investigated the ways in which predators mediate chemical defences of prey have concentrated on specialised predators. For example, grasshopper mice (*Onychomys* spp.) have demonstrated prey specific specialisations that neutralise the threats posed by various defended prey. They have been observed biting the heads off stink beetles (*Eleodes longicollis*) while pushing their quinone producing tails into the ground (Eisner & Meinwald 1966; Langley 1981). When attacking lubber grasshoppers

(*Brachystola magna*) which have spine-tipped legs that are used like saw blades, they immobilise the grasshopper and bite these appendages until they break before then consuming the rest of the body (Whitman 1986). Other well known examples of animals mediating the dangerous prey defences behaviourally are found in black-headed orioles (*Icterus galbula*) feeding on hibernating monarchs butterflies (Fink & Brower 1981, Brower & Calvert 1985, Brower 1988), bee-eaters consuming hymenoptera (Fry *et al.* 1992), the loggerhead shrikes (*Lanais ludovicianus*) impaling the lubber grasshoppers and other noxious prey (Yosef & Whitman 1992; Yosef *et al.* 1996), and finally the different predatory strategies employed by *Portia labiata* (a spider-eating jumping spider [Salticid]) when hunting *Scytodes pallidus* (a spider that hunts jumping spiders). Normally *Portia* stalk *Scytodes* stealthily from behind because *Scytodes* spits sticky gum to ensnare their prey. However, when females are carrying egg sacs, and so cannot spit effectively, *Portia* will attack *Scytodes* without hesitation from the front (Jackson *et al.* 2002). Quite a feat for an animal with a cephalic ganglion smaller than the size of a pinhead!

The documentation of behavioural characters that can mediate the ingestion of toxic compounds has lagged behind the similar documentation of physiological traits that mediate the effects of defence chemicals. While physiological adaptation may indicate a long evolutionary relationship between predator and prey, behavioural mechanisms may also have a similarly long evolutionary history. Behavioural mediation of chemical defences may also be more common than physiological adaptations as they might be less costly and there may be less inertia to their selection. This is because behaviour may develop from a general tendency for behavioural plasticity animals which helps them take advantage of changeable environments (Barnett 2004). However, because there are still few well-documented cases of these behavioural mechanism in different species of predators, it is far too early to make any meaningful comparisons between physiological and behavioural mechanisms or the selective pressures that favour their respective selection. However, the demonstration in this study that even generalised predators can utilise sophisticated behavioural sequences that may decrease the levels of defensive compounds of prey is interesting in itself and worthy of further investigation.

There is a suggestion that generalist species may be more likely to innovate and there is a growing literature which examines the ecological and morphological relationships in feeding innovations (see Lefebvre & Bolhuis [2003] and Sol [2004] for reviews). However, it is unknown weather this behaviour was a true innovation or merely a behavioural exapation. I have seen free-living birds using similar behaviour when handling prey that are too vigorous to eat or too large to swallow (personal observation). Therefore, it is reasonable to expect the birds to have used these behaviours in other contexts given that they are wild caught. However, the birds did not utilise these behaviours when they were first encountered models and mimics in Chapter 6. Moreover, the behaviour was not universally used. However, the birds that did use this behaviour would use it often repeatedly within a daily trial and it was predominantly associated with the models.

8.4.1 Conclusions

Traditional treatments of Batesian mimicry theory stipulate that the levels at which predators avoid a mimicry system is dependent on the three factors of the relative frequency of mimics to models the strength of the models chemical defence, and the availability of undefended alternative prey in the system. This experiment tested a recent SDP model that predicted that increasing mimic frequency would reduce the attack rate on the mimicry complex. This is the first study to confirm this prediction in a Batesian mimicry system, thus challenging the long-held view that increases in mimic frequency would also increase the predation costs to models. These data also indicate that the birds became more cautious (i.e., the proportion of prey consumed declined) at times of higher mimic density. The implications of the results of this chapter are far reaching as they challenge a number of assumptions that are held about Batesian mimicry and the mechanisms that might be responsible for predators preferences of prey.

The handling behaviours that birds developed are also interesting as they suggest that behavioural mediation in prey chemical defences may be easily learnt and used by predators in response to changes in prey density. Behavioural modification is also a mechanism that has not been often considered as a method of reducing the cost of consuming chemically defended prey in a generalist predator. Most of the previous examples of behavioural mediation of prey chemical defences have come from predators that are more specialised in their hunting strategies. Therefore, these results have implications for how we view the costs and benefits of developing sophisticated prey handling behaviours. This might mean that the costs for consuming chemically defended prey may have been over estimated in studies that expose predators to prey for only short periods of time.

The results of this study are of interest to all evolutionary biologists because they indicate that the relationship between mimic frequency and survival may be more complex than had been previously thought. The results of this study also question the basis of how animals decide to consume chemically defended prey and demonstrate the need for further investigation in this area in order to document further cases of this phenomenon.

Chapter 9

GENERAL DISCUSSION

The link between physiological state and reproductive success is well founded through an abundance of empirical research (see Clutton-Brock 1988; Newton 1989 for reviews). However, the links between short-term feeding behaviour, physiological state, and fitness have proven more elusive to quantify. It was the aim of this thesis to generate data that would help elucidate this relationship as this would help confirm the association between short-term behavioural strategies and energetic and physiological state. The results of this thesis provide evidence that the foraging decisions of birds can at times be related to energetic state. However, I also found that it is important to validate the assumptions of experiments in order to avoid problems as to the interpretation of results.

9.1 Rationality and energetic state in European starlings (*Sturnus vulgaris*)

First, I was interested in energetic state and how this affected rationality. The results of the experiment indicated that the birds did not breach rationality and that the decisions of the birds were not affected by their energy reserves. However, this result cannot be taken as a definitive refutation of the Schuck-Paim *et al.* (2004) model. This is because the birds were probably weighting their decisions in favour of the dimension of the payoff that they received. Therefore, the birds were probably construing the problem that I presented to them in a manner that was not intended.

9.2 The energy budget rule in rufous hummingbirds (*Selasphorus rufus*)

The second topic I chose to examine was the energy budget rule (EBR) of risk-sensitive foraging behaviour. I attempted to replicate the result of Caraco *et al.* (1990) in an environment subject to large daily and seasonal temperature changes. I found that the birds as a group displayed little indication of risk-sensitive foraging behaviour. In addition, the birds' preferences did not change with the temperature or their behaviour. There are many possible explanations for this result such as: (1) lack of costs to switch between flower type, (2) birds utilising torpor on days when the temperature was extremely cold, and (3) the birds utilising micro-climates that were warmer than the average ambient temperature when it was cold. Therefore, there is no way to conclusively attribute the birds' behaviour to any factor as the study either presented the problem in a manner that was not interpreted in the manner I had intended, or the result was confounded by a behavioural or environmental factor that I did not consider.

The implications of Chapters 2 and 3, which are where I addressed these two questions, are that more care needs to be taken in the design, analysis, and interpretation of foraging experiments. Care also needs to be taken that the experiment is construed in a manner that is intended by the subject so that the researcher is sure that the experiment is interpreted in the correct manner by the subject.

9.3 The energy-toxin trade-off in European starlings

Finally, I was interested in the energy-toxin trade-off that birds might make at times of energetic stress. I found unequivocal evidence that birds do increase the number of chemically defended prey they consume when their energetic states fall. The results support the notion that birds may utilise mildly and moderately defended prey at times of nutritive need. My novel findings indicate that the processes involved in the predation of chemically defended prey may be much more complex than has previously been thought. First, I found that predators are able to learn prey associations between more than two prey types and that they can consistently rank prey in accordance with the levels of chemical defence that they contain. Second, I found that if the levels of a toxin vary in a mimicry complex, then this might give the co-mimics an advantage over another prey of similar average toxicity. Third, I found evidence that suggests that birds might be able to strategically control their intakes of chemically defended prey. Fourth, I found that even generalist predators, such as European starlings, can develop sophisticated prey handling behaviour that could help mediate their intakes of toxins. Fifth, I found that birds may be able to taste the quinine with a general gustatory or trigeminal nervous response. Finally, and perhaps most interestingly, I found that the increasing the frequency of Batesian mimics in a mimicry system can increase the rate of survivorship of both mimics and models in a Batesian mimicry system. This is the first time that the this effect has been empirically demonstrated although it has been predicted in stochastic dynamic programming models of the evolution of mimicry (Sherratt *et al.* 2004).

9.4 Implications and future research in aposematism and mimicry

Energetic state needs to be considered more in the design and analysis of experiments that examine the consumption of chemically defended prey in the laboratory. Care needs to be taken that the energy levels of subjects are controlled between individuals. Also, care needs to be taken in pre-experimental food deprivation as even a two-hour fast could represent a significant amount of time to a small passerine. For example, a two-hour fast has far greater implications for a small species such as a great tit (*Parus major*) than a larger species such as a European starling because smaller species use up their fat reserves more quickly than larger species (Newton 1998).

The confirmation of energy toxin trade-off has implications for the wider debate on mimicry because if moderately defended prey can be consumed, then this makes the quasi-Batesian effects more likely. The data from Chapters 5 to 7 suggest that predators might strategically manage their intakes of quinine. If birds are able to strategically trade-off their intakes of toxins in relation to their physiological state, then this also indicates that educated predators could be a much more important factor in the evolution and maintenance of aposematism.

Although learning the properties of chemically defended prey is important, a predator's experience with chemically defended prey might not stop once it has become educated as to the chemical properties of a chemically defended prey. Therefore, educated predators may represent a significant selective pressure to aposematic prey because the learning period is likely to be much shorter than the educated period. Therefore, more thought needs to be given to the role of educated predators in the predation of aposematic prey species.

The results of Chapter 8 are important because they suggest that an increase in the frequency of undefended mimics does not lead to an increase in predation on the models and mimics in the system. This finding challenges the view that increases in undefended mimics are always parasitic on their models. I think it will be important for others to replicate the results of Chapter 8. This is because these results are important and challenge the previously held view that increased frequencies of Batesian mimics have a parasitic effect on the models.

Signal reliability may play a role in the in deterring predators. This is because if predators are able to strategically manage their intakes of chemically defended prey, then as they approach their critical body burdens of body toxins, birds might need to be more careful about which prey they consume. Therefore, they may choose prey with constant levels of chemical defence so that they can better manage their toxin intakes. Signal reliability could be an extremely important consideration for a predator attempting to strategically manage their body burdens of toxins. More unreliable signals such as the case in mimicry systems may interfere with predators abilities to manage their body burdens of toxins. Therefore, I think it would be important to conduct further research on the effects that unreliable signals may have on birds as they attempt to strategically manage their levels of body burdens of chemical defences. Moreover, it might be interesting to note if predators utilise other sensory modalities (such as taste) when a visual signal conveys less information as to the chemical defences of a prey animal.

Trade offs have not been examined in much detail in aposematism and mimicry systems. However, they are likely to widespread and pervasive in these systems as any other biological system (e.g., Bennett & Owen 2002). I would encourage further theoretical and empirical research because this is an interesting field which could greatly enrich our understanding of the costs and benefits involved in aposematic traits and especially intra- and inter-specific mimicry.

To pursue these issues an integrative approach is needed. A wealth of physiological techniques are becoming available for use in the field and the laboratory and combined with behavioural assays, these techniques offer new ways to approach the study of animal behaviour.

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