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**DETECTING BIRDS FROM  
BAITS BY MANIPULATING  
COLOUR AND ODOUR**

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A thesis submitted in  
partial fulfilment  
of the requirements  
for the degree of

Doctor of Philosophy  
at  
The University of Waikato

by Lynette J. Hartley

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The University of Waikato  
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Abstract of a thesis submitted in partial fulfilment of  
the requirements of the degree of Ph.D

## **Detering birds from poisonous baits by manipulating colour and odour**

Lynette Hartley

Poisonous baits are used extensively in New Zealand to control introduced mammalian pest species particularly brushtail possums (*Trichosurus vulpecula*) and rats (*Rattus rattus*, *R. norvegicus*, *R. exulans*). This thesis examined bait characteristics that could be manipulated to make poisonous baits less attractive to native birds. The first characteristic examined was colour as the appearance of foods was believed to be important to foraging birds. The target mammalian species may be more attentive when foraging to other aspects of food such as taste and smell. A six colour choice method for testing colour preference in birds was developed using chickens (*Gallus domesticus*). The procedure involved testing birds individually, on six occasions, with novel food of six different colours presented simultaneously. All three species of birds tested; captive New Zealand weka (*Gallirallus australis*), wild New Zealand robins (*Petroica australis*), and free range domestic chickens showed similar colour preferences. The red and yellow novel food was preferred to the blue novel food. Baits in New Zealand are currently dyed green to deter birds but this study suggested blue may be a more effective deterrent.

There was considerable individual variability in colour preference suggesting one colour is not likely to deter all birds in a population from poisonous baits. Both weka and chickens showed a pattern of increasing consumption from test to test and the implications of this behaviour pattern are discussed in terms of poisonous bait consumption.

Free range chickens were used to investigate whether chickens avoided some shades of blue more than others but no preferences were detected. The relative luminance of the colours offered to the chickens was calculated, however, and there was an indication that chickens preferred lighter rather than darker colours. I would suggest, therefore, that light coloured poisonous baits should not be used.

It was suggested that natural predator-prey systems may provide useful concepts for the design of bird deterrents. Many toxic insects have a combination of several conspicuous characteristics, all of which assault different senses at the same time. I investigated combinations of colour, novelty and methyl pyrazine, an odour used by toxic insects to deter birds. Wild robins were not deterred from familiar food by methyl pyrazine odour and the addition of methyl pyrazine did not alter chickens' colour preferences or their consumption of novel food. Pyrazine, however, was very effective in deterring captive weka from red and blue novel food. The weka ate very little of either colour when it was associated with pyrazine odour.

The combination of pyrazine and blue, which was effective in captivity, was tested in the field using real (non toxic) baits. Consumption over six days was compared with that of the green-coloured, cinnamon-flavoured, baits commonly used in poisoning operations. Fewer of the blue-pyrazine baits were eaten on the first day and there was an indication that consumption of blue-pyrazine baits was lower overall. Methyl pyrazine may be useful in deterring birds from poisonous baits and is certainly worthy of more investigation.

**Keywords:** Alerting colours, bird foraging behaviour, choice tests, colour vision, feeding deterrent, food preferences, insect defensive odour, methyl pyrazine, New Zealand robins (*Petroica australis*), repellents, spectral reflectance, warning signals, weka (*Gallirallus australis*).

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Lynette Hartley

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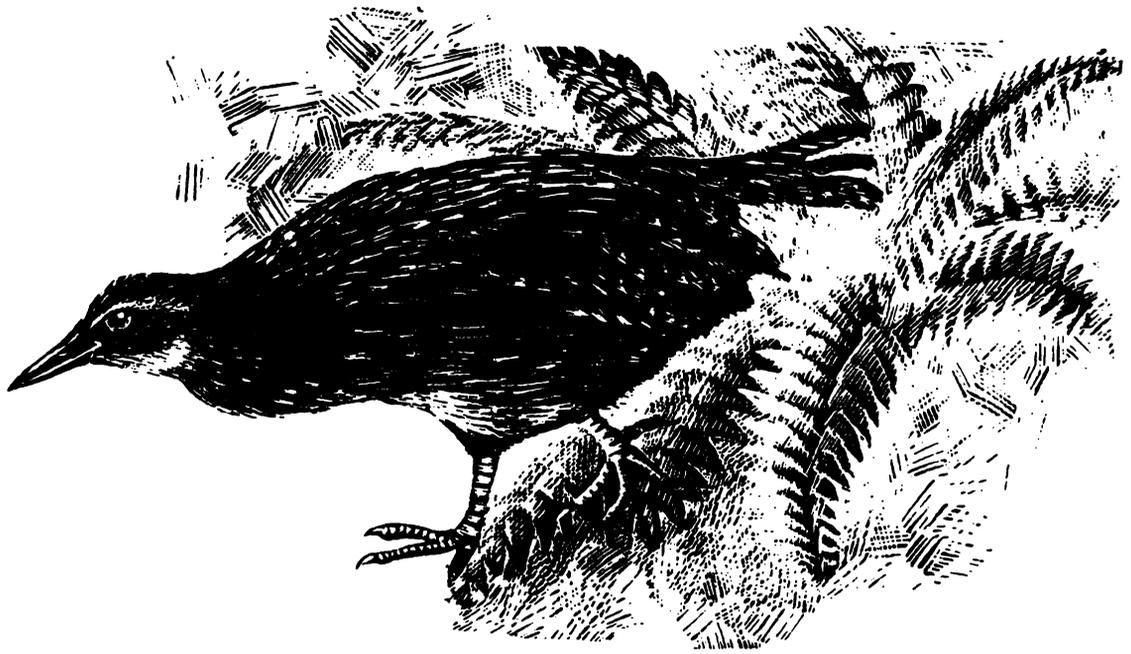
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## CHAPTER 1

### General Introduction

This Ph.D study is concerned with the interactions between native New Zealand birds and poisonous baits. This introductory chapter discusses why poisonous baits are used in New Zealand and the considerable benefits they bring. Some of the concerns associated with the use of poisons are highlighted and the effects of poisons on native bird species are discussed. There has been research in the past, in New Zealand, investigating methods of deterring birds from poisonous baits and this research is described along with the measures that are currently in place to protect birds. The chapter finishes with a discussion on the species that were chosen for the study, followed by the aims of the thesis research.

### Poison use in New Zealand

#### The pest problem

Over one thousand years ago the first humans arrived in New Zealand (Green, 1975; Holdaway, 1996) to find a rich and unusual vertebrate fauna composed of birds, reptiles, marine mammals and three species of bat. Polynesian, and later European immigrants, brought with them a range of terrestrial mammals which the indigenous New Zealand flora and fauna had not encountered previously. Many introduced mammalian species are now firmly established and several have had a detrimental effect on the indigenous New Zealand ecosystems.

Introduced mammalian predators include ferrets (*Mustela furo*), stoats (*M. erminea*), rats (*Rattus rattus*, *R. norvegicus*, *R. exulans*), and pigs (*Sus scrofa*). Introduced predators have been implicated in the decline of much of New Zealand's indigenous bird and reptilian fauna (O'Donnell, 1996; Brown, 1997b; Empson and Miskelly, 1999). For example pigs have been implicated in the

decline of native land snails (Meads, Walker and Elliott, 1984), and stoats in the decline of kaka (*Nestor meridionalis*; Beggs and Wilson, 1991) and yellowheads (*Mohoua ochrocephala*; Elliott and O'Donnell, 1988). In addition, browsing mammals such as deer (*Cervus* sp) have also had an adverse effect on New Zealand's ecosystems (Anon, 1997a).

Brushtail possums (*Trichosurus vulpecula*) are both predators of native species, such as birds, and competitors with native species for food (Livingstone and Nelson, 1994). Possums were introduced from Australia, and now occupy more than 90% of New Zealand's land area. In 1994 there were an estimated 70 million possums with a further 20 million born each year (Livingstone and Nelson, 1994).

An additional problem associated with introduced mammals is that several, including ferrets, possums, deer, stoats and hedgehogs (*Erinaceus europaeus*) are known to carry bovine tuberculosis (Tb; *Mycobacterium bovis*; King, 1990). Tb is a problem when it occurs in domestic cattle and deer herds because of the threat it poses to New Zealand's \$5 billion (NZ) agricultural export trade (Livingstone and Nelson, 1994). The presence of self sustaining pockets of Tb in wild animals, particularly possums, deer (Hickling, 1995) and ferrets (Livingston, 1996), has been recognised as a potential source of re-infection for domestic herds (Carter, 1995).

There is considerable interest in New Zealand in removing possums and ferrets from both farmland and forest areas adjacent to farmland to assist Tb control. There is also considerable interest in removing pests such as possums and rats from areas with conservation value in order to protect the indigenous flora and fauna.

### **The use of poisons for pest control**

A common method for controlling possums in New Zealand is by aerial broadcast of carrot or cereal baits containing sodium monofluoroacetate (compound 1080;

Powlesland, Knegtmans and Marshall, 1999). This method is used because it is very effective and large areas can be covered. An alternative to aerial distribution of 1080 baits is manual placement of the baits in bait stations. These stations are plastic containers which are fixed at regular intervals throughout the area to be poisoned. Bait station control is also very effective. The anticoagulant brodifacoum is also widely used (Innes and Barker, 1999) particularly for rodent eradications on offshore islands. Recent concern over secondary poisoning risks associated with this poison (Eason *et al.*, 1999) has resulted in a decline in its use on the mainland. Other poisons that are routinely used to control possums include phosphorous, cholecalciferol and cyanide. Anticoagulants such as brodifacoum and pindone are primarily used to control rodents, mustelids and rabbits (Innes and Barker, 1999).

Research is continuing on improving current control techniques and the development of those that do not involve the use of poisons, such as immunocontraception. Poisons are, however, still the key method for controlling pest species, particularly possums. There is no current poison that is specific to the mammalian target pests.

Control using poisons is also very cost effective. New Zealand Department of Conservation figures suggest an aerial drop of 1080 costs around \$15 (NZ) per hectare and bait stations cost \$60 - \$70 (NZ) per hectare. Trapping costs around \$60 (NZ) per hectare for easily accessible country and up to \$100 (NZ) per hectare for less accessible country (Penny, 1994). Fencing and shooting are only suitable in some locations and for very large or rugged areas, aerial poisoning may be the only practical option (Penny, 1994). At present there is no cost effective alternative to using poisons to control mammalian pests.

### **Environmental effects**

The positive environmental benefits of removing pest species have been well demonstrated. The conservation benefits include an improvement in forest and

ecosystem quality (Atkinson *et al.*, 1995; Rose and Pekelharing, 1995; Avis, 1997; Parkes, Baker and Ericksen, 1997; Empson and Miskelly, 1999) and benefits to native animal species (Powlesland, Knegtmans and Marshall, 1998; Innes *et al.*, 1999). Positive effects have also been recorded for the control of Tb with the incidence of Tb in cattle falling following the control of ferrets (Caley, Morley and Thomas, 1998) and possums (Hickling, 1995; Pannett, 1995).

There has been considerable research into the possible negative environmental effects associated with poisoning operations including residue studies (Dowding, Murphy and Veitch, 1999) and studies of the possible effects on non-target species (e.g. Spurr and Powlesland, 1997; Booth and Wickstrom, 1999; Powlesland *et al.*, 1999; Spurr and Drew, 1999) including humans (Eason *et al.*, 1999). Such research is ongoing and the organisations involved in pest control are responsive to research findings.

### **Public concern over the use of poisons**

Some of the public are very distrustful of poisons, particularly 1080. There was, for example, considerable public discussion on the use of 1080 in a poisoning operation at Pureora, in the central North Island, in which robins died (Anon, 1996; Field, 1996; Fox, 1996; Anon, 1997b; Edwards, 1997), and public protests in Northland during a control operation (Pullman, 1994; Parsonson, 1996). Recently, an activist received sympathetic and dramatic media coverage when he hijacked a helicopter in a protest against the use of 1080 (McLauchlan, 1996). The issue is an emotional one and managers are responsive to public concerns. The Department of Conservation, for example, altered the methods used in a poisoning operation at Karioi, Waikato, following public opposition to the operation (Penny, 1994).

It is important that issues such as the effect of poisons on the environment, and whether non-target species are adversely affected by poisoning, are addressed to the satisfaction of both managers and the public. Both New Zealand's agricultural

industry and the natural New Zealand environment would suffer if the use of poisons, particularly 1080, was banned since at present there are no other cost effective methods available for large scale pest control.

## **The effect of poisoning operations on native birds**

### **Monitoring techniques**

Since poison use started in New Zealand in the early 1950s there has been concern about the effects of poisons on birds. Prior to 1993 monitoring of bird populations during poisoning operations relied on either the five minute bird count technique (Spurr and Powlesland, 1997) or searching for bodies (Harrison, 1978). Neither technique is satisfactory. The five minute bird count is of questionable validity because 1) the conspicuousness of birds varies considerably over time irrespective of the numbers present, 2) differences in an observer's experience and ability can affect the results and 3) only large differences can be detected (Atkinson *et al.*, 1995; Powlesland *et al.*, 1999). Anomalous results have been obtained using this technique. On several occasions, for example, the number of birds recorded increased significantly immediately following a poisoning operation (e.g. Pierce and Montgomery, 1992; Powlesland *et al.*, 1999). Searching for bodies is very labour intensive and it is likely that ill birds retreat to places where their bodies would not be found. Occasionally, over the years, bird deaths were reported but it was believed that few individuals of any one species were affected and that the consequences for populations were minimal (Spurr, 1981; Eason and Spurr, 1995).

Researchers now use monitoring techniques such as radio telemetry and 'roll calling' (e.g. Powlesland *et al.*, 1998). Roll calling involves individually identifying birds, and, in the months preceding an operation, compiling a list of all the individuals having home ranges in an area to be poisoned and in a non-poisoned control area. After the poisoning operation it is possible to determine how many individual birds are missing from the poisoned area relative to the non-

poisoned area. This technique has been used effectively for North Island robins (*Petroica australis longipes*; Powlesland *et al.*, 1998) and kokako (*Callaeas cinerea*; Innes *et al.*, 1999). Radio telemetry and roll calling are expensive and time consuming, but accurate, and researchers can now quantify bird deaths during poisoning operations.

### **Species vulnerability**

Although many bird species have not, as yet, been adequately monitored, several stand out as being particularly vulnerable to poisoning.

Weka (*Gallirallus australis*) populations have been severely reduced in some poisoning operations, with the entire population of one island exterminated and reductions of 80 - 98% in other instances (Eason and Spurr, 1995). Weka populations have, however, survived other operations apparently without such huge losses (Walker, 1997). New Zealand robins have also been found to be vulnerable. Poisoning operations have resulted in a loss of robins at Pureora (54%; Powlesland *et al.*, 1998), Kapiti Island (24 - 64%; Empson and Miskelly, 1999), Maruia, Westland (48%; Brown, 1997a), Wanganui National Park (30 - 35%; Jim Campbell, *pers. comm.*, Department of Conservation, Wanganui, New Zealand), and the Chetwode Islands, Marlborough Sounds (up to 30%; Walker and Elliott, 1997).

The vulnerability of other species is less well documented. Pukeko (*Porphyrio porphyrio*) appear vulnerable with more than 90% of the population killed on Tiritiri Matangi Island, in the Hauraki Gulf (Eason and Spurr, 1995) and 50% killed on Motuihe island, also in the Hauraki Gulf (Dowding *et al.*, 1999).

Tomtits (*Petroica macrocephala*) also appear vulnerable as all individuals of this species disappeared during a poisoning operation at Pureora (Powlesland *et al.*, 1998). More than 80% of fernbirds (*Bowdleria punctata*) died in an experimental operation in Southland (Ranum, Dawson and Elliott, 1994) and approximately 500 black-backed gulls (*Larus dominicanus*) were killed on Kapiti Island in the rat

eradication mentioned above (Empson and Miskelly, 1999). It remains to be seen whether these were isolated incidents or whether such large losses frequently occur in these species.

Variable responses to poisoning have been reported for the New Zealand parrots. Four out of 20 kaka died following the poisoning operation on Kapiti Island (Empson and Miskelly, 1999) whereas all 21 monitored kaka survived an operation in Waihaha, Pureora Forest Park (Greene, 1998). Kea (*Nestor notabilis*) may be vulnerable as well, as they are known to eat carrot baits and have been found dead after a poisoning operation in the Dobson Valley, in the Southern Alps (Spurr and Powlesland, 1997). The status of the parakeets (*Cyanoramphus* sp.) is unclear because they have not been adequately monitored.

Numerous other species have been found dead occasionally, but have not been monitored adequately through enough poison operations to draw conclusions on their susceptibility. Brown kiwi (*Apteryx australis*), for example, have eaten non-toxic cereal baits in the wild but monitored individuals have also survived operations (Pierce and Montgomery, 1992). Two of the nine little spotted kiwi (*Apteryx oweni*) that were monitored on Kapiti Island died during the poisoning operation (Empson and Miskelly, 1999), but all nine little spotted kiwi that were monitored during an operation on Red Mercury Island, off the Coromandel Peninsula, survived (Eason and Spurr, 1995).

Other species, notably kokako, have been monitored adequately and have survived repeated poisoning operations (Innes *et al.*, 1999) although four out of 15 kokako disappeared following the rat eradication on Kapiti Island (Empson and Miskelly, 1999). Blue ducks (*Hymenolaimus malacorhynchos*) have also been carefully monitored during operations without loss (Greene, 1998).

If poison use in New Zealand forests ceased, birds would no longer be killed by poison, but it must be remembered that the target pest species frequently compete

with birds for foods and may also be direct predators on adult birds or their eggs and chicks. Bird populations therefore may continue to decline if the pest species remain at high numbers. When predator numbers are reduced by poisoning some bird populations have been shown to recover from severe losses associated with the poisoning. Powlesland *et al.*, (1998) showed that deaths of over 50% of the robins in a population at Pureora were recouped during the following breeding season. Within a year the Pureora population had increased to 28% above the pre-poison number, while the population in the non-poisoned area had remained static. Pukeko numbers also recovered over three years on Tiritiri Matangi after a 90% reduction (Spurr and Powlesland, 1997). While some species may recover from substantial losses due to poisoning, rarer species may be adversely affected by small losses. A way of reducing the number of birds lost in poisoning operations is needed.

### **Protecting birds**

When baits are distributed in aerial operations many ground feeding bird species have access to them. In addition, a proportion of pellets are caught in the trees exposing canopy-feeding birds (Lloyd and Hackwell, 1993). Bait stations are designed to reduce the number of birds exposed to baits by making access difficult but their use is restricted by the cost involved. Bait stations do not completely remove the risk to birds as the target species, rats and possums, can lift baits out of bait stations as they feed and leave whole baits and crumbs on the forest floor where birds may encounter them. A dead robin was found in an area where poisonous baits were presented in bait stations in Te Urewera National Park and it tested positive for brodifacoum poison (Brent Beaven, *pers. comm.*, Department of Conservation, Te Urewera National Park).

In order to completely protect vulnerable birds during poisoning operations the birds must be taken into captivity or removed from the area to be poisoned. The Department of Conservation, for example, took individuals of two species, weka and brown teal (*Anus aucklandica*), into captivity during the aerial poisoning

operation on Kapiti Island. Robins were also captured and transferred to a neighbouring island (Empson and Miskelly, 1999). The weka on Mokoia Island, Lake Rotorua, were captured prior to a rodent eradication on the island and held in captivity for five months before being released. While in captivity two of the weka died (6%; Owen, 1998).

Not only is it logistically difficult, expensive and risky to capture and maintain wild birds in captivity, there are also issues associated with the birds' adjustment to captivity. The weka off Kapiti Island held at Karori Sanctuary Trust, for example, rapidly habituated to people and to the food provided (*pers. obs.*). Birds may remain in captivity for a lengthy period as the Department of Conservation generally wait two years, for example, before declaring a rodent eradication successful.

There would be considerable savings if a bird-safe poisonous bait were developed. Birds would not need to be individually protected during poisoning operations and one of the public's concerns about the use of poisons would be addressed.

My study looked at particular characteristics of baits that could be altered to make poisonous baits safer for birds. The study is timely given the recent increased awareness of bird deaths. In addition, several researchers are currently investigating new poisonous bait formulations to make baits more attractive to rats (Veitch, 1995) and possums (Morgan and Henderson, 1996). A knowledge of the characteristics of baits that may make them attractive to birds would be invaluable when designing new baits and may prevent the development and promotion of baits that are dangerous for birds. The following sections describe which bird species were chosen for this study and why, how success was measured, and some of the characteristics of baits that have been investigated in New Zealand to date.

## Study species

It is possible that a proportion of birds die during poisoning operations, not by eating baits directly (primary poisoning) but by eating insects or other animals that have eaten the baits (secondary poisoning). This study is concerned with primary poisoning since making baits less attractive to birds will not address the issue of secondary poisoning. It was important, therefore, to focus the study on birds that eat baits directly.

It has been suggested that a species' natural diet may be a predictor of how likely that species is to eat baits (Spurr, 1993). Insectivores and honey eaters may, for example, be less likely to eat baits than fruit eaters. Many native New Zealand birds have mixed diets (Heather and Robertson, 1996). Even birds such as bellbirds (*Anthornis melanura*) that eat nectar, and blue ducks (*Hymenolaimus malacorhynchos*) that consume aquatic invertebrates, are known to supplement their diet seasonally with fruit (Heather and Robertson, 1996). Many New Zealand species, then, may be susceptible to eating baits.

As discussed earlier the native bird species that have been found to die in substantial numbers during poisoning operations are weka, robins, tomtits, and fernbirds. Weka are omnivorous and have been observed eating baits directly on numerous occasions (Brown, 1993; Eason and Spurr, 1995; Spurr and Powlesland, 1997). They are active scavengers of dead rats and possums so both primary and secondary poisoning are likely to occur. Robins are insectivorous but feed on fruits in summer, autumn (Heather and Robertson, 1996) and winter (*pers. obs.*). They have also been observed eating baits directly (Brown, 1993; Eason and Spurr, 1995). Tomtits and fernbirds are described as mainly insectivorous (Heather and Robertson, 1996) but are known to supplement their diets with fruit seasonally: tomtits in autumn and winter (Heather and Robertson, 1996), and fernbirds when breeding (Barlow and Moeed, 1980). These two species have not been observed eating baits.

In this study, I concentrated on weka and robins because these species appear most vulnerable and they have both been recorded eating baits. It was hoped that an understanding of the characteristics that influence foraging in these species would also be applicable to other native birds.

Poisoning operations traditionally take place in winter or early spring in New Zealand. Because bird diets, in general, are known to vary seasonably (Berthold, 1976) with, for example, food availability and seasonal activities such as nesting, it was important to conduct trials during the appropriate season. All field trials in this study were conducted in winter and early spring.

### **How safe is safe?**

It is possible that some level of poisoning is acceptable in a bird population if that population will ultimately benefit numerically from the removal of the targeted pest. On offshore islands poisoning is usually designed to be a one-off event because the aim is to completely eradicate the targeted pest species. In this situation bird populations would be likely to recover over time from any losses associated with the eradication. On the New Zealand mainland the situation is more complicated because repeated poisoning operations are needed to maintain the target species, e.g. possums, at acceptably low levels. A balance point must exist for each bird population between the proportion of birds killed in an operation and the length of time needed to restore that population to its pre-poisoning level or higher. This balance point is unknown for any of the native bird species at present. It is possible that even a low number of deaths for some species with a low rate of recovery would be unsustainable in a repeat poisoning situation.

Because the level of poisoning that a given bird population can endure was unknown, it was not possible to define how safe a 'bird-safe' bait needed to be. There was no acceptable published threshold above which a deterrent could be regarded as safe. I therefore sought a combination of bait characteristics that

resulted in a lower level of consumption by native birds than the bait characteristics in current use in New Zealand.

## **Bait characteristics that may affect bait consumption**

### **Bait size**

In the 1970s poisoning for possums in forests became more frequent and the New Zealand Wildlife Service became suspicious that small birds were eating fragments of bait. Extensive trials (Harrison, 1978) showed that poisonous baits that had been sieved to remove fragments smaller than 16 mm in diameter posed less of a risk to birds. Recent monitoring has supported this conclusion; a lower proportion of robins died during an operation in which baits (carrot) were carefully screened, than in an otherwise similar operation, in which there was a less thorough screening (Powlesland *et al.*, 1998).

Despite screening being compulsory there is concern that 1) the effectiveness of screening may vary between operations, 2) baits may break into fragments during aerial distribution, and 3) other animals may break baits up as they eat them and leave fragments. Other studies have considered bait size (Harrison, 1978) and bird consumption and it is worthy of further investigation but this study does not investigate this bait attribute.

### **Repellents**

An obvious approach to deterring birds from baits is the incorporation of a repellent into the baits. The catch is that for a repellent to be useful in the New Zealand pest control situation it must first repel native birds, and second, not adversely affect bait palatability for the target species.

Early repellent research in New Zealand optimistically sought a lure for possums that would also act as a bird repellent. Over 40 flavours were tested on Kapiti Island (Pracy, Robertson and Udy, 1982). Only one species, weka, showed an

interest in the test sites but the study resulted in a list of additives regarded as repellent to birds in general. Acceptability to possums was also investigated and the list was narrowed to almond, bayleaf, clove, cinnamon, eucalyptus, lemon, peppermint, bergamot, gingerine, nutmeg, pimento leaf and spearmint. Possum controllers were free to use any of these lures and, with the exception of cinnamon, the effectiveness of these additives as bird repellents was not investigated further. Cinnamon was tested on two more species: kaka on Kapiti Island (number not stated) which refused to eat cinnamon lured dates, and mallard ducks in Virginia lake (Wanganui city) which were offered bread with, and without, cinnamon (Udy and Pracy, 1981). The ducks rejected the cinnamon treated bread. It is likely that the individuals of both these species were familiar with dates and bread respectively as kaka on Kapiti were regularly fed dates and the ducks were on a lake in a popular picnic area. If both species were familiar with eating the non-treated food before encountering the treated food, then rejection of the cinnamon flavoured test food may have been related to neophobia not preference.

Despite the lack of firm evidence that cinnamon deterred birds, cinnamon oil has been routinely incorporated into commercial carrot and cereal bait formulations in New Zealand since 1983 (Spurr and Powlesland, 1997). Cinnamon is added partly to mask the smell and taste of 1080 from possums and partly to repel birds (Spurr, 1993). The effectiveness of cinnamon oil as a bird repellent has since been tested on a handful of captive native bird species with mixed results. Spurr (1993) found some birds were only deterred for a day, and most not at all, by cinnamon. Hickling (1997) found the deterrent effect of cinnamon relative to plain baits increased with repeated exposures when the cinnamon oil was fresh each day and McLennan, Porter and Cowan (1992) found that kiwis were undeterred when their regular food was treated with cinnamon.

There is considerable interest world wide in finding bird repellents. These would be useful in repelling birds from crops, animal feed stores, airports, and hazardous

substances such as insecticides. Surprisingly few effective bird repellents have been identified (Shah, Clark and Mason, 1991). Of 43 products registered as bird damage control chemicals in the USA, only seven were promoted as repellents i.e. non-lethal deterrents (Mason and Clark, 1997).

Effective bird repellents are generally chemicals that are painful or cause sickness in birds (Mason and Clark, 1997). Methiocarb, for example, causes post-ingestional malaise in birds (Mason, 1989) and methyl anthranilate (Mason, Clark and Shah, 1992) and cinnamamide (Gill *et al.*, 1997) cause immediate unpleasant irritation or pain to birds. All such chemical bird repellents tested in New Zealand to date have also been found to deter the target mammalian species, particularly rats, making the chemicals unsuitable for use in a pest control situation. Methyl anthranilate, for example, at a concentration known to repel birds (2.5%) also deterred rats (Spurr, Porter and Thomson, 1995). Concentrations of 0.5% cinnamamide were found to repel weka and kea but, at this concentration, cinnamamide significantly reduced food consumption by rats making this chemical also unsuitable for use (Spurr and Porter, 1998). Other repellents have been tested in New Zealand including, dimethyl anthranilate, ortho-aminoacetophenone, tannic acid (Spurr *et al.*, 1995; Spurr and Porter, 1998), amyl acetate, and optamint oil (McLennan *et al.*, 1992) but none of these warranted further investigation.

It seems that chemicals that have unpleasant effects for birds, and are consequently effective bird repellents, also adversely affect consumption by the target species, particularly rats. Given this difficulty and the lack of likely repellents to test, an alternative approach was taken in this study. Two bait characteristics were investigated that may affect the acceptability of baits to birds without physically repelling them: colour and odour. Colour has been investigated in New Zealand previously but the particular odour tested in this study has not.

## Bait colour

Following deaths of birds in rabbit control operations in the early 1950s the New Zealand Wildlife Service initiated bait colour trials to determine whether colour could deter birds from poisonous baits. Kalmbach (1943) had already shown that North American birds avoided grains dyed 'unnatural' colours particularly green and yellow, and Caithness and Williams (1971) examined colour preferences in a single group of New Zealand domestic chickens (*Gallus domesticus*) using grains dyed three colours (yellow, green, and blue). When these chickens were found to eat less of the green grain Caithness and Williams (1971) tested another three species: wild sparrows (*Passer domesticus*), wild California quail (*Callipepla californica*) and wild finches (species not specified) in mixed flocks at 10 separate sites. The birds were only offered a choice between green-dyed grain and plain grain, with the exception of one trial, in which sparrows were offered yellow-dyed grains as well. The plain grain was eaten consistently in preference to the green-dyed grain. The chickens were pre-fed with plain grain prior to the trials but it is unclear whether the wild birds were. If birds were pre-fed or had experienced the grain in the past then the birds' avoidance of green-dyed grain may have been due to neophobia rather than a dislike of green food. At no time do wild birds appear to have been offered a full range of colours to choose from. Caithness and Williams (1971) concluded that dyeing grain discouraged birds from eating it and that green was the most effective colour.

One potential difficulty with many colour preference studies, including those by Kalmbach (1943) and Caithness and Williams (1971) was that colours were presented to a limited number of flocks of birds. The feeding choices of birds are known to be affected by the choices and behaviour of their flock mates (Ward and Zahavi, 1973; Reidinger and Mason, 1983) and this factor may not have been considered in the design of these studies.

In 1956 and 1958 McQueen and Pracy conducted an unpublished study which followed on from Caithness and Williams' work. They offered coloured wheat

and coloured pellets in three colours (red, yellow and green) to an unstated number of weka in two separate trials on Kapiti Island (described in Udy and Pracy, 1981). It was concluded that the weka preferred yellow over red over green and it was stated that no other bird species touched the foods. The methods used in these tests are not adequately described, and Udy and Pracy (1981) noted that weka were later observed eating green-dyed pellets in poisoning operations.

Despite the deficiencies in the studies described and the limited tests on native birds it became mandatory for poisons to be dyed an approved shade of green (colour within the range 221-225 as specified in British Standard 381 C 1964) with an approved dye (Erio Green B or Lissamine S. F. 150; Caithness and Williams, 1971. The dye current specified is Hexacol green V200A).

Udy and Pracy (1981) later conducted trials with new dyes. They offered weka and kaka pellets dyed three different greens, orange, blue, yellow and black. They did not state which of these two species or how many individuals ate baits, or the methodology of the trial, but they reported that orange, yellow and blue were the most preferred colours followed by black and finally green. This was the first and, I believe, only trial in which wild birds in New Zealand were offered a full range of colours. The trial was conducted at least 10 years after it became obligatory to dye poisonous baits green. There has been no further published research into the colour preferences of New Zealand birds.

### **Bait odour**

Until recently, it was believed that birds, in general, had a poor sense of smell and therefore little research worldwide has focused on repellent odours. Since the 1960s however, evidence has accumulated on the complexity of avian olfactory structures and the likely importance of olfaction in many diverse bird species (Jones and Roper, 1997). Chickens, for example, are known to detect and respond to at least 31 odours (Jones and Roper, 1997). Many of the original flavours tested in New Zealand such as almond, clove and cinnamon have strong odours to

humans and may have detectable odours to birds as well, but their effectiveness as general bird deterrents is doubtful. Birds have been shown to respond to some odours such as predator odours and the odour of conspecific blood with behavioural changes (Funk *et al.*, 1996). Odour research may allow a new approach in the search for cues that reduce consumption of baits by birds.

In summary, of the characteristics investigated in the past in New Zealand as a means of deterring birds from baits, colour shows promise but has not been studied with native New Zealand birds, cinnamon is in general use but its effectiveness has not been established, no repellents have been found to be useful and size appears to affect bait consumption and is currently controlled.

## **Aim**

The general aim of this thesis was to investigate two characteristics of poisonous baits, colour and odour, that could be manipulated to make baits safer for native birds.

This aim was achieved by:

- i) using choice tests to determine the colour preferences of three species of bird, two of which were native, when they were offered a novel food
- ii) determining how useful colour preferences may be as bird deterrents by a) assessing whether two separate populations of the one species had similar preferences, b) assessing whether different species of bird had similar preferences and c) assessing whether altering the shade of a colour altered its attractiveness
- iii) determining whether the addition of a second attribute, odour, in addition to colour made preferences stronger or more long lasting.

The structure of the thesis is as follows:

**Chapter 1: *General Introduction.*** This chapter describes the background to, and structure of, the thesis.

**Chapter 2:** *An investigation into the effect of colour on the willingness of chickens to eat a novel food.* This experiment has three sections. In the first, two methods were tested for determining colour preferences in chickens. In the second, one of these methods was used to determine the colour preferences of 12 free range chickens for a standard six colours. In the third, chickens were offered four shades of a single colour to determine whether the shade of colour offered affected preference. The relationship between the relative luminance of the colours for chickens and preferences was examined.

**Chapter 3:** *Colour preferences and coloured bait consumption by weka (*Gallirallus australis*), an endemic New Zealand rail.* The colour preferences of individual captive weka were tested by offering them the standard six colours used with the free range chickens in Chapter 2 using the same method.

**Chapter 4:** *Colour preferences in North Island robins (*Petroica australis*): Implications for deterring birds from poisonous baits.* The colour preferences of two populations of wild North Island robins were determined for the standard six colours used in Chapters 2 and 3 to determine whether a) robins had colour preferences, b) colour preferences were consistent between two separate populations and c) colour preferences were similar to those of the other species tested previously.

**Chapter 5:** *The effect of insect defensive odour on the consumption of a familiar food by New Zealand robins (*Petroica australis*) and novel food by domestic chickens (*Gallus domesticus*).* In the first section of this chapter robins were used to investigate the effect of adding pyrazine odour to a familiar food. The second section investigated which colours, when combined with pyrazine, were the least preferred by chickens and whether the addition of pyrazine affected consumption.

**Chapter 6:** *Can colour and a insect defensive odour deter native New Zealand birds from eating baits?* The first section in this chapter examined whether the combination of novelty, blue colour and pyrazine was more effective at dissuading captive weka from eating than novelty and colour alone. The second section was

conducted in the wild and examined whether baits that were blue and contained pyrazine were less consumed than baits with the cues commonly used in poisonous baits in New Zealand, green and cinnamon.

**Chapter 7: *General conclusions.*** In this chapter I discuss the implications of the findings presented in previous chapters for poisonous bait design and identify directions for future research.

This thesis presents work started in May 1996 under the supervision of Dr Joseph Waas and Dr Cheryl O'Connor. The chapters contribute to the overall aim of the thesis and all but the general introduction and the general conclusion, have been written as scientific papers for submission to journals. The relevant reference lists follow each chapter and there may be some repetition between chapters. Two papers have been accepted for publication (Chapters 3 and 4) and the style of these papers reflects the style of the journals to which they were submitted. The layout and style of the papers that have not yet been submitted were kept consistent throughout the thesis. For each chapter I was responsible for the conceptual development, planning, fieldwork, data collection and writing. My supervisors and co-authors, Cheryl O'Connor and Joe Waas, provided advice on thesis direction and trial design and commented on all the chapters. Dave Duganzich did the statistical analysis in Chapters 2, 3 and 4 and the bulk of Chapter 5 while I was responsible for the remaining analysis with guidance from a statistician.

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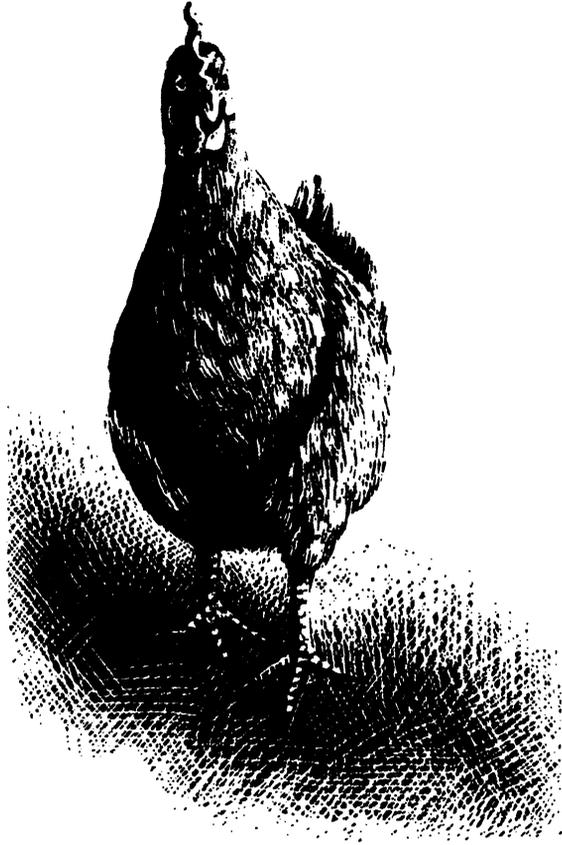
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## CHAPTER 2

### **An investigation into the effect of colour on the willingness of chickens to eat a novel food.**

Lynette Hartley, Cheryl O'Connor, Joseph Waas

#### **Abstract**

If birds have colour preferences it may be possible to deter them from poisonous baits by dyeing the baits suitable colours. We used domestic chickens (*Gallus domesticus*) to assess two methods of determining colour preferences in birds. Both methods allowed identification of colour preferences and chickens ate more yellow and white novel food than green, orange, or pink novel food using both methods. We then used the method that was the most suitable for use in the wild to test the colour preferences of twelve free range chickens for six colours (red, yellow, green, mid blue, light blue, and brown). The chickens showed colour preferences in both the amount eaten and the time elapsed until they ate pellets of each colour for the first time. They showed the greatest preference for the yellow pellets and the least for the mid blue pellets. As the visual system of birds is not completely understood we were concerned that chickens may have chosen pellets on factors other than hue, such as lightness. We calculated the relative luminance of the colours offered to chickens based on the sensitivity of chickens' double cones. A good correlation was observed with chickens preferring the two lightest colours white and yellow, however, mid blue was least preferred but not the darkest colour. We also offered free-range chickens a choice of pellets in four shades of blue but chickens did not show a preference.

We concluded that chickens had colour preferences when offered coloured, novel food and that these preferences could be detected experimentally. Blue was one of the least preferred colours overall for chickens, suggesting it may be a suitable

colour for dyeing poisonous baits. Caution should be shown, however, in the choice of the shade of blue and very light colours should be avoided.

**Keywords:** Chickens, colour preference, colour vision, deterrents, hue, luminance.

## Introduction

Poisons are used in New Zealand to control introduced mammalian pests. The objective of these operations is generally to kill either rats (*Rattus rattus*, *R. norvegicus*, *R. exulans*) or brushtail possums (*Trichosurus vulpecula*) for conservation reasons, or possums alone, because they are a vector in the maintenance of tuberculosis (*Mycobacterium bovis*) in domestic cattle and deer. Some of these operations also lead to the death of native birds (Powlesland, Knegtmans and Marshall, 1998; Empson and Miskelly, 1999). While bird populations may benefit in the longer term from the removal of possums and rats (Brown, 1997; Innes *et al.*, 1999), there remains considerable public concern about the effect of poisons on non-target species (Dick and Wallace, 1994; Watts, 1994; Frater, 1995; Speedy, 1995; Wright, 1995). Some native bird species, such as weka (*Gallirallus australis*) and New Zealand robins (*Petroica australis*), are particularly vulnerable to poisoning (Chapter 1).

Research in New Zealand has failed to identify a repellent that will deter birds from baits without also deterring the target mammalian pest species. The key to deterring one group, but not the other, may lie in identifying differences in the perceptual abilities or physiology of birds and the mammalian pests. One difference between mammals and birds lies in their visual abilities. Mammals have mono- or, more often, dichromatic colour vision (Jacobs, 1993) whereas birds generally have sophisticated and complex tetra- or pentachromatic colour vision (Varela, Palacios and Goldsmith, 1993; Vorobyev *et al.*, 1998). Bird foraging is frequently guided, almost entirely, by visual rather than taste or odour stimuli (Avery and Nelms, 1990) whereas rodents, at least, are more attentive to taste and odour than the visual features of food (Reidinger and Mason, 1983).

It has been suggested that birds may avoid foods of some colours and that they may avoid eating poisonous baits if the baits are dyed these colours (Kalmbach, 1943; Caithness and Williams, 1971). If mammals are less aware of, or concerned about, the colour of food, then changing the colour of baits may not affect

consumption by the target pest species. It is known that the colour of a bait does not affect bait consumption by a range of mammals including possums (Day and Matthews, 1999), rabbits (*Oryctolagus cuniculus*; Caithness and Williams, 1971) and several North American mammals (Kalmbach, 1943).

In this study we aimed to determine whether birds had colour preferences for coloured food and which colours were the least preferred. In Experiment 1 we used chickens (*Gallus domesticus*) to test two different methods of determining colour preferences in order to establish a method that would be suitable for testing the colour preferences of native birds. In Experiment 2 we used one of the methods established in Experiment 1 to test the colour preferences of a group of free range chickens. In Experiment 3 we tested whether free-range domestic chickens preferred particular shades of a single colour and, as a consequence, how specific instructions to managers would need to be on the colour of baits distributed during poisoning operations.

## **EXPERIMENT 1: ESTABLISHING A METHOD FOR TESTING COLOUR PREFERENCES**

Previous studies have investigated colour preferences in birds in a variety of ways. Some of these studies have little relevance to a pest control situation. For example, many researchers have offered birds different coloured objects. The motivation of a bird in approaching or pecking at an object such as a coloured disc (e.g. Salzen, Lily and McKeown, 1971), or a coloured illuminated panel (e.g. Fischer, Morris and Ruhsam, 1975; Kovach, 1977), is difficult to assess and any choices made may be different from the choices made in a feeding situation. We therefore investigated methods of identifying colour preferences by offering birds coloured foods.

Several studies have tested colour preferences in day old domestic chicks (e.g. Fischer *et al.*, 1975; Roper and Marples, 1997). The native New Zealand birds exposed to poisonous baits are predominantly adult birds with a wide range of

experiences and, presumably, colour associations. Studies on young chicks may, therefore, have little relevance and so we investigated a method suitable for adult birds.

Colour preferences in the past have often been assessed using flocks of birds (e.g. Caithness and Williams, 1971; Slaby and Slaby, 1977; Brunner and Coman, 1983; Bryant, Hone and Nicholls, 1984; McPherson, 1988). The feeding choices of birds are known to be affected by the choices and behaviour of their flock mates (Ward and Zahavi, 1973; Reidinger and Mason, 1983) and this factor must be considered in experimental designs. The native birds we ultimately wanted to test, weka and robins, do not flock in the wild so we wanted to establish a method for testing birds individually.

Many colour preference studies in the past have pre-fed birds with an undyed version of the test food that was to be used in the experiment (e.g. Kalmbach, 1943; Brunner and Coman, 1983; McPherson, 1988; Willson, Graff and Whelan, 1990; Mastrota and Mench, 1994; Willson, 1996; Roper and Marples, 1997). While pre-feeding ensures birds will approach the test area the disadvantage with pre-feeding is that birds may select colours during testing that they perceive as similar to the familiar pre-feed or they may generalise from the pre-feed to the test foods and eat colours they would not otherwise have eaten. Results obtained, therefore, may not be applicable to a wild situation where baits are novel for birds. We wanted to use a method for testing birds that did not involve pre-feeding.

It is essential to describe the colours used in trials objectively. A bird's colour vision differs from a human's in several ways. Birds have at least four, or frequently five, classes of cone in comparison to the three in humans (Bowmaker *et al.*, 1997). In addition, the maximal sensitivity of these cones falls at different wavelengths across the electromagnetic spectrum. Because colour vision is believed to depend on the interaction between cones (Thompson, Palacios and Varela, 1992) colour matching systems, such as the Munsell system (Wyszecki

and Stiles, 1982; Munsell, 1986), are not suitable for describing the colours offered to birds (Endler, 1990). Similarly, many electronic devices (e.g. photographic light meters and the Minolta Chroma meter) are calibrated to human vision and are, therefore, also unsuitable. Many, if not all, diurnal birds have a cone in, or close to, the near ultraviolet region of the electromagnetic spectrum (Chen, Collins and Goldsmith, 1984; Bennett and Cuthill, 1993; Finger and Burkhardt, 1994). Humans, in contrast, are not sensitive to ultraviolet light (Thompson *et al.*, 1992) and this difference also makes human based colour measuring and matching systems inadequate to describe the colours offered to birds. We used spectral reflectance curves to record the colours offered as these provide a quantitative description of the light reflected from a coloured surface that is unbiased by the human visual system (Endler, 1990; Bennett *et al.*, 1997). Human colour descriptions such as blue and red have been used in the text to differentiate the colours offered.

The aim of Experiment 1 was to establish a method of testing colour preferences with chickens that would be appropriate to use later on native species.

## Methods

Two possible methods were trialed for testing colour preferences, both of which met the criteria discussed in the introduction. One group of chickens was offered six colours presented simultaneously and another group was offered pairs of the six colours presented on successive days. The two methods differed in how pellets were presented, and in the number of colours that were presented at one time, but not in the colours themselves or the type of pellet. We used domestic chickens in this work because, unlike the native birds of interest, weka and robins, domestic chickens were readily available. They are similar to weka and robins in that they feed on the ground on a variety of foods including fruits and invertebrates.

### *Test birds*

All chickens (Shaver starcross and White shaver breeds) were over 2.5 years of age and were part of a group which had been raised outdoors from chicks, for at least 4 months in a free-range flock. During this time they received chick crumbs and a wide variety of other foods including fresh fruit and vegetables. The chickens were then housed in individual mesh cages and fed regular commercial poultry layer pellets (pale brown in colour) supplemented with oyster grit and vitamins. The lighting in the housing area was a combination of daylight and incandescent bulbs on a 12:12 h light:dark cycle. Chickens received their regular feed throughout the experimental period but were maintained at 80% of their free-feeding body weight (for unrelated trials). All tests were conducted in the mornings between 0800 and 1100 h, prior to feeding, between May and July.

### *Colours*

The coloured dyes (Hansels, New Zealand Ltd) were selected from commercially available food colours and were distinguished by humans as pink, orange, yellow, green, blue and white. The spectral reflectance curves of the colours (Fig. 1) were measured by Daniel Osorio (Sussex University, UK) using an Ocean Optics spectrometer. It was assumed that the food colours had no significant taste to chickens although it would be difficult to test this. The food dyes had no discernible taste to humans and the flavours associated with other components in the pellets such as sugar, lard and salt were likely to have overpowered any taste associated with the dye. The same small ( $64 \text{ mm}^3$ , *ca.* 0.05g) solid cylindrical, coloured pellets were used for both groups. These pellets were made from flour, lard and water. They were baked at  $110^\circ\text{C}$  for 8 minutes and were novel to the chickens.

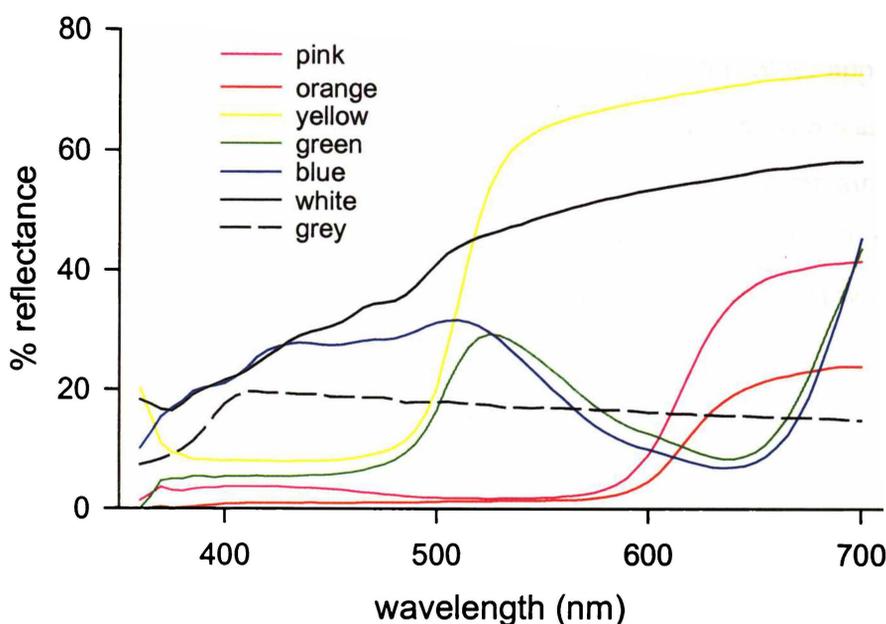


Figure 1: *Spectral reflectance curves of the coloured pellets and the background presented in Experiment 1. The spectral reflectance curve of the grey background is included. Spectral reflectance curves show the relative proportion of light reflected off a coloured surface at each wavelength. The reflectance between 350 and 750 nanometres (nm) is presented as this includes the region of the electromagnetic spectrum in which birds are believed to have visual sensitivity.*

### *Test procedure*

#### Six colour test method

Six chickens were tested individually in a test box (0.9 x 0.9 x 0.5 m) which had solid grey painted walls and floor, and a mesh top. The grey was matched visually to photographers' standard grey (18% tone; spectral reflectance curve, Fig. 1) in order to limit differences in contrast that would have occurred between the coloured pellets and a background such as black or white. The test room had white walls and was lit with two fluorescent tubes (40 W) mounted on the ceiling 2.3 m above the test box.

Once a day for six days each chicken was offered six piles of the coloured pellets presented 7 cm apart and arranged in an arc with the open end facing the entrance to the test box. Each pile contained 30 pellets of a single colour and the layout ensured each colour was equidistant from the chicken when it entered. The order and position of the colours was varied according to a Latin square design in such a way that the chickens received all six colours each day and no colour appeared in the same location twice for any chicken during the trial. The chicken was free to move among, and sample from, the piles of pellets. Each chicken remained in the test box for 3 minutes which was sufficient time to eat all the pellets offered if it chose. The number of pellets of each colour remaining after each test was recorded and the number of pellets eaten calculated.

#### Paired colour test method

Six chickens were offered pairs of colours while they were in their individual home cages (0.3 x 0.5 x 0.4 m). Cages had mesh walls, ceiling and floor and were in rows. The walls of the room were painted white. Ten grams (*ca.* 200 pellets) of each of two colours were presented to each chicken, each day, on a randomised schedule. Each chicken received each of the 15 possible paired combinations of the six colours during the 15 days of the trial. The coloured pellets were presented mixed together in a small tray (150 x 100 mm) which was painted the same grey as the floor of the test-box used in the six colour test method (Fig. 1). Chickens in adjacent cages may have been able to watch one another eating during the tests but they did not receive the same paired combinations on any one day. The pellets covered most of the bottom of the tray which was held, by hand, at the entrance to each chicken's cage for 30 seconds so that the chicken could easily see and eat from all parts of the tray. When presented with food in this fashion preliminary trials, with other chickens, showed that the chickens ate very rapidly and that 30 seconds was sufficient time for an average chicken to eat half of the pellets offered. For optimal results we chose a weight of pellets and a corresponding time that resulted in a chicken eating half the available pellets so that chickens did not switch to the less preferred colour when the more preferred colour was depleted.

Switching would have made preferences more difficult to detect. At the end of 30 seconds the tray was removed and the remaining pellets were sorted into their respective colours, weighed, and the amount eaten calculated. The lighting in this trial was a mixture of daylight and incandescent bulbs.

### *Analysis*

One chicken ate only white pellets and no pellets at all after Day 2 in the six colour method so was omitted from the analysis. Data from this experiment were analysed as the percentage of pellets consumed of those available. Data from all chickens and all days were used in the analysis of the two colour method and were analysed as the percentage consumed of that available, by weight.

The empirical logit transformation (Sokal and Rohlf, 1995) was applied to the consumption data from both tests to reduce the built in non-normality and heteroscedasticity. Because the data were of a repeat measures type, in both experiments, observations from day to day were expected to be non-independent due to consumption values being strongly correlated with consumption values on previous days. Data were thus examined by testing various models using the residual maximum likelihood procedure (REML) in the Genstat 5.3 statistical package (Patterson and Thompson, 1974).

The fixed effects considered in the six colour test were: colour, the position of a colour in the layout and day (of test). We took account of the pattern of increasing consumption observed from day to day by fitting straight lines to the logit transformed data. These linear timetrends allowed us to analyse differences in consumption on Day 1 (i.e. y intercept) and differences in the rate at which consumption increased during the trial (i.e. slope). The effect of colour that we tested, therefore, was that which occurred on Day 1. Linear timetrend and the interaction between linear timetrend and colour were also retained as fixed effects in the model because of their statistical significance ( $P < 0.05$ ).

The non-independent nature of the observations was taken into account by the terms considered in the random model. In the six colour test these comprised variability due to chicken, the interaction of chicken with colour, chicken with day and colour with day. A spline term was fitted to the logit data (Verbyla *et al.*, 1999). This term allowed for serial correlation between consecutive observations in the same bird or bird-colour combination during the experiment (e.g. it allowed us to account for the variation of one bird eating more, or less, than expected for several days). We also tested for the interaction of chicken with the spline term and the three way interaction of the chicken, the colour, and the spline term, in the random model.

In the paired colour method each chicken was tested for 15 days during which time she received each colour five times. There were, therefore, three measures associated with time: 1) the number of times a chicken had been offered that a particular colour, to date (up to five), 2) the number of times a chicken had been tested to date (up to 15) and 3) a residual effect due to day to day variation (day 15). Consumption increased steadily from day to day over the 15 days of the experiment so we fitted a linear timetrend to the first measure. Both linear and quadratic timetrends were fitted to the second measure. All curves were fitted in the logit scale. The timetrend terms were considered in the fixed effects model along with the number of times a colour had been offered (offer) and colour. The effect of colour was considered at the middle of the trial i.e. significance testing was done on the estimated level of consumption for each colour in the middle of the trial. The interactions considered in the fixed effects model were those between colour and the time terms related to the five times a colour was offered.

The terms considered in the random model for the paired colour method were chicken, and the interactions of chicken with colour, day (of year), and with the linear timetrend associated with the five days a colour was offered. We also considered the spline term and the interactions between chicken, colour and both

the spline term and the linear timetrend associated with the 15 days of the experiment.

The REML procedure provided likelihood ratio tests (LRT) and Wald tests for the components of the random and fixed models respectively, both of which are asymptotically distributed as Chi-square with appropriate degrees of freedom.

## Results

### *Six colour test method*

Colour had a significant effect on the number of pellets consumed by chickens on the first day colours were offered using the six colour method (Table 1). White pellets were the most consumed (42%), yellow and orange pellets were intermediate (9% and 6% respectively), and green, blue, and pink pellets were the least preferred with consumptions of 3% or less (Fig. 2a; all the bar-graphs in this chapter are presented with the colours ranked from the most preferred to the least preferred to allow easy comparison of colour preferences). Different chickens had different colour preferences (chicken  $\times$  colour, Table 1) with two of the chickens preferring white, two preferring yellow and one preferring blue.

Consumption increased steadily from the first day pellets were offered and by Day 6 all of the chickens were eating all of the pellets offered. This resulted in a significant linear timetrend (Table 1, Fig. 3a). While the amount consumed increased over time for all colours the rate at which consumption increased was found to differ between colours (linear timetrend  $\times$  colour, Table 1) with the consumption of the less preferred colours increasing more sharply than that of the more preferred colours (Fig. 3a). These linear time trends accounted for almost all the differences in colour consumption from day to day (colour  $\times$  day, Table 1). Not only did chickens eat more pellets they also ate more colours each day (mean number of colours eaten each day; Day 1: 1.6; Day 2, 2.6; Day 3, 4.0; Day 4, 4.6; Day 5, 6.0; Day 6, 6.0).

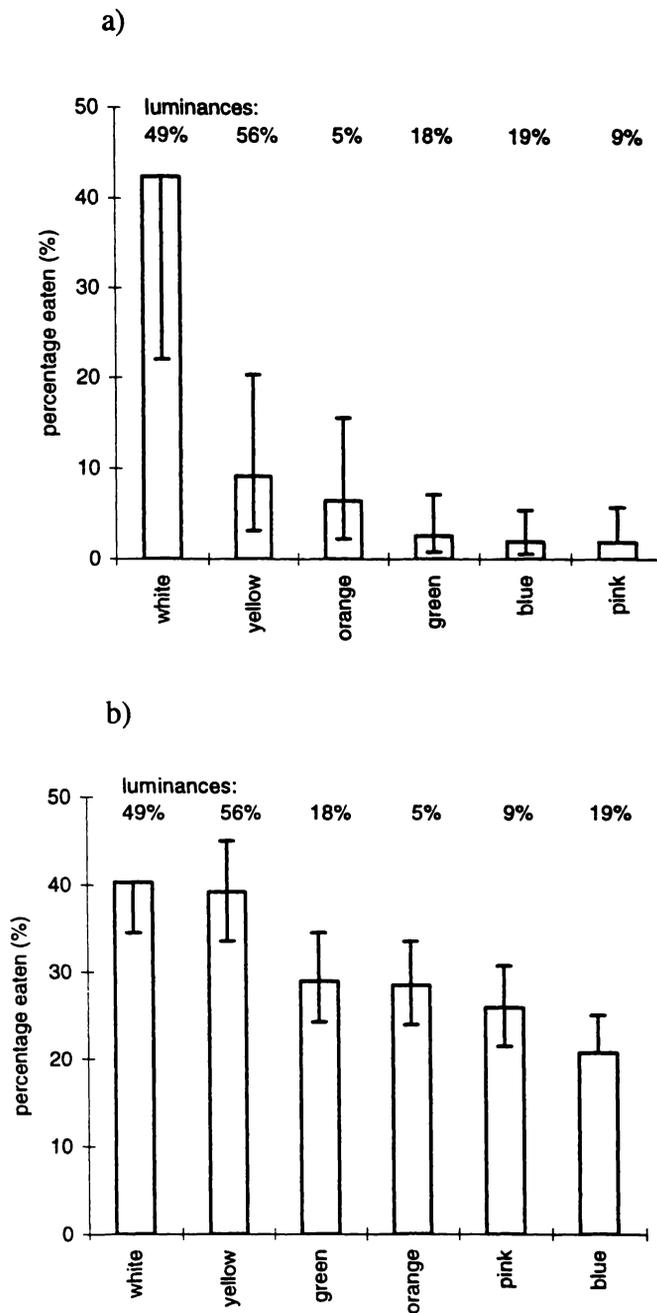


Figure 2: The adjusted mean consumption of coloured pellets by chickens in Experiment 1, ranked from most preferred to least preferred when a) the six colours were offered simultaneously and b) when the colours were presented in pairs. Preference is shown on Day 1 in a) and in the middle of the Experiment in b). The error bars represent half least significant differences. Means are different at  $P < 0.05$  if the error bars do not overlap. The luminances of the coloured pellets for chickens relative to a perfect white are presented as above each bar (Experiment 3).

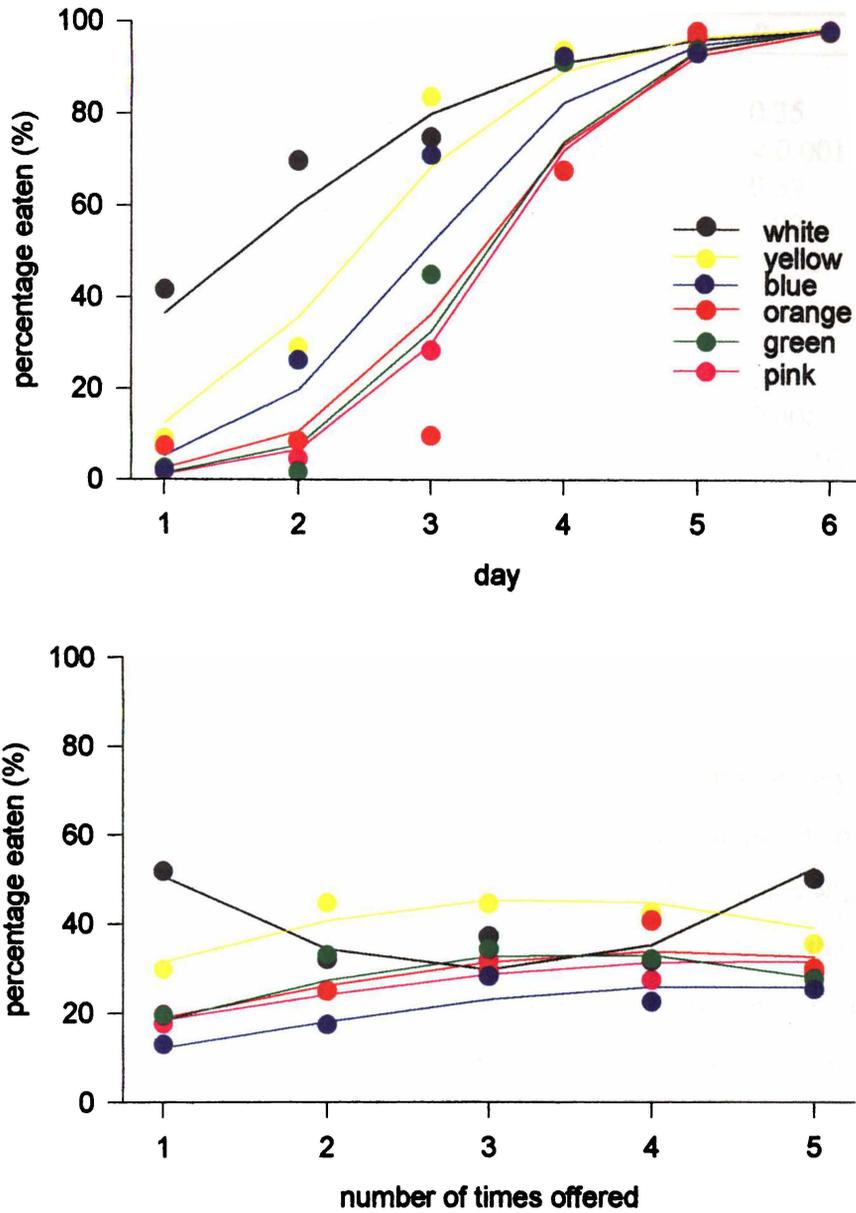


Figure 3: The percentage of pellets eaten per day by chickens in Experiment 1 when a) the six colours were presented simultaneously for the six days of the trial and b) the six colours were presented in pairs for the five days on which each colour was offered. The lines present the predicted values backtransformed from the original logit model and the symbols present the backtransformed mean observed values.

Table 1: *Statistical results for fixed and random effects generated by likelihood ratio (LRT) and Wald tests from the REML analysis of Experiment 1 for the amount consumed when all six colours were offered simultaneously.*

Source	d.f.		P
<i>Fixed effects</i>		Wald	
Position	5	5.6	0.35
Linear timetrend	1	178.0	< 0.001
Day	4	2.8	0.59
Colour	5	13.0	0.02
Linear timetrend × colour	5	19.5	0.002
<i>Random effects</i>		LRT <sup>a</sup>	
Chicken × spline	1	3.8	0.03
Chicken × colour × spline	1	6.4	0.006
Chicken	1	10.0	< 0.001
Chicken × colour	1	21.9	< 0.001
Chicken × day	1	7.8	0.003
Colour × day	1	0.7	0.20

<sup>a</sup>The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The P value is thus 0.5 times the probability for a  $\chi^2$  with one degree of freedom (Stram and Lee, 1994).

There was considerable variability between chickens in the amount they consumed overall (Table 1). One chicken, for example, was eating all the pellets offered by Day 3 while others did not eat all the pellets offered until Day 5 or 6 which resulted in different overall consumptions. Some chickens ate more than others on some days (chicken × day, Table 1). The chicken by spline term interaction was significant as was the interaction between chicken, day and the spline term (Table 1) which also suggested that chickens showed some variability in consumption from day to day and that this variability differed from chicken to chicken. There was no indication that chickens ate from some positions in the arc of pellets more than others (Table 1) and there was no indication that chickens ate more on some days of the week than others once the linear effect had been removed (Table 1).

It was noticed that chickens showed consistency in the order in which they ate pellets, even after they started eating everything offered. Chickens ate white

pellets first in over half of the 30 tests (number of times a colour was eaten first: white, 18; yellow 6; green 3; orange 2; blue 1; pink 0).

*Paired colour test*

When we offered chickens a choice between pairs of colours the colour of the pellets had a significant effect on the amount the chickens consumed (Table 2). Intake fell into two clusters. White and yellow pellets were the most consumed with mean consumptions of 40% and 39% respectively while green, orange, pink and blue were least consumed (29%, 28%, 26% and 2% respectively; Fig. 2b).

Table 2: *Statistical results for fixed and random effects generated by likelihood ratio tests (LRT) and Wald tests from the REML analysis in Experiment 2 for amount consumed when chickens were offered pairs of colours..*

Source	d.f.		P
<i>Fixed effects</i>		Wald	
Linear timetrend (15)	1	16.5	< 0.001
Offer	4	2.0	0.7
Linear timetrend (5)	1	1.2	0.3
Quadratic timetrend (5)	1	0.2	0.7
Colour	5	21.3	< 0.001
Colour × offer	20	37.8	0.01
Colour × linear timetrend (5)	5	5.3	0.4
Colour × quadratic timetrend (5)	5	17.7	0.003
<i>Random effects</i>		LRT <sup>a</sup>	
Spline	1	31.3	< 0.001
Chicken × colour × spline	1	14.5	< 0.001
Chicken	1	3.5	0.03
Chicken × colour	1	15.4	< 0.001
Chicken × linear timetrend (5)	1	1.8	0.09
Colour × day (15)	1	12.9	< 0.001
Chicken × colour × linear timetrend (15)	1	4.9	0.01

<sup>a</sup> The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The P value is thus 0.5 times the probability for a  $\chi^2$  with one degree of freedom

The percentage of pellets eaten by chickens increased steadily from day to day during the 15 days of the experiment (linear timetrend (15), Table 2) and this pattern of increasing consumption differed for some chickens for some colours (chicken × colour × linear timetrend (15), Table 2). Different chickens also ate

different amounts overall (Table 2). Once the effect of the increasing consumption over the 15 days of the experiment was accounted for by the 15 day linear term the time related terms associated with the five days each colour was offered were not significant (offer, linear timetrend (5), quadratic timetrend (5), colour  $\times$  linear timetrend (5), chicken  $\times$  linear timetrend (5), Table 2).

The amount of the different colours eaten differed from day to day over the five days colours were offered (colour  $\times$  offer, Table 2, Fig. 3b). The consumption of most of the colours remained relatively constant over the five days they were offered but the amount of white consumed dropped in the middle of the experiment relative to the other colours (Fig. 3b) although it was still one of the most preferred colours overall. While chickens showed an overall colour preference, the preference shown differed from day to day (colour  $\times$  day (15), Table 2) and different chickens had different colour preferences (chicken  $\times$  colour, Table 2).

The spline term was significant (Table 2) which suggested that chickens' consumption fluctuated about the linear timetrends that were fitted to the data. The inclusion of the spline term allowed the fluctuations to be removed and the underlying trends analysed. The fluctuations about the timetrends differed between colours and chickens (chicken  $\times$  colour  $\times$  spline, Table 2).

## Discussion

Colour preferences were detected using both the six colour and the paired colour methods. It appears that chickens do have colour preferences and that we can experimentally detect these preferences. We found green to be one of the least preferred colours of those offered and this result is similar to that obtained by Caithness and Williams (1971) and Kalmbach (1943).

The colour preferences identified were very similar using both methods, with yellow and white being the most preferred colours and green, pink and orange and

blue the least preferred. The similarity in preferences observed in both trials was reassuring as it suggested that real preferences were detected rather than the peculiarities of either test method or test environment. We noticed that the chickens preferred the two colours that were the lightest, judging by human vision. This may indicate chickens were choosing on lightness rather than colour.

In the six colour method, we observed that chickens entering the test box each day tended to move straight to their preferred colour. Once this colour had been eaten they would quickly shift to eating colours adjacent to their first choice in the line up. A possible consequence of a chicken eating its preferred colour first, but being less particular about subsequent consumption, was that colours next to the preferred colours may have been consumed more than would otherwise have been the case. The number of times colours appeared adjacent to other colours in the layout was not considered when a Latin square was chosen for the six colour tests. As it happened, blue appeared next to yellow or white, more often (on 5 occasions) than green (3 occasions), red (2 occasions) or orange (2 occasions). Future colour preference experiments should consider this problem and design layouts in which each colour is presented next to each other colour an even number of times. We found that colours were not preferentially eaten from any positions in the six colour method, for example either end of the arc, although this possibility should not be ignored in future trials.

A strong pattern of increasing daily consumption emerged over the six days of the six colour method and the 15 days of the paired colour test. This pattern of increasing consumption made statistical analysis complicated but it has implications for poisonous bait design since it gives an indication of how a bird's behaviour toward a bait may change over time. Changes in consumption from day to day are therefore worth recording in future trials. Fitting linear timetrends to the consumption data allowed analysis of the underlying colour preferences. Consumption each day was affected by a chicken's experience the day before so it

was necessary to use a statistical analysis that took account of both this factor and the pattern of increasing consumption.

Chickens were variable in the amount they consumed. Consumption not only differed from chicken to chicken, in both methods, but also between chickens from day to day. In addition to variability in consumption, individual chickens showed variability in their colour preferences. Most previous studies examining colour preferences in individual birds have also commented on variability in colour preference between individuals (Willson *et al.*, 1990; Willson and Comet, 1993; Puckey, Lill and O'Dowd, 1996; Willson, 1996). A measure of variability in colour preferences between individuals would be valuable if recommendations are to be made on colours to dye poisonous baits as it may give an indication of the relative effectiveness of each colour. Variability in colour preferences also extended to different consumption patterns for the different colours over time. This variability was reflected in the significant interactions in the analyses between the time related terms in both methods and colour.

The two test methods were conducted under different lighting conditions in this preliminary trial. The light given off by a fluorescent tube (Experiment 1) is different spectrally from that given off by an incandescent bulb or daylight (Experiment 2; Billmeyer and Saltzman, 1981; Wyszecki and Stiles, 1982). A colour may look different under different lighting conditions for a bird if, for example, one light source contained a different proportion of ultraviolet light (e.g. Hunt *et al.*, 1997). None of the colours offered reflected substantially in the ultraviolet range (Fig. 1) but the slight differences in colour preferences between the two methods may have been due to the colours appearing different under the two different lighting conditions. If comparison between colour preferences is an objective then future experiments should take place under standardised lighting conditions. As wild birds are exposed to poisonous baits outdoors, experiments on bait acceptance should also be conducted outdoors under natural light.

We concluded that the six colour method would be more useful of the two methods with wild birds in a field situation for the following reasons. First, this method would provide useful information on colour preference even if a bird was not present for all the days of an experiment as each bird received all the colours each day. The paired method depended on a bird being present for all tests in order to receive all colour combinations or, failing that, access to a considerably larger number of birds all of which would need to be individually recognisable. Neither of these requirements may be practical in a field situation. The six colour test method took fewer days than the paired colour method and was not dependent on estimating in advance how long it would take for a bird to consume half the available food. Finally the six colour method had an advantage in that the order and time a bird touched each colour each day, and a dynamic measure of how consumption of each colour changed from day to day relative to the other colours, could be obtained.

## **EXPERIMENT 2: COLOUR PREFERENCES IN FREE RANGE CHICKENS TESTED OUTDOORS**

In this experiment we used the six colour method established in Experiment 1 to assess colour preferences in free range chickens for the specific colours that would be used later in trials with native birds. We were interested in seeing how general colour preferences were between different bird species as an indication of how useful colour may be in deterring birds, in general, from poisonous baits. Testing the same colours with each species allowed this comparison. We used free range domestic chickens as they resembled the native birds of interest in that they foraged outdoors and were familiar with a wide range of foods but they were more readily available and could be handled without difficulty. Because native birds, in later experiments, would be tested outdoors under natural light it was important to test the chickens outdoors as well to ensure that comparison of colour preferences between the chickens and the other species would be valid.

## Methods

### *Test birds*

The twelve chickens tested were 15 - 18 months old and lived in a group of approximately 100 birds on Benzies free-range egg farm (West Melton, Canterbury, New Zealand). They had been raised on the farm since hatching and were fed indoors daily at 0800 h on poultry pellets (pale brown in colour) supplemented with garlic and vitamins. The chickens normally consumed all the feed provided by 1000 h and foraged out of doors throughout the rest of the day for natural foods. They also received a wide range of fruit and vegetables fortnightly, outdoors, depending on the season. Coloured leg bands allowed identification of individuals and they were tested outdoors between 1400 and 1900 h from September to November. There was no artificial lighting in the housing area or outdoors.

### *The colours*

Six colours were offered to the chickens (Fig. 4). The spectral reflectance curves of the colours (yellow, green, mid blue, light blue, red and brown (dark red); Fig. 5) were measured by Justin Marshall, University of Queensland, Australia, using a Subspec spectrometer (Marshall, Jones and Cronin, 1996). Red, mid blue, yellow and green were chosen as these colours are well spaced across the human visual spectrum and consequently should be discernibly different to any species with colour vision similar to, or better than, our own. The light blue and brown were included as they offered birds different shades of the blue and red, respectively. White was not included as chickens in the previous trial showed such a strong preference for it. By removing white we hoped the preferences for less preferred colours would become clearer. We could not find a food safe dye that reflected ultraviolet light so could not test chicken preferences for a colour in this range.

Spectral reflectance curves allowed us to examine the inherent properties of a colour prior to processing by human or bird visual systems. We wanted to offer

the chickens (and native birds in later trials) colours that were uniform. An examination of the spectral reflectance curves of the coloured pellets (Fig. 5), however, showed that the blue and green pellets reflected considerably less light than the yellow and red pellets and that the yellow pellets reflected more light than any of the other colours (i.e. comparing the areas under the curves for each colour). The spectral reflectance curves also showed that the difference in height between the non-reflecting section and the reflecting section of each curve was greater for the red and yellow than the blues and green.

We investigated the interaction of the blue and green food dye with several white foods such as rice, flour and pasta at several different concentrations in an effort to make the colours offered more uniform. We could not find a food that, when dyed, produced blues and greens that had curves that resembled the curves of the red and yellow in magnitude. We therefore continued to use the flour recipe used in the previous experiments since we knew that chickens would eat it. By presenting coloured lights or coloured objects to the birds we may have been able to control the colours offered more accurately but we felt this would compromise the purpose of the trial which was to determine the birds' preferences when eating coloured novel food. Coloured pellets, were the same formulation, size, and shape as those used in Experiment 1. Powdered food colours were supplied by Bush Boake Allen, NZ Ltd., Auckland, New Zealand.

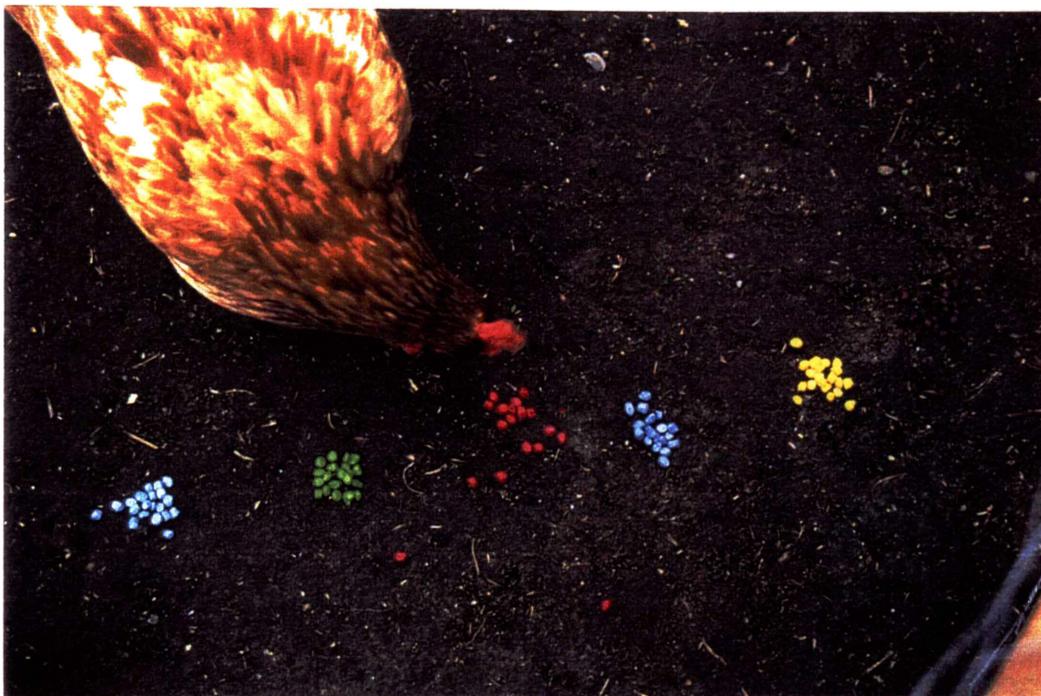


Figure 4: A domestic free range chicken eating the coloured pellets offered during the colour preference test described in Experiment 2.

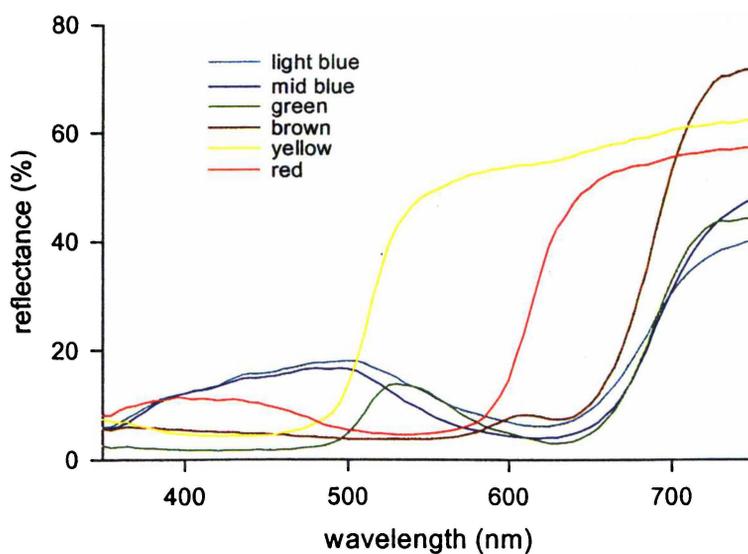


Figure 5: Spectral reflectance curves of the coloured pellets offered to free-range domestic chickens in Experiment 2. Spectral reflectance curves show the relative proportion of light reflected off a coloured surface at each wavelength.

### *Test procedure*

Twelve chickens were tested outside using the six colour method. Chickens were placed one at one time in the same test box used in Experiment 1 but it was found that the free range chickens became distressed in the test box with solid walls so we used a test box the same size with mesh walls and top. Chickens were tested in the box for two minutes each day, for six consecutive days, weather permitting. Two minutes was sufficient time for a chicken to eat all the colours offered if it chose. The test box was set on bare soil in a field immediately adjacent to the chickens home field. Other chickens' could not see the test chicken's choices during tests but the test chicken could see out to familiar surroundings. All chickens were familiarised with the test box by placing them in it for a few minutes prior to the start of testing.

Each chicken was presented with six piles of coloured pellets 7 cm apart arranged in an arc as in Experiment 1. Each pile contained 20 pellets of a single colour. The position of colours in the arc was changed daily between birds, and days, using a Latin square design to ensure that 1) all colours were offered each day, 2) all colours appeared in every position for each bird, 3) all colours appeared in every position each day and 4) each colour appeared next to each other colour twice and only twice for each bird. This criteria overcame the problem encountered in the previous six colour experiment where particular colours were presented next to some colours more often than others. A movable panel ensured all the colours were presented in shadow. The dampness, and therefore colour, of the ground was kept constant using water applied with a mist sprayer.

The total time elapsed until each chicken first ate each colour was recorded as an alternative measure of colour preference to consumption. This time was the total time a chicken was exposed to the coloured pellets before eating. For example, if a chicken did not eat yellow pellets until three seconds into the third test then the time elapsed was 120 seconds for each of Day 1 and 2 plus three seconds for Day 3 (i.e. 243 seconds).

Despite familiarity with the test box, it was found that chickens, when placed in the test box alone, would eat familiar but not novel food. Various methods were used to persuade chickens to eat the novel food when alone including changing the time of day (and hence hunger levels), feeding the chickens in the test box with familiar food before starting tests, altering the test box appearance, altering the test box location and placing the test box where the test chicken could see other chickens eating familiar food. Because the chickens were still reluctant to consume pellets we trained them to eat the novel food by putting up to three chickens at a time in the test box and offering them black and white pellets of the same formulation used in the main test (See Fig. 9 for the spectral reflectance curves of the black and white pellets.) By familiarising chickens to both black and white pellets it was hoped their choices, during tests, would not be biased by the pre-feeding. Most chickens needed up to three, five-minute, sessions in the test box with other chickens before they would eat there alone. All chickens were eating at least half the black and white pellets offered when alone in the test box before testing commenced.

### *Analysis*

#### Amount consumed

Two chickens ate sporadically during the experiment and were omitted from the consumption analysis. The majority of the chickens (eight out of the remaining 10) were eating all the pellets offered by Day 4 so only the first three days of data were used in the analysis. Consumption data were analysed as the percentage of pellets consumed of those available and logit transformed before being analysed as in the six colour method in Experiment 1. The fixed effects considered were consumption of each colour on Day 1, the position of a colour in the layout, the timetrend associated with the pattern of increasing consumption from day to day (linear timetrend), and the interaction between the linear timetrend and colour. The random effects considered were chicken, the variation between individual chickens in their colour consumption (chicken colour) and the interaction between chicken and the linear timetrend.

### Time to eat

All chickens were included in the analysis of the time to eat a colour and the data were square root transformed to stabilise the variance and then analysed as in Experiment 1. The only random effect considered was chicken, with colour and position as fixed effects. In this case position refers to the position of the colour when a chicken first ate it.

## Results

The chickens were initially unwilling to eat the novel pellets offered. Once a chicken in a group started eating the black and white pellets during the pre-feeding the other chickens present watched intently, often with their eyes within a few centimetres of the eating chicken's beak. They would then try the novel food themselves.

Chickens consumed different amounts of the pellets depending on the colour of the pellets (Table 3). The chickens consumed the most yellow pellets (75%) while consumption of red, brown, light blue and green pellets was intermediate (60%, 48%, 43% and 34%, respectively) and few mid blue pellets (8%) were consumed (Fig. 6).

Once again chickens showed a strong pattern of increasing consumption over time (linear timetrend, Table 3, Fig. 7). While consumption was low for the less preferred colours, such as mid blue, and relatively high for the more preferred colours such as red and yellow on Day 1, by Day 3 chickens were consuming nearly 100% of all the colours including mid blue (Fig. 7). This resulted in a tendency for consumption to increase at different rates depending on the colour of the pellets (linear timetrend  $\times$  colour, Table 3).

The colours that were consumed most on the first day were also eaten the most quickly. Yellow pellets were eaten, on average, sooner (6 s) than pellets of the

other colours, except brown (100 s) and red (106 s), Mid blue pellets (237 s) were eaten, on average, later than any of the other pellets (Table 4, Fig. 8).

Table 3: *Statistical results for fixed and random effects generated by likelihood ratio (LRT) and Wald tests from the REML analysis in Experiment 2 for amount consumed.*

Source	d.f.		P
<i>Fixed effects</i>		Wald	
Position	5	8.0	0.16
Colour	5	13.4	0.02
Linear timetrend	1	54.7	< 0.001
Linear timetrend × colour	5	11.5	0.04
<i>Random effects</i>		LRT <sup>a</sup>	
Chicken	1	23.3	< 0.001
Chicken × linear timetrend	1	2.7	0.05
Chicken colour	1	22.4	<0.001

<sup>a</sup>The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The P value is thus 0.5 times the probability for a  $\chi^2$  with one degree of freedom.

There was considerable variability in consumption between individual chickens with different chickens consuming different amounts overall (chicken, Table 3) and different chickens increasing their consumption at different rates (chicken × linear timetrend, Table 3). There was also variability between chickens in the time elapsed until they ate the pellets (Table 4).

Different chickens ate different amounts of the different coloured pellets (chicken colour, Table 3). This was, at least partly, due to different chickens having different colour preferences although it may also have been due to some chickens preferences for the more preferred colours being stronger than those of other chickens. No chickens showed a preference for the least preferred colour, mid blue. Chickens did not eat more pellets or more quickly from any positions in the layout (Tables 3 and 4).

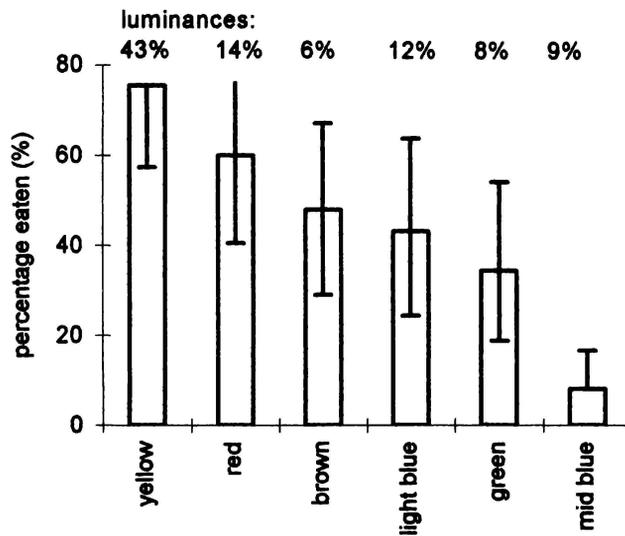


Figure 6: The adjusted mean percentage consumed of each of six colours by free range chickens on day 1 in Experiment 2 from least preferred to most preferred. The error bars represent half least significant differences. Means are different at  $P < 0.05$  if error bars do not overlap. The luminances of the coloured pellets for chickens relative to a perfect white are presented as a percentages above each bar (Experiment 3).

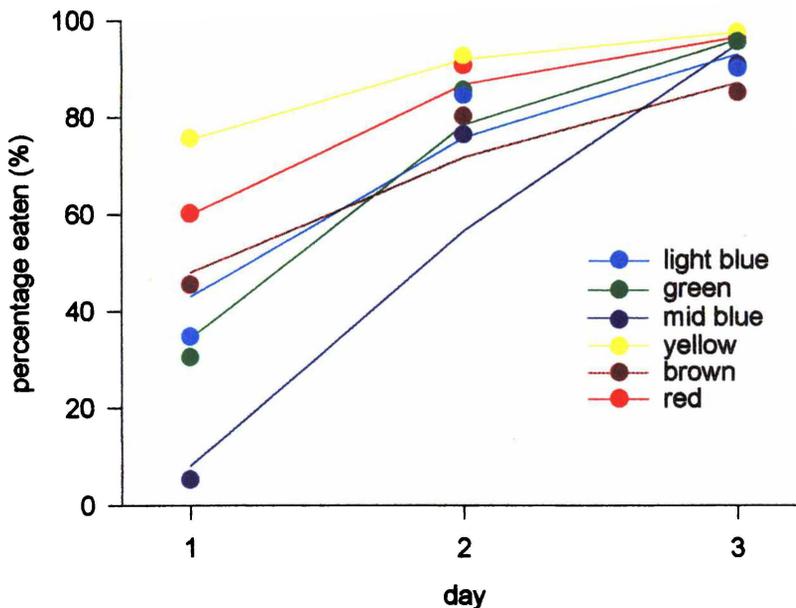


Figure 7: The mean percentage consumed of the six colours offered to free-range chickens in Experiment 2 over the first three days of the experiment. The symbols present the backtransformed mean observed values and the lines present the predicted values backtransformed from the original logit model.

Table 4: *Statistical results for fixed and random effects generated by likelihood ratio (LRT) and Wald tests from the REML analysis in Experiment 2 for time elapsed to eat.*

Source	d.f.	Wald	P
<i>Fixed effects</i>			
Position	5	3.5	0.6
Colour	5	27.1	< 0.001
<i>Random effects</i>			
Chicken	1	LRT <sup>a</sup> 14.4	< 0.001

<sup>a</sup> The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The P value is thus 0.5 times the probability for a  $\chi^2$  with one degree of freedom.

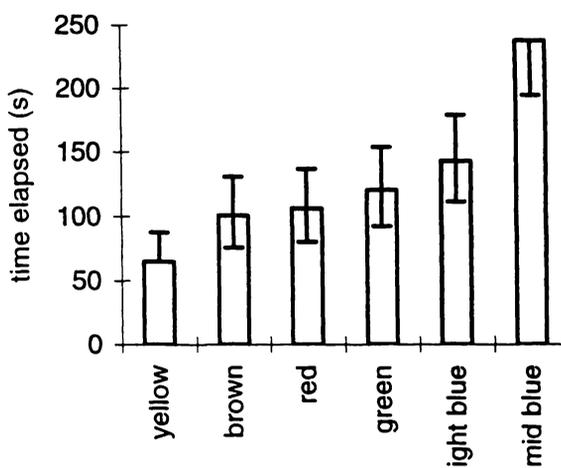


Figure 8: *The adjusted mean time elapsed until the free range chickens in Experiment 2 ate each of the colours offered ranked from most preferred to least preferred. The error bars represent half least significant differences. Means are different at  $P < 0.05$  if error bars do not overlap.*

## Discussion

When we offered red, yellow, green, light blue, mid blue, and brown novel pellets to free range chickens they showed a preference for the yellow and red pellets and did not eat the mid blue and green pellets as readily. The preferences for pellets of other colours were intermediate. This result was consistent for both the amount consumed and the time elapsed until the chickens first ate the pellets of each colour. The exact colours offered differed between this and the first experiment but both groups of chickens showed a preference for the yellow pellets. They ranked mid blue pellets among their least preferred in this experiment and in the

paired colour method. The only other colour in common between Experiments 1 and 2 was green which was a less preferred colour in both experiments.

The colour preferences found in our experiments were similar to those shown in the majority of earlier studies where adult birds have been offered coloured food. In six out of the 12 studies we examined birds preferred red and/or yellow food to blue and/or green food (Caithness and Williams, 1971; Slaby and Slaby, 1977; Brunner and Coman, 1983; McPherson, 1988; Willson and Comet, 1993; Marples, Roper and Harper, 1998). The remaining studies did not find a preference (Bryant *et al.*, 1984; Mastrota and Mench, 1994), did not rank the colours of interest (Willson *et al.*, 1990) or found differing preferences (Kalmbach, 1943; Willson, 1996; Moran, 1999). Not all the studies offered all colours.

The willingness of chickens to try the coloured novel food differed between the current experiment and Experiment 1 despite the similarity in methods and the fact that the same food was presented in both trials. We were forced to pre-feed the free range chickens in groups in this experiment before they would eat the coloured novel food when they were alone. This behaviour differed from that of the chickens in Experiment 1 where the majority of birds (five out of six) readily ate the coloured food while alone in the test box. The chickens in Experiment 1 were familiar with being placed alone in boxes and were regularly offered food while alone as they were the subjects of unrelated psychology experiments. The chickens in Experiment 2, in contrast, lived and ate in a large flock from which they were never separated. These chickens would eat familiar, but not novel, food when alone. The difference in behaviour observed suggests that a species social system and a bird's individual history may both influence the situations in which an individual is willing to sample novel food. It is unclear whether pre-feeding the chickens in Experiment 2 affected their colour preferences although it may have increased the amount the chickens ate on the first day of the experiment.

We were unable to dye pellets to reflect in the ultraviolet region of the spectrum so we were unable to examine chickens' responses to ultraviolet food. Burkhardt (1982) proposed that ultraviolet reflectance may enhance fruit attractiveness to foraging birds although there is little experimental evidence for this. Willson and Whelan (1989) surveyed the fruits of 53 species in central east Illinois, USA, and found that 17 reflected in the ultraviolet range. Field and aviary trials however showed that birds did not consistently prefer the ultraviolet reflecting fruit in comparison to the same fruit from which the ultraviolet reflectance had been removed. Similarly Lee, Hodgkinson and Johnson (1990) examined the fruit of 41 indigenous New Zealand plants and found that only one reflected in the ultraviolet range. While it is tempting to imagine that objects that reflect ultraviolet would appear somehow unusual or particularly conspicuous to birds, it seems more realistic to expect that ultraviolet wavelengths would be processed visually by birds in a similar way to other wavelengths and ultraviolet reflecting colours would have similar properties to any other colours for birds (Thompson *et al.*, 1992; Bennett *et al.*, 1997). As a result we predict that ultraviolet reflecting colours are unlikely to elicit exceptionally strong preferences or avoidances in birds, but we were unable to test this.

In this study, we could not offer colours that were uniform as judged from the spectral reflectance curves. As discussed earlier, some of the colours of the pellets reflected more light than others (area under the curves) and some had greater height changes between the non-reflecting and reflecting wavelengths. Generally speaking, the total amount of light reflected by a surface affects how light (Billmeyer and Saltzman, 1981) or bright (Wandell, 1995) a colour will appear and the height of the change in reflectance between wavelength segments affects how saturated or intense (Hubel, 1998) a colour will appear (see Billmeyer and Saltzman, 1981)). (It should be noted, however, that exactly how light or saturated a colour will appear relative to another colour will depend on the spectral sensitivities and visual processing of the species, or individual, looking at the colour; Endler and Thery, 1996).

Because we were unable to offer chickens colours that had uniform spectral reflectance curves we were concerned that chickens may have chosen pellets, in this and Experiment 1, on the basis of one of these attributes (lightness or saturation) rather than hue. (Hue is the attribute which permits a colour to be classified as red, yellow, green and so on. Hues with spectral reflectance curves that reflect, for example, between roughly 480 and 560 nanometers (nm) appear green, between 560 nm and 590 nm appear yellow and between 590 nm and 630 nm appear orange etc. to humans (Billmeyer and Saltzman, 1981). Hue, then is the wavelength at which reflectance changes from high to low and the sign of the change; Endler, 1990). We noticed that chickens may have been choosing the colours that had the greatest overall reflectance. The chickens in Experiment 1, for example, showed a strong preference for white and the chickens in both Experiments 1 and 2 preferred yellow. In addition, the chickens in Experiment 2 preferred the lighter rather than the darker of the two blues offered.

If birds avoid eating foods that appear dark, but eat foods that appear light, irrespective of hue, this has implications for poisonous bait design. It would be insufficient to specify the colour (hue) baits should be dyed. It would be necessary, instead, to specify the exact shade of colour to dye baits. In the following experiment we investigated whether chickens preferred some shades of blue to others and whether there was a correlation between the preferences displayed and the lightness of a colour for a chicken.

### **EXPERIMENT 3: THE EFFECT OF SHADE AND LUMINANCE**

Mid blue was the least preferred colour in Experiment 2 and, therefore, the most promising colour to dye poisonous baits. We investigated the effect on consumption of altering the shade of blue offered.

Birds, unlike mammals, have double cones which appear to have an important role in vision since they make up 45-50% of the cones in a bird's eyes (Bowmaker and Knowles, 1977). Evidence suggests double cones perceive luminance rather than

colour (Vorobyev *et al.*, 1998). Luminance describes the visually effective radiance of a surface (Boynton, 1990), that is, the amount of light reflected off a surface weighted by the species' visual sensitivity at each wavelength. If the luminance (or lightness) of the colour of a novel food is important to chickens when they forage, we might expect a correlation between the luminance of the colours offered and the preferences observed. We examined the colours used in all three experiments in this paper, calculated their relative luminances for chickens, and compared these to the preferences observed.

## Methods

### *Effect of shade*

Twelve chickens were tested at the same study site used in Experiment 2. The chickens came from the same flock as the chickens used in Experiment 2 but they were different individuals. Three different shades of blue pellet were obtained by adding increasing amounts of blue food dye with the same white flour-based pellet mix used earlier. A fourth darker shade was obtained by adding black food dye to the strongest of the blue mixes. Black food dye was used rather than carbon (soot), the other option, as carbon has been implicated as a bird repellent when in the form of activated charcoal (Mason and Clark, 1994) and powdered animal charcoal (Pank, 1976). The spectral reflectance curves of the colours are shown in Fig. 9 and were measured by Justin Marshall. The blues reflected progressively less light as they became darker. The height change between the reflecting and non-reflecting wavelengths also reduced progressively but, as discussed earlier, we were not able to make a blue with a large height difference between the reflecting and non-reflecting sections of the spectral reflectance curve. Each chicken was presented with four piles of coloured pellets arranged in an arc as previously. Each pile contained 30 pellets of a single colour. Using 30 pellets per pile meant that the number of pellets offered was consistent between all three of these experiments (120) even though the number of colours offered, and hence number of piles, differed.

### *Analysis*

The number of pellets consumed of each colour and the time elapsed until a colour was eaten for the first time were analysed as reported for Experiment 2. Only the data from the first three days were included and the fixed and random effects considered in the model were the same. Data from all chickens were included in the analysis.

### *Luminance*

To calculate the spectral sensitivity curve for the chickens' double cones we used Dartnall's nomogram (Wyszecki and Stiles, 1982) with a maximum sensitivity at 597 nm (Bowmaker and Knowles, 1977). We used this sensitivity curve to weight the spectral reflectance curves (Figs. 1, 5, and 9) of each of our colours (by multiplying the values from the curves at 5 nm intervals against the chicken sensitivity curve at each wavelength). From this value we calculated the luminance for each of our colours relative to a white that reflected maximally across the spectrum. In daylight the spectral curve of the incident light is essentially flat between 460 and 750 nm (see Wyszecki and Stiles, 1982) so we did not include this factor for the experiments conducted in daylight or partial daylight. The six colour method (Experiment 1) was tested under a fluorescent light and we used the colour spectra of the ambient light at the test box to calculate relative luminance.

## **Results**

### *Shades of blue*

The chickens did not show a preference for any of the shades of blue offered judged by either the amount consumed on the first Day (Table 5, Fig. 10) or the time elapsed until a chicken first ate pellets of the different shades (Table 6, Fig. 11). As in the previous chicken trials, the most noticeable effect was the increasing proportion of pellets consumed each day. This pattern of consumption resulted in a linear time trend (Table 5, Fig. 12). Chickens did not increase their consumption of the different colours at different rates (linear timetrend  $\times$  colour,

Table 5). As previously, different chickens consumed different amounts of pellets (Table 5) and first ate pellets at different times (Table 6). There was considerable variability between chickens in the amount eaten of the different colours (chicken colour, Table 5) but little evidence of variability between chickens in the rate at which their consumption increased (chicken  $\times$  linear timetrend, Table 5).

Table 5: *Statistical results for fixed and random effects generated by likelihood ratio (LRT) and Wald tests from the REML analysis in Experiment 3 for amount consumed.*

Source	d.f.		P
<i>Fixed effects</i>		Wald	
Position	3	18.6	< 0.001
Colour	3	3.5	0.32
Linear timetrend	1	51.1	< 0.001
Linear timetrend $\times$ colour	3	3.4	0.33
<i>Random effects</i>		LRT <sup>a</sup>	
Chicken	1	18.2	< 0.001
Chicken $\times$ linear timetrend	1	1.9	0.09
Chicken colour	1	19.5	< 0.001

<sup>a</sup> The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The P value is thus 0.5 times the probability for a  $\chi^2$  with one degree of freedom.

Table 6: *Statistical results for fixed and random effects generated by likelihood ratio (LRT) and Wald tests from the REML analysis in Experiment 3 for time elapsed to eat.*

Source	d.f.		P
<i>Fixed effects</i>		Wald	
Position	3	1.5	0.68
Colour	3	5.3	0.15
<i>Random effects</i>		LRT <sup>a</sup>	
Chicken	1	4.7	0.02

<sup>a</sup> The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The P value is thus 0. times the probability for a  $\chi^2$  with one degree of freedom

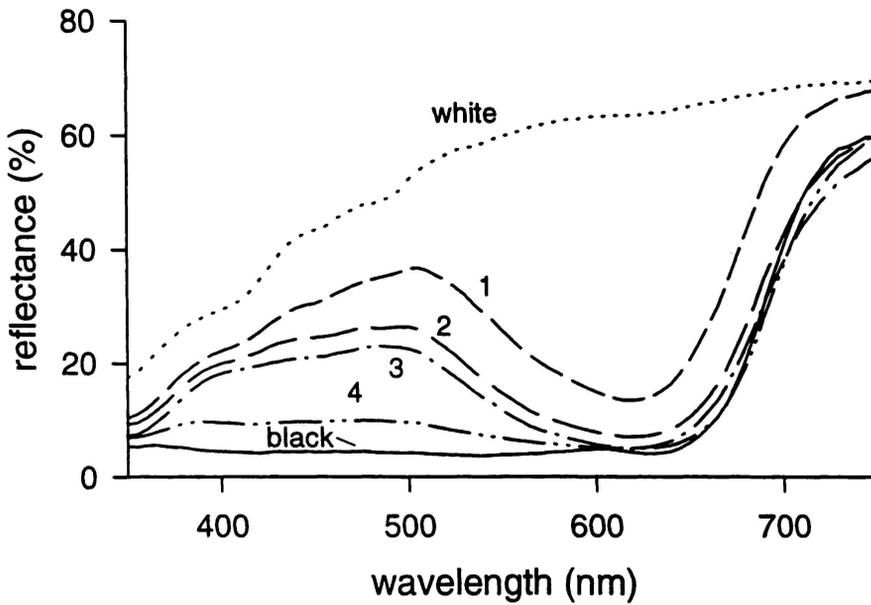


Figure 9: Spectral reflectance curves of the four shades of blue pellet (1 = lightest, 24 = darkest) offered to free range domestic chickens in Experiment 3. The spectral reflectance curves of the black and white pellets used to familiarise the chickens with the test procedure are also presented.

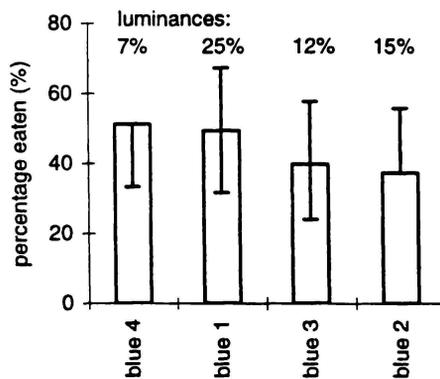


Figure 10: The adjusted mean percentage consumed by chickens on day 1 of the four shades of blue (1 = lightest, 4 = darkest) offered in Experiment 3, least preferred to most preferred. The error bars present half least significant differences. Means are different at  $P < 0.05$  if error bars do not overlap. The luminance of the coloured pellets for chickens relative to a perfect white are presented as percentages above each bar (Experiment 3).

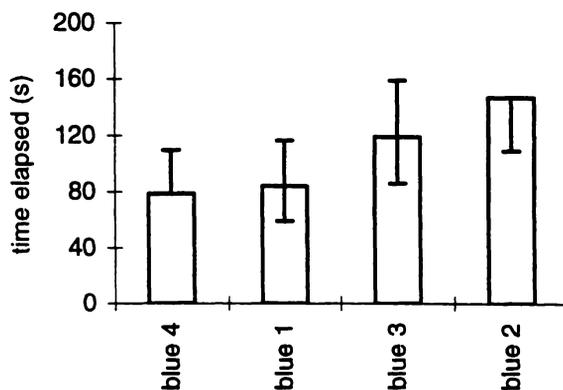


Figure 11: *The adjusted mean time elapsed until the free range chickens in Experiment 3 ate each of the four shades of blue offered. The error bars represent half least significant differences. Means are different at  $P < 0.05$  if error bars do not overlap.*

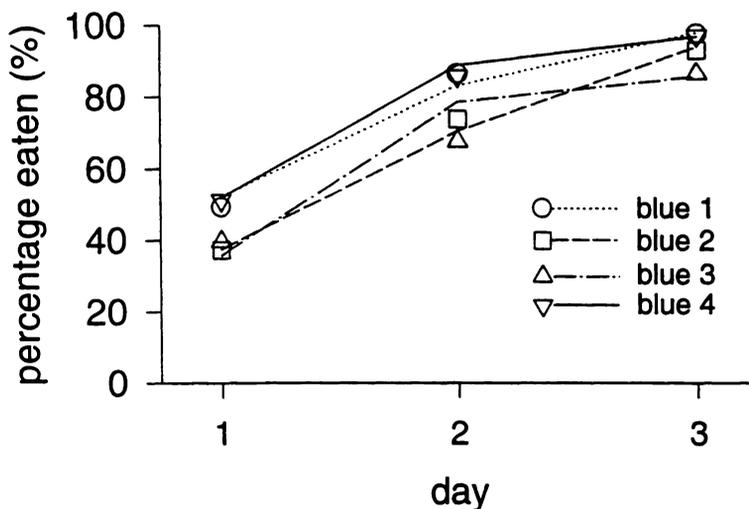


Figure 12: *The mean percentage of pellets consumed by free range chickens in Experiment 3 over the first three days of the experiment. The symbols present the backtransformed mean observed values and the lines present the predicted values backtransformed from the original logit model.*

In contrast to the results reported in Experiment 2 chickens ate more pellets from some positions than others when presented with different shades of blue (Table 5). They ate fewer pellets from the pile furthest to their left and more pellets from the next pile in. There was no evidence for a position effect, however, in the time elapsed until a chicken first ate a colour (Table 6).

### *Luminance*

The relative luminances of the coloured pellets offered to chickens are presented on the bar graphs showing the mean amount consumed of each colour in Experiment 1 (Fig. 2), Experiment 2 (Fig. 6) and Experiment 3 (Fig. 10) to allow comparison between preference and luminance. Colours had the same luminances to chickens, relative to a perfect white, regardless of which of the two lighting conditions they were tested under in Experiment 1, daylight and a fluorescent light. There was a good correlation between preference and luminance overall with the yellow and white being more preferred and more luminant (lighter) for chickens in Experiment 1 and the yellow being the most preferred and most luminant in Experiment 2. It is unclear whether there was any correlation between luminance and preference for the other colours offered since there was little difference between the chickens' preferences for the other colours. Mid blue, which was the least preferred colour for the free range chickens in Experiment 2, was not the darkest of the colours offered. The luminance of the lightest blue offered in Experiment 3 was not as high as the luminance of the yellow or white offered in earlier trials.

## **Discussion**

We found no evidence that the free range chickens had preferences for pellets of any of the shades of blue we offered judged by either the proportion of pellets they consumed on Day 1 or the time elapsed until they ate each shade.

It is possible the chickens did not prefer any of the shades of blue offered because they were unable to differentiate between them. This seems unlikely because

chickens have cones with sensitivities at both 418 nm (violet) and 455 nm (blue) (Bowmaker and Knowles, 1977) suggesting they should have good sensitivity to blues. Osorio, Jones and Vorobyev (1999) found that young chicks were extremely accurate in their differentiation of colours which appeared very similar shades to human observers including shades of blue. In addition, we have already shown, in Experiment 2, that chickens can differentiate between two shades of blue and preferred one more than the other. The range of blues offered in this trial was greater than the range offered in Experiment 2. In Experiment 2 the blue pellets had luminances of 9% and 12% whereas the luminances of the blue pellets in Experiment 3 ranged from 7% to 25%. The test method was the same in both experiments so we do not know why the chickens did not show preferences in this experiment. The same number of pellets was offered in both experiments so the lack of preference observed in Experiment 3 is not likely to be related to hunger. It is possible that blue on its own is a poor trigger for eating compared to the more preferred colours present in Experiments 1 and 2.

There was sufficient correlation between luminance and the colour preferences observed in Experiments 1 and 2 to suggest that luminance may be a factor in the colour preferences of chickens. It is possible that, had we offered a blue that was very light, with a luminance equal to that of the yellows offered in Experiments 1 and 2, chickens may have shown a preference for it. It may be prudent, therefore, to avoid dyeing poisonous baits colours that are very luminant (light) for chickens.

## **General Discussion**

Overall the chickens preferred red and yellow novel food to blue and green novel food and this preference agreed well with those found by previous researchers (Caithness and Williams, 1971; Slaby and Slaby, 1977; Brunner and Coman, 1983; McPherson, 1988; Willson and Comet, 1993; Marples *et al.*, 1998). There are many possible reasons why birds may prefer some colours to others. Some colours may, for example, appear more conspicuous or attractive to birds. Alternatively it is possible that birds choose to eat particular coloured foods to

meet their dietary requirements. Carotenoids, for example, are coloured pigments which animals can only obtain through their diet. Carotenoids are believed to be important for animals for disease prevention and general health (Stahl and Sies, 1996) and it is possible animals seek out carotenoid-rich foods (Olson and Owens, 1998). Most yellow, orange and some red foods owe their colour to the presence of carotenoids (Fox and Cameron, 1989) and these pigments are also found with chlorophyll in green plants. Because red and yellow foods are often rich in carotenoids, birds may form a preference for these colours, although this does not explain the lack of preference for green observed in this study. It remains to be seen whether other bird species show a preference for red and yellow and eat less blue and green food.

The chickens did not show preferences for any of the shades of blue offered. The issue of whether birds prefer to eat some shades of a colour more than others is an important one because we are wanting to identify the colour and shade that is most likely to discourage birds from eating poisonous baits. Few studies have attempted to examine the effect of shade on colour preference. Puckey *et al.* (1996) found that silvereyes (*Zosterops lateralis*) preferred red artificial fruit of two brightness levels over white artificial fruit and ate very few of any of the grey artificial fruit they were offered. The researchers concluded from this that the silvereyes selected fruit on the basis of hue rather than brightness. The individual silvereyes used were already familiar with red and white, but not grey, fruit from a previous trial. We suggest that Puckey *et al.* (1996) showed that silvereyes could differentiate the red from a series of greys but that their choice of reds over the greys may have been due to the birds choosing familiar food over novel food. In order to test how important factors such as intensity are in chicken foraging it would be necessary to offer chickens food in a range of hues, luminances, intensities and any other characteristics of interest. These factors could be quantified by measurements off the spectral reflectance curves of each colour and analysed using principle component analysis to determine their relative importance in chickens' foraging decisions.

When we compared chickens' colour preferences to the luminance of the colours offered in all three experiments we found a good correlation with the lightest colours for the chickens being the most preferred. The spectral sensitivity curve for humans for luminance has a maximum sensitivity of 555 nm (photopic luminous efficiency function, Boynton, 1990). This compares with the curve for chicken double cones with a maximal sensitivity of 597 nm. Human luminance vision, therefore, may approximate chicken luminance vision and colours that appear light to humans should be avoided for use in poisonous baits since birds may be more inclined to eat such colours.

Our study did not satisfactorily test whether the intensity of a colour affected colour preferences. We were unable to produce an intense blue or green (i.e. a blue or green with a spectral reflectance curve that showed a large difference in height between the reflecting and non-reflecting sections). It appears, however, that such blues and greens may not exist or may be rare in dyed objects because the pigments that produce intense blues are not available (Laird, 1994). An examination of the spectral reflectance curves of common colour standards published in Wandell (1995), Wyszecki and Stiles (1982) and Billmeyer and Saltzman (1981) support this conclusion. None of the curves of the blues or greens presented by these authors have equal heights to the red and yellow curves.

This observation raises another possible explanation for the colour preferences observed in our, and previous, studies. If intense blues are difficult to achieve it is probable that previous researchers also offered birds choices between reds and yellows that were more intense than the blues and greens offered. Previous studies did not, unfortunately, present the spectral reflectance curves of the colours offered so we cannot confirm this. The generalised preference among birds in previous studies for red and yellow food over blue and green food may have been related to birds preferring more intense colours over less intense colours rather than birds preferring red and yellow over blue and green per se. In a poisoning situation, however, it does not matter whether a bird is avoiding blue

because of the hue or because it is less intense as long as the two characteristics always go together. Should further studies indicate that blue is a promising colour for deterring birds from poisonous baits, consideration should be given to whether intensity may be a factor.

The method developed in this study proved effective in quantifying colour preferences and it allowed us to measure 1) the variability in colour preference between individuals and 2) how preferences changed over time. Both these measures are important in identifying a colour to dye poisonous baits as they provide an indication of what proportion of individuals may avoid eating a particular colour and which colour may be avoided for longest. Native birds should be tested for colour preferences using the method developed in this study.

## **Acknowledgments**

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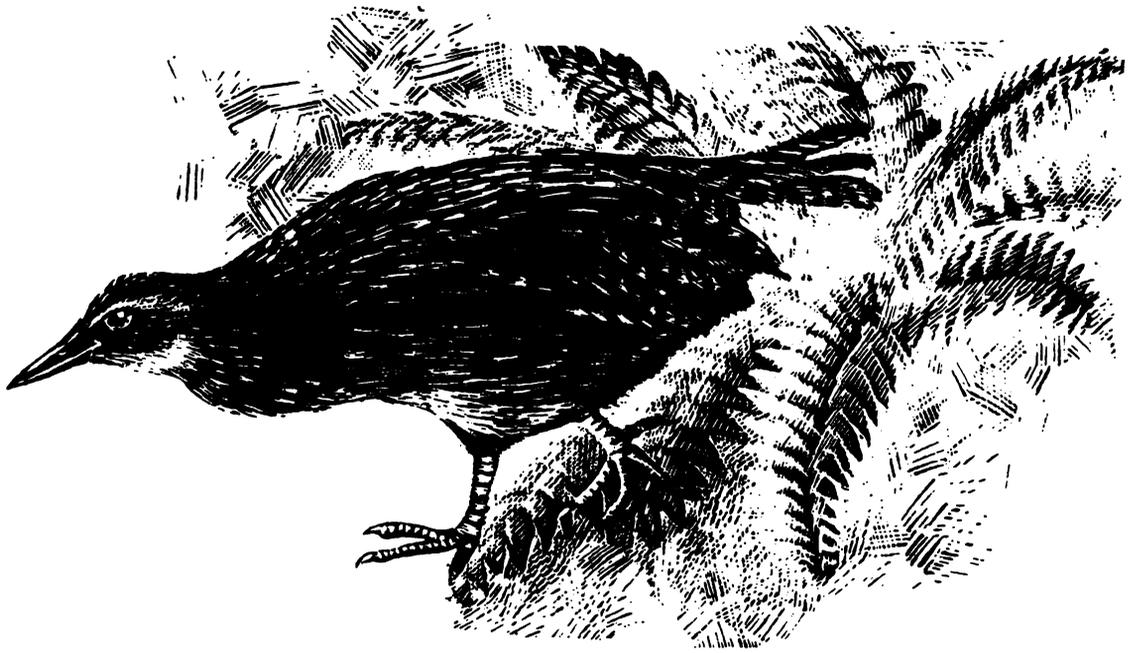
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## CHAPTER 3

### **Colour preferences and coloured bait consumption by weka (*Gallirallus australis*), an endemic New Zealand rail**

Lynette Hartley, Joseph Waas, Cheryl O'Connor & Lindsay Matthews

#### **Abstract**

Native birds are among the non-target species that are killed in poisoning operations directed at introduced mammalian pests in New Zealand. By identifying colours that birds find unattractive, and incorporating them into poisonous baits, some deaths may be avoided. The colour preferences of weka, *Gallirallus australis*, an endemic New Zealand rail, were tested over 6 days by offering individual weka a choice between six different colours of a novel food pellet. Weka ate significantly more red and yellow pellets on the first day than green, mid blue, light blue, or brown pellets but consumption of all colours increased sharply on subsequent days. The colour preferences found agree well with published studies on other bird species. If colour preferences are general across species colour may be useful in deterring birds from poisonous baits. Weka however with their opportunistic and adaptable feeding style rapidly increased their consumption of baits, even those colours they initially avoided. It appears that a more active deterrent than colour will be needed for weka.

**Keywords:** bird deterrents, choice tests, non-target species, poisonous baits, spectral reflectance.

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## Introduction

Poisons are used with increasing frequency in New Zealand in operations to kill introduced mammalian pests. There are conservation benefits from pest control operations, for example, populations of both New Zealand robin *Petroica australis* (Powlesland, 1998) and kokako *Callaeas cinera* (Innes et al, 1999) recover following the poisoning of pest species.

Non-target species such as native birds are also known to be killed in poisoning operations (Harrison, 1978; Spurr, 1993), with weka *Gallirallus australis* being particularly vulnerable (Eason and Spurr, 1995). These medium-sized flightless rails are endemic to New Zealand. Their omnivorous and adaptable feeding habits may predispose them to poisoning. Weka diet includes invertebrates, fruit, carrion, and eggs (Carroll, 1963; Heather and Robertson, 1996; Bramley, 1994) and, when in captivity, weka adapt readily to a wide range of novel foods (G. N. Bramley, pers. comm.). Weka live and forage in a wide range of environments from open areas to shrubland and dense forest. They are territorial throughout the year (Heather and Robertson, 1996) and do not flock.

At present, in New Zealand, two strategies are mandatory to discourage birds from taking poisonous baits (Pesticides Act, 1979): (1) all poisonous baits are required to be dyed green, a colour believed to be unattractive to birds; and (2) baits must be sieved to remove particles smaller than 16 mm (Caithness and Williams, 1971; Spurr, 1981). Green was chosen following a New Zealand study by Caithness and Williams (1971) using introduced bird species. An earlier American study (Kalmbach and Welch, 1946) had indicated that birds avoided dyed food in general and green and yellow dyed food in particular. The aim of our study was to determine if the colour of a novel food affected the likelihood of a weka eating it.

While weka vision has not been studied specifically, the retinas of birds usually have at least four spectrally distinct classes of retinal cone (Chen and Goldsmith,

1986; Bennett and Cuthill, 1994) as compared to three classes in humans. It is reasonable to assume, then, that weka have good colour vision but there are differences between human vision and bird vision both in the sensitivity maxima of the visual pigments and in the neurophysiology of visual information processing (Varela et al., 1993). We cannot, therefore, assume that weka, or other birds, see the world in the same way that we do. Pigeons, for example, may not categorise colours into the same colour groupings as human viewers (Wright and Cummings, 1971).

In many previous studies of colour preferences, particularly with wild birds, pre-feeding with the undyed form of the test food was used to ensure a sufficiently large number of birds visited the test site. There are two potential problems with this: 1) any reduced consumption of dyed food relative to plain food may simply be a measure of short term neophobic response rather than colour preference; and 2) the preference for particular colours may be confounded by the similarity between that colour and the colour of the familiar pre-feed. Birds in studies involving pre-feeding have generally preferred plain food to any of the coloured foods (Kalmbach and Welch, 1946; Caithness and Williams, 1971; Brunner and Coman, 1983).

In the current study, we examined the individual food colour preferences of weka that were not pre-fed with the test food. Weka were offered the coloured food daily for several days to investigate changes in colour preference and consumption over time. The overall goal was to identify the colours weka least prefer to eat and to see how long colour preferences lasted. This is part of a series of trials with native species determining the usefulness of colour in deterring native birds from eating poisonous baits and hence reducing deaths of non-target bird species in poisoning operations.

## Methods

### *Test birds*

The weka used in this trial were held in captivity at Karori Reservoir, Wellington, New Zealand. They had been captured from the wild (Kapiti Island, New Zealand) by the Karori Sanctuary Trust and were maintained by the Trust's team of volunteers until they could be returned to Kapiti Island following a poison operation there. The weka had been in captivity for 6-7 months when the study began. Birds had coloured leg bands which allowed identification of individuals.

The weka lived in a 1100 m<sup>2</sup> enclosure (the home enclosure) surrounded by a mesh fence but open to the sky. The weka, since capture, had become familiar with people and were fed daily at 0930 h. The food provided varied from day to day but always contained at least three of the following: rolled oats, sardines, peas, sweetcorn, minced ox-heart, raisins, grated cheese and chicken pellets. The weka ate all the food provided within approximately 30 min and were occasionally observed to take natural food in the form of insects and worms (pers. obs.).

Three groups of weka were isolated, six weka at a time, in an adjacent triangular test enclosure which measured 13.5 by 12.5 by 12.5 m. The vegetation in both the enclosures consisted of knee high blackberry *Rubus fruticosus*, long grass and broom *Cytisus scoparius*. The six weka were chosen randomly from those in the home enclosure with the qualification that each group consisted of three males and three females. Weka were allowed to settle into the test enclosure overnight without disturbance, before being tested individually, daily, for 6 days. The weka were fed their normal diet immediately after the test each day. Water was provided for bathing and drinking.

### *Test procedure*

The test enclosure was further divided by a partition to form a sub-enclosure (triangular, 4.5 by 4.0 by 4.0 m) in which testing took place. The lower metre of the partition was covered with opaque cloth so weka could not see in or out during tests.

Individual weka were presented with a choice between pellets of six colours (Fig. 1): red, brown, yellow, green, and two shades of blue (mid blue and light blue). The spectral reflectance curves for each of the colours are shown in Fig. 2 (as measured with a portable dual spectrometer, PSD 1000, Ocean Optics, Florida, USA). Spectral reflectance curves show the percentage of light scattered from the surface of an object at a each wavelength. They therefore provide a measure of the raw signal available to the viewer that is independent of the human visual system or visual range (Boynton, 1990; Endler, 1990; Bennett et al., 1997). Providing the spectral reflectance curves of the colours used describes the colours more accurately than is possible with colour names such as red, light blue etc.

The non-toxic pellets (5 x 5 x 2 mm, mean weight 0.4 g) were handmade from flour, water, sugar, lard and food-colouring (powdered dyes from Bush Boake Allen, New Zealand Ltd) and were baked (15 min, 120°C). It was assumed that the coloured dyes had no significant taste to weka although it was not possible to test this. They were diluted (at least 1:200 by weight with flour) and had no discernible taste to humans. No attempt was made to simulate existing poisonous baits in size, shape, colour or texture. Rather, weka were presented with pellets that were completely novel to them, differing only in colour.



Figure 1: A weka eating the coloured pellets offered during the colour preference test.

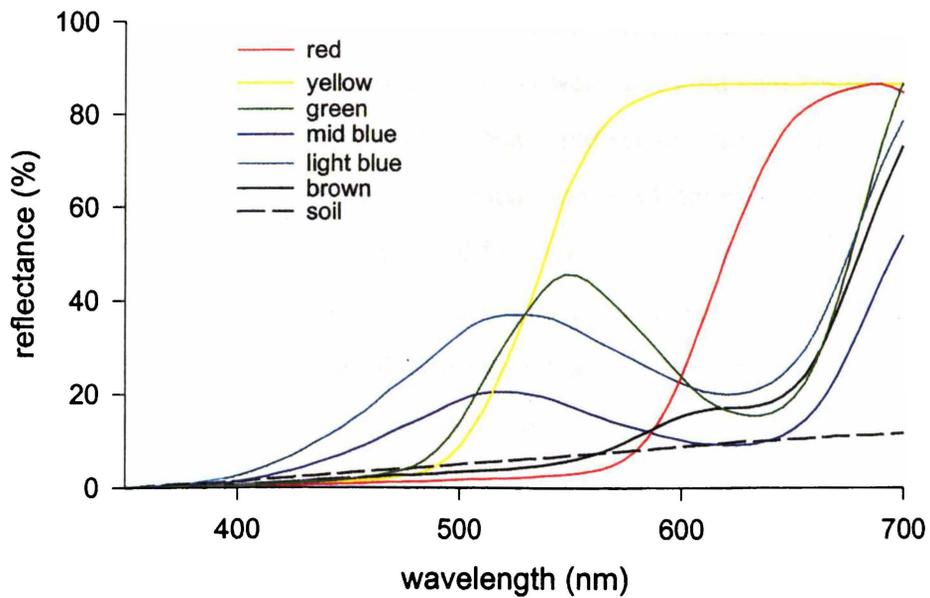


Figure 2: Spectral reflectance curves of the six colours of pellets offered to weka and the background soil. Spectral reflectance curves show the relative proportion of light reflected at each wavelength.

In the sub-enclosure, an area of approximately 1m<sup>2</sup> of vegetation was cleared to expose the soil (greyish-brown in colour, see Fig. 2). The pellets were laid out on the exposed soil in piles of ten pellets of each colour, 100 mm apart, to form an arc. As the test area was located in one corner of the sub-enclosure weka tended to approach the arc from the open end. The position of the colours in the arc was changed between birds and days using a Latin square design to ensure that each colour was offered each day, each colour appeared in each position during the trial and each colour appeared next to each other colour twice, and only twice, for each bird during the trial. This procedure controlled for the possibility that colours adjacent to particularly attractive colours would be chosen more often and controlled for a position bias in the hens' choices. The dampness, and therefore colour, of the ground was kept constant with water applied with a mist sprayer. The pellets were always presented in shade due to overcast weather or the use of a movable opaque panel which cast a shadow across the test area. All trials were completed between 0800 and 1200 h.

Each weka was individually isolated in the sub-enclosure for 14 minutes per day. The weka were found to enter willingly. A video camera was mounted over the pellets to record the bird's behaviour. Videos were analysed later for the time elapsed until each weka touched with its beak, and started eating, each of the colours for the first time each day. The total number of pellets each bird consumed of each colour was calculated from those remaining after each test. If weka failed to touch or eat a colour on any given day, the time elapsed was recorded as 840 seconds, the total duration of that test. Four individuals did not eat any pellets and were excluded from the analysis (leaving  $n=14$ ).

#### *Data Analysis*

The empirical logit transformation (Sokal and Rohlf, 1995) was applied to the consumption data as it contained numerous 0 and 100% values. Because the data were not independent the resulting variate was then analysed using the residual maximum likelihood procedure (REML) in the Genstat 5.3 statistical package,

each observation being weighted by the inverse of its estimated variance (Patterson and Thompson, 1974). The fixed effects we considered were position of colour in the layout, colour, linear and quadratic time trends (across the six days of the test period) and the interaction of colour with time trends. Colours were compared at Day 1. The non-independent nature of the observations was recognised by terms considered in the random effects model. These comprised day (of year), individual bird effects and the interaction of individual bird effects with time trend, colour and time trend by colour. The interaction between individual bird and residual test day was also considered. The REML procedure provides likelihood ratio tests (LRT) and Wald tests for the components of the random and fixed models respectively. Both are asymptotically distributed as Chi-square with appropriate degrees of freedom.

The same approach was used to analyse the time elapsed before weka touched pellets and before weka started eating. In this case time elapsed was used, not percentage data and values were log transformed to stabilise the variance prior to analysis.

## Results

Colour had a highly significant effect on the number of pellets consumed by weka on Day 1 (Table 1). Intake on Day 1 fell into two clusters, red and yellow with mean consumptions of 48% and 62%, respectively, and a cluster of colours that were little consumed (green 28%, brown 15%, light blue 11%, and mid blue 9%; Fig. 3). The colours that were most consumed on Day 1 (red and yellow, Fig. 3) were also the colours that weka touched with their beaks and started eating most quickly on Day 1 (Figs 4a & 4b). The two blues, green and brown were in the cluster of least consumed colours on Day 1 and were also the colours weka were least likely to touch and least likely to eat (Figs 4a & 4b).

Table 1: *Statistical results for fixed and random effects generated by likelihood ratio (LRT) and wald tests from the REML analysis.*

Source	df	Consumption		Time to touch		Time to eat	
		Wald	P	Wald	P	Wald	P
<b>Fixed effects</b>							
position	5	3.7	0.59	3.4	0.64	3.1	0.68
colour	5	30.0	<0.001	53.4	<0.001	37.1	<0.001
linear	1	29.8	<0.001	9.3	0.002	8.4	0.004
quadratic timetrend	1	6.2	0.013	2.9	0.089	3.5	0.061
colour x linear timetrend	5	34.6	<0.001	38.7	<0.001	21.8	<0.001
colour x quadratic timetrend	5	7.2	0.21	10.7	0.058	6.7	0.24
<b>Random effects</b>							
bird	1	7.3	0.003	0	ns	0	ns
bird x linear timetrend	1	8.9	0.001	0	ns	0.1	0.38
bird x quadratic timetrend	1	0	ns	0	ns	0	ns
bird x colour	1	86.7	<0.001	29.3	<0.001	50.1	<0.001
bird x colour x linear timetrend	1	2.8	0.047	1.9	0.084	4.8	0.014
bird x colour x quadratic	1	0	ns	0	ns	0	ns
bird x residual testday	1	1.7	0.096	0	ns	0	ns
day of year	1	17.5	<0.001	0	ns	12.1	<0.001

<sup>a</sup> The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The *P* value is thus 0.5 times the probability for a  $\chi^2$  with one degree of freedom (Stram and Lee, 1994).

Table 2: *Consumption of coloured pellets by weka. Ten pellets of each of six colours were offered for six consecutive days.*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mean no. pellets eaten per weka	17.1	31.9	45.5	52.7	50.9	52.7
Mean number of colours eaten per weka	2.7	3.9	5.4	5.8	5.6	5.7

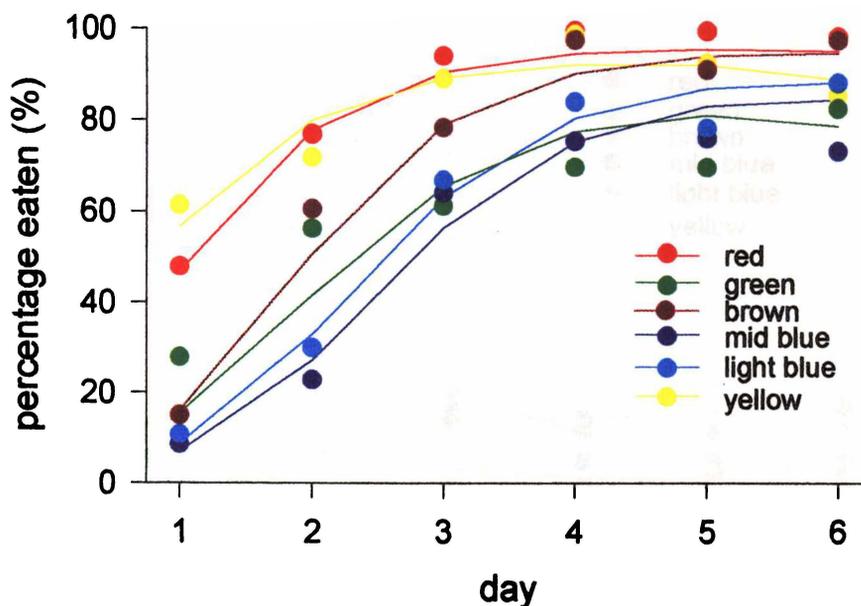


Figure 3: *Daily consumption of coloured pellets by weka. The symbols present the back-transformed mean observed values and the lines present the predicted values back-transformed from the original model.*

The mean number of pellets eaten per weka increased steadily from Day 1 to Day 4 after which consumption stabilised at just over 50 pellets per day per weka (out of a possible 60) (Table 2). This resulted in a significant linear time trend for amount eaten (Table 1). Time to first touch and time to start eating also showed the same trend with weka being slower to touch and to start eating on Day 1 and becoming faster in subsequent days (Table 1, Figs 4a & 4b). The mean number of colours eaten per weka each day also increased from Day 1 to Day 4 after which consumption stabilised around 5-6 colours per day (Table 2). By Day 5 (i.e. after 46.9 min of exposure to the test colours in total) all the weka had tried all the colours, with the exception of one weka that never ate green. Half of the weka were eating 90% or more of every colour.

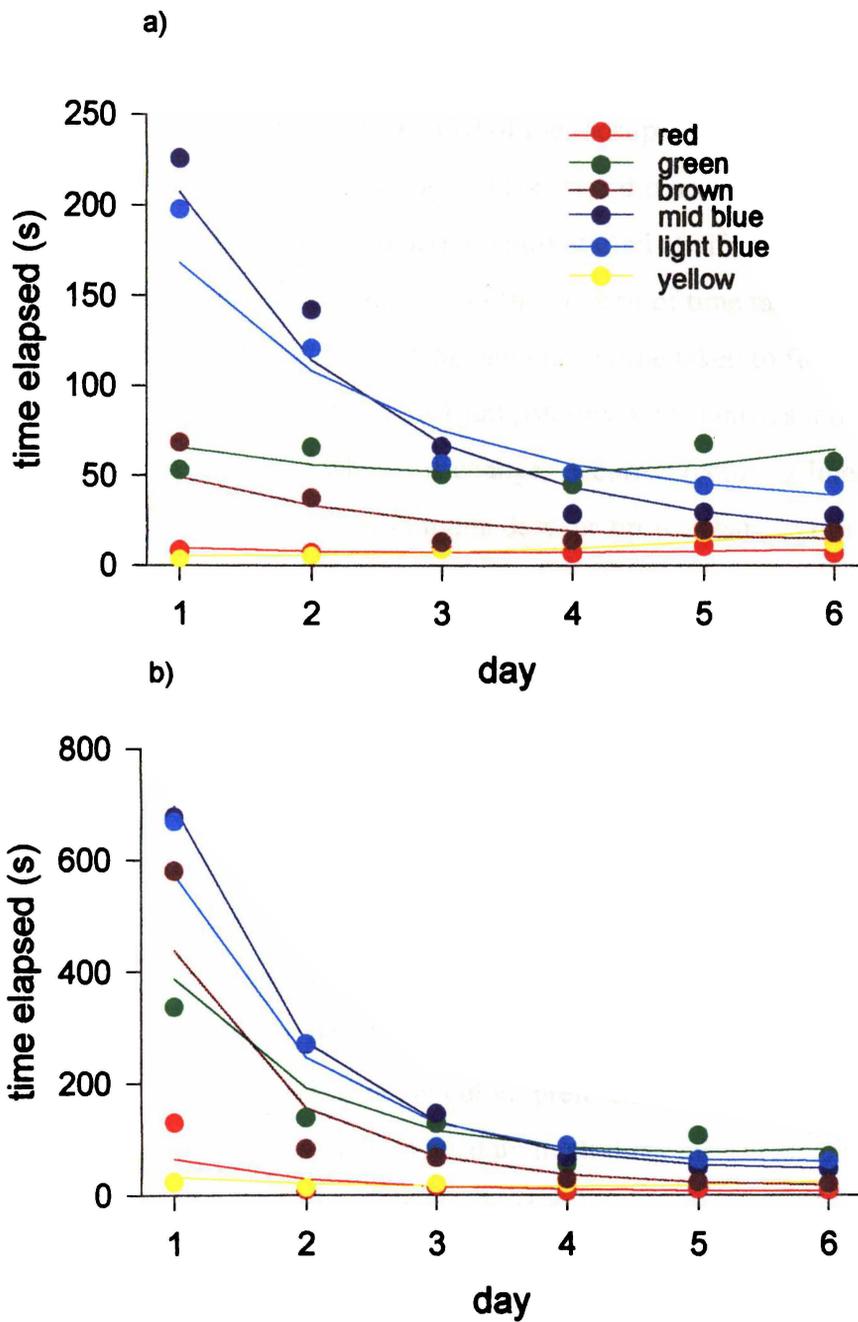


Figure 4: Time elapsed daily until weka first a) touched coloured pellets with their beaks and b) ate coloured pellets. The symbols present the back-transformed mean observed values and the lines the predicted means back-transformed from the original model.

While the amount consumed increased over time for all colours the rate at which consumption increased was found to differ significantly between colours (colour x linear timetrend, Table 1). Brown, for example, was eaten little on Day 1 but by Day 4 consumption of brown matched that of the most preferred colours. Consumption of green and the two blues, in contrast, did not increase as rapidly (Fig. 3). In other words, consumption of colours started at different levels and increased at different rates. Our analysis of the amount of time taken until a colour was first touched (Fig. 4a) and the amount of time taken to first start eating a colour (Fig. 4b) showed similar significant patterns with significant differences between colours in the rate at which the changes occurred (colour x linear timetrend, Table 1). As in the consumption data the brown changes quickly from being a colour that was approached and eaten slowly to one that was approached and eaten quickly. The green in contrast shows little change in the rate it is approached and eaten at throughout the trial (i.e. the green and brown lines diverge in Figs. 4a and 4b).

Individual weka differed in the amount they consumed and in the rate at which their consumption increased (bird effect, bird x linear timetrend effect, Table 1) but no differences between birds were detected in the measures of time at which they approached and time at which they first started eating pellets. Individual weka were also found to have different colour preferences on Day 1 (bird x colour effect, Table 1) and this difference showed up in all three measures: amount consumed, time to first touch, and time to start eating a colour. One weka out of the 14, for example, showed a preference for mid blue and light blue (but not green) in amount consumed, time to first touch and time to start eating on Day 1. This individual's preference persisted throughout the six day trial with mid blue or light blue consistently chosen first and second (in 90% of tests). Another weka ate more green than any other colour throughout the trial (mean number of pellets eaten per day: green 9.7, brown, 9.5, red 8.2, yellow 8.0, light blue 4.9, mid blue 3.0) and a third weka consumed green second only to yellow. No colour was ranked consistently lower than all others by all birds.

The rate at which consumption increased per colour was not found to differ significantly from bird to bird (bird x colour x linear timetrend, Table 1) in the measures of consumption and time to eat. No effect was found for position of a colour in the layout (i.e. weka did not show left, right or centre biases).

As weka were tested in 3 groups, it was possible to differentiate between the effects associated with the day of test (ie 1-6) and the day of year. Once test day effects had been removed, a significant day of year effect remained in amount consumed and time to first start eating (Table 1). Some days all the weka on average ate more or less than other days.

## Discussion

The colour of a novel food influences the likelihood of a weka eating it. Weka showed significant colour preferences on the first day of exposure dividing the food into two clusters: a more preferred cluster of red and yellow pellets and a less preferred cluster of the two blues, green and brown (Fig. 3). Previous studies have found similar colour preferences. Domestic fowl offered yellow and green grain, preferred yellow to green (Caithness and Williams, 1971); a range of ground feeding Australian birds offered red, yellow, green, blue, and black grain ranked blue and green as least preferred (Brunner and Coman, 1983); cedar waxwings *Bombycilla cedrorum* offered red, blue, green, and yellow banana mash showed a strong and significant preference for red and yellow over blue and green (McPherson, 1988); and Steller's jays *Cyanocitta stelleri* preferred red peanuts over yellow over blue over green ones (Slaby and Slaby, 1977). In all the above studies, the birds were pre-fed which suggests, when compared to our results, that not only do a variety of bird species show similar colour preferences but that the preferences are similar irrespective of whether or not birds have been pre-fed.

Wild weka on Kapiti Island, the source of the birds in this study, were tested for colour preferences in 1956 (unpublished data reported in Udy and Pracy 1981). The weka showed a similar preference to those in this study (yellow over red over

green) although comparison is difficult as the colours and the actual methodology used in the early study were not presented in detail. In summary, the most promising colours for dyeing poison baits to deter weka would appear to be blue or green and, based on the consumption on the first day of exposure alone, this study would suggest the use of blue.

It is surprising that weka showed such strong preferences. They are opportunistic omnivores with considerable dietary plasticity (Bramley, 1994) and are able to use mechanisms other than vision to evaluate novel foods. Weka, for example, were frequently observed to vigorously investigate the pellets with their beaks before picking them up and investigating them with their tongues. Pellets could be rejected after either of these steps (pers. obs.). Puckey et al. (1996) also found silvereyes *Zosterops lateralis*, which are generalist frugivores feeding on fruits of many colours in the wild, to show a strong preference for red over white over yellow when offered artificial fruit. Puckey et al's (1996) silvereye study is, to our knowledge, the only other published study that has determined colour preferences in individual fledged birds using coloured food without pre-feeding.

Unfortunately the direct comparison of preferences with those found in the current study is precluded by the use of different colours.

There was some variability between birds in terms of preference. On the first day, out of the 14 birds, four ate mid blue, and three ate light blue, the two least preferred colours that day. Puckey et al. (1996) also found significant variability in colour preferences between individual birds. For example, eight silvereyes preferred red, three preferred white and none preferred yellow artificial fruit. Given the variability evident between individual birds it seems unrealistic to expect a colour to deter 100% of birds. Research, rather, should concentrate on determining which colours will deter as large a proportion of birds as possible from a novel food.

The 1080 concentration in baits is usually 0.08 or 0.15% by weight (Spurr and Powlesland, 1997). The average weight of a male weka is 1000 g and of a female weka is 700 g (Heather and Roberston, 1996). If we assume the LD<sub>50</sub> for weka is 5.5 mg/kg (the average for three similar sized birds for which the LD<sub>50</sub> is known; ring-necked pheasants, *Phasianus colchicus*, mallards, *Anus platyrhynchos*, and sulphur crested cockatoo, *Cacatua galerita*, McIlroy, 1993) and the 1080 concentration is 0.15% then an average female in this trial would have received a lethal dose if it ate between six and seven pellets and the average male if it ate between nine and ten pellets. On Day 1 therefore 57% of males consumed a lethal dose in yellow pellets and 64% of females, 14% and 29% respectively consumed a lethal dose of green pellets, 7% of both males and females consumed a lethal dose of light blue pellets and 0% and 7% respectively consumed a lethal dose of mid blue pellets. While mid blue appears the most promising colour to dye poisonous baits judging on consumption on Day 1 pellets this colour would still have killed up to 7% of weka on the first day of this trial.

Unfortunately, while birds showed significant colour preferences on the first day of exposure the effect did not last for the full 6 days of the trial. Eventually 13 out of the 14 birds sampled all of the colours. The implications of this result for birds in the field are unclear. It is possible that the test weka, on a novel diet, were more inclined to try the pellets than wild birds, surrounded by their natural food sources, would be. Additionally, as all colours were presented together weka may have been more inclined to try less preferred colours once they found the more preferred colours were edible and safe. Alternatively, wild weka may be even more inclined to try novel foods than captive birds if they live under a higher level of hunger. Further trials in a wild situation are needed to clarify this.

In the current study, consumption increased rapidly from the first day until weka were eating over 70% of the pellets of every colour (Fig. 3). Our earlier colour study with adult chickens (Chapter 2) showed a similar pattern of consumption. Bryant et al. (1984) in a large study with flocks of wild birds also observed a

tendency for the intakes of birds visiting feed trays to increase from Day 3 onward suggesting this process of progressively adopting novel coloured food is not unique to weka. Bryant et al. (1984) suggested the birds were initially wary of coloured grain but on finding there was no adverse physiological reaction the avoidance waned. This process has been observed in many species (Provenza, 1997).

The rate at which birds increased their consumption over the 6 days of the test differed significantly from colour to colour (Fig. 3). As poisonous baits are expected to have a finite lifespan in the field, the rate at which individuals take to baits of a particular colour over time is an important and, until now, unexplored factor in designing appropriate baits. Weka, for example, showed a moderate interest in green on the first day as measured by the amount of time they took to touch green pellets and the number of green pellets eaten. Green, however, had the lowest rate of increase in consumption over the duration of the trial. This resulted in fewer green pellets being eaten than any other colour. A potential dilemma exists then in choosing between a colour for which weka show little interest on the first day but will quickly adopt over time (eg brown or blue), and a colour in which they show more interest on the first day (eg green) but are less inclined to eat more of over time. In field trials with promising colours, it will be important to consider not only the immediate response of birds but also the response to coloured baits left in the field for the expected lifespan of the poison.

The apparent neophobia to coloured food observed in our trial and by Bryant et al (1984) could protect birds from toxic foods such as poisonous berries and potentially even from quick acting poisons such as 1080 but slow acting poisons such as brodifacoum are specifically designed to circumvent neophobia in the target species and, as such, present a hazard even to cautious birds.

Green was identified as a promising deterrent in this study yet the green baits currently used in New Zealand do not deter wild weka from eating them. Deaths

of 80-100% of weka have been recorded (Eason and Spurr, 1995), although some of these deaths may be due to secondary poisoning. Weka, with their considerable dietary plasticity, may be poor candidates for deterring solely with colour under field conditions.

Comparison between colour preference studies is hampered by difficulties in determining the exact colours offered as written descriptions are not sufficiently specific. Presenting spectral reflectance curves of colours used is a solution not only to the problem of specifying a colour but also allows the reader an unbiased indication of the nature of the light reflected off a coloured surface allowing comparison between the colours used in this and other studies.

While this study attempted to manipulate the colour of pellets alone we cannot be sure that weka, or other birds, see objects the same way humans do (Endler, 1990). We do not know what aspects of reflected light are important in bird vision, or bird decision processes. For example, wavelength, polarisation properties (Cameron and Pugh Jr, 1991), intensity and brightness (Billmeyer and Saltzman, 1981; Endler, 1990; Wandell, 1995) and contrast to background (Pank, 1976; Willson et al., 1990) are all potentially important factors in bird vision. The possibility that the weka's choices in the current study are based on these or other attributes cannot be ruled out. Nevertheless, the consistent preferences found by a variety of researchers suggests that colour may play an important role in birds' foraging choices. It is important to remember that changing the surface of a bait, and hence its reflectance properties may dramatically alter the appearance of that bait to the bird, even though the colour remains constant to us. It is also unclear how far colour preferences can be generalised. In this study, the two blues were found to be the least preferred colours on the first day but can this conclusion be extended to all the colours we call blue?

In summary, weka did show colour preferences with light blue, mid blue and green being the most promising colours for deterring weka from eating poisonous

baits. There was some variability in colour preferences between birds suggesting no colour will deter all birds. A more realistic aim, then, is to identify a colour that will deter as large a proportion of birds as possible. Given the weka's opportunistic and adaptable feeding style, it appears that a more active deterrent than colour alone will be needed for this species. The consistency of colour preferences found in this study in comparison to previous studies by a variety of researchers with a variety of bird species offers hope that other native bird species will show similar colour preferences. Species with less adaptable dispositions, may show a more lasting avoidance of the less preferred colours.

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## CHAPTER 4

### **Colour preferences in North Island robins (*Petroica australis*): Implications for deterring birds from poisonous baits**

Lynette Hartley, Cheryl O'Connor, Joseph Waas, and Lindsay Matthews

**Summary:** There is growing awareness and concern in New Zealand about native birds eating poisonous baits intended for pest species such as brushtail possums (*Trichosurus vulpecula*) and rats (*Rattus rattus*, *R. norvegicus*, *R. exulans*). We investigated the colour preferences of North Island robins (*Petroica australis*), a species known to be vulnerable to poisoning. The main aims were to determine if: 1) robins had colour preferences, 2) the preferences were consistent between two separate populations and 3) the preferences were similar to those found previously in weka (*Gallirallus australis*), another native species.

Robins in Pureora Forest Park and Te Urewera National Park were individually offered a choice between differently coloured versions of a novel food (red, yellow, brown, green, light blue and mid blue) daily, for six consecutive days. Robins showed food colour preferences pecking more at the red, yellow and green cake than the mid blue, light blue or brown cake. No difference was evident in the colour preferences of the two populations. Dyeing poisonous baits may be sufficient to stop a proportion of robins from eating them. Further work is needed to determine how colour preferences vary across seasons, populations and species.

**Keywords:** Bird repellents; robin; non-target species.

**Status:** Published in New Zealand Journal of Ecology (1999) 23(2): 255-259

## Introduction

Poisons are used with increasing frequency in New Zealand in operations to kill introduced mammalian pests. The conservation benefits of these control operations have been documented. For example populations of North Island robins (*Petroica australis longipes*; Powlesland, Knegtmans and Marshall, 1998), and North Island kokako (*Callaeas cinerea wilsoni*; Innes *et al.*, 1998) recover following the poisoning of pest species. However a wide range of native birds are known to die during poisoning operations (Harrison, 1978; Powlesland *et al.*, 1998; Spurr, 1993; Empson, 1999) and there is considerable concern and pressure from the public to make poisonous baits safer for non-target species (Wright, 1995).

Overseas research on bird repellents has often focussed on repelling birds from crops and storage areas. The challenge in New Zealand is to produce a bait that will repel birds while remaining attractive to target species, generally brushtail possums (*Trichosurus vulpecula*) and rats (*Rattus rattus*, *R. norvegicus*, *R. exulans*). Two possible approaches for accomplishing this goal are 1) incorporation of a bird-specific chemical repellent into existing bait formulations and 2) identification of bait characteristics, such as colour, that may make baits less attractive to birds. Suitable colours, for example, could discourage birds from eating baits because they make the baits inconspicuous or because the baits no longer look like food. Unfortunately all repellents that have been effective against birds in New Zealand have also been found to adversely affect consumption by target species, notably rats (e.g. Spurr and Porter, 1998). Altering the colour of poisonous baits, however, had no adverse effect on bait consumption by possums (Day and Matthews, 1999).

Birds are currently discouraged from taking poisonous baits by size and colour (Pesticides Act, 1979): all baits are required to be larger than 16 mm and dyed green. Work by Kalmbach and Welch (1946) showed that some North American

birds avoided dyed food, particularly if it was green or yellow. In New Zealand in the 1950s Caithness and Williams (1971) conducted trials leading on from Kalmbach and Welch's work. These trials resulted in the recommendation that poisonous baits be dyed green. Caithness and Williams tested only introduced bird species. Once poisoning of possums started in forests and forest margins, many more native bird species were exposed to poisonous baits. In addition operations targeting rats on conservation land expose a wide range of native birds to baits, but the colour preferences of these native New Zealand bird species have not been established.

We tested the colour preferences of the North Island robin. This species was chosen as robins are particularly vulnerable to poisoning (Brown, 1997; Powlesland *et al.*, 1998; Empson, 1999). Two geographically isolated groups of robins were tested at the same time to see if different populations showed the same colour preferences. The methods used and colours offered followed those in a previous trial with weka (*Gallirallus australis*, Chapter 3), an omnivorous, ground feeding, New Zealand rail (Heather and Robertson, 1996), allowing comparisons of colour preference and behaviour between these two species of native birds.

## Methods

The North Island robin is a small, predominantly insectivorous passerine that generally feeds on or near the ground (Heather and Robertson 1996). Two geographically isolated populations of robins were tested for colour preferences, one at Tahae, Pureora Forest Park, 38° 25' S, 35° 10' E, 600 m a.s.l., and the other at Otamatuna, Te Urewera National Park, 38° 20'S, 177° 10' E, 500-700 m a.s.l. Both study sites were in mixed forest with emergent podocarps over a mainly tawa (*Beilschmiedia tawa*) canopy. Seasons appeared synchronous between sites judging by general observation of fruiting and nesting (*pers. obs.*).

Robins were presented individually with a choice of six colours offered simultaneously, for six consecutive days (Fig. 1). The colours were red, yellow, green, mid blue, light blue, and brown in the form of dyed, non-toxic cake (Edmonds pre-mixed Madeira cake) soaked in lard. Non-toxic powdered dyes (Bush Boake Allen, New Zealand Ltd) were used to colour the cake and care was taken to ensure that the colours offered were similar to those in the previous trial with weka. The spectral reflectance curves of the colours (measured by Justin Marshall, University of Queensland, Australia) are shown in Fig. 2. Cake was a completely novel food for the robins which were wild birds.

Test areas, consisting of an area 1.0 m x 0.5 m scraped clear of leaf litter, were set up in the central areas of 18 robin territories at both Pureora and Te Urewera. Most territories were occupied by a pair of robins, although a few were occupied by lone males. The robins were offered a teaspoon of finely chopped cake of each colour laid out in a line of piles each 10 cm apart. The position of the colours in the line was changed between birds and between days using a Latin square design to ensure that each colour appeared next to each of the other colours twice, and only twice, in each area. All test areas were positioned in shade.

The robins had been trained to approach researchers for mealworms (pale brown in colour, *Tenebrio* sp. Powlesland *et al.*, 1998). Once present, robins showed considerable interest in the test areas as clearing the leaf litter exposed insects that the birds foraged for. The tests lasted 30 min each day and a video camera was used to record the behaviour of each bird. Tests were completed in blocks of six territories alternating between the two study sites so both populations were tested during the same time period (7 July - 18 November 1997, austral winter - spring).



Figure 1: A robin eating the coloured cake offered during the colour preference test

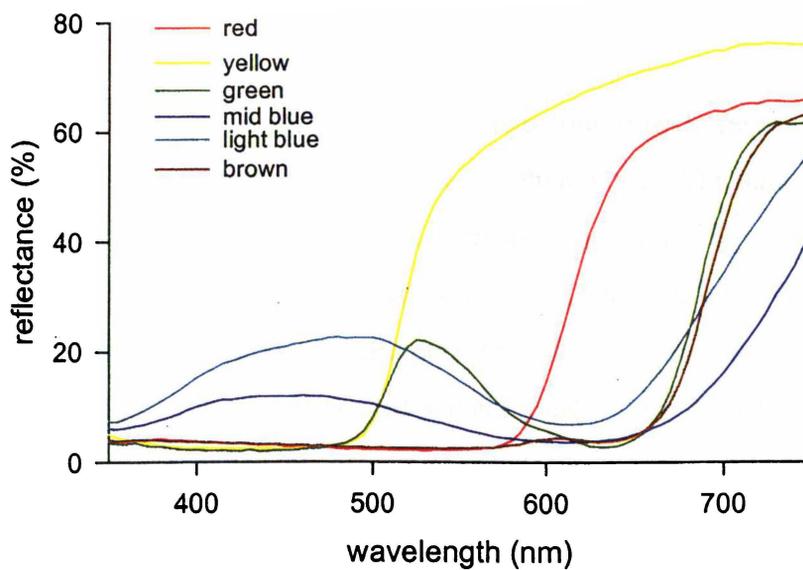


Figure 2: Spectral reflectance curves of the six colours of the cake offered to robins. Spectral reflectance curves show the relative proportion of light reflected off a coloured surface at each wavelength.

Robin interest in the colours was measured by the number of pecks directed at cake of each colour. The resulting numbers were analysed as a Poisson variate using the Generalised Linear Mixed Model (GLMM) in the Genstat 5.4 statistical package (Breslow and Clayton, 1993). GLMM combines residual maximum likelihood analysis (Patterson and Thompson, 1974) for multiple area strata with log-linear modelling for response variates which are not normally distributed. Thus underlying effects are modelled as additive on the log scale and, consequently, are multiplicative as observed counts. Fixed effects comprised colour, location, run number (i.e. 1st, 2nd, etc. occasion on which a bird responded), plus their interactions. Terms were retained when the related Wald statistic in GLMM gave a Chi-square value corresponding to  $P < 0.05$ . Sources of variation considered for the random model comprised test-day, test-area and bird (within area), their interactions with colour, and the interaction of area and bird with test-day.

Means resulting from the GLMM analysis were compared using the Least Significant Ratio (LSR). If the ratio of the larger to the smaller mean is greater than the LSR then the means are different at the 5% level using Fisher's LSD test.

## Results

Six of the 71 robins that visited the test areas (one from Pureora and five from Te Urewera) were present on the sites for less than 5 min. The data for these individuals were excluded from the analysis. The remaining robins foraged for mealworms and natural food on and around the test areas. Of the foragers, 24 did not peck at any coloured cake (Pureora 35%, Te Urewera 39%, Table 1). Only data from those robins that pecked at cake (Pureora  $n = 22$ , Te Urewera  $n = 19$ ) were used in the statistical analysis of colour preference. Over 40% of these robins pecked at yellow and green cake and a smaller percentage pecked at either of the blue or the brown cakes (Table 1). An average of only 2-3 colours were sampled by each robin and only two individuals pecked at all the colours.

Table 1: Percentage of robin eating novel cake of each of six colours including those that ate natural food off the test area but did not eat the cake ( Pureora n=34, Te Urewera n=31).

	None	Any colour	Yellow	Red	Green	Mid blue	Light blue	Brown
Pureora	35	65	47	29	29	6	9	21
Te Urewera	39	61	45	29	42	39	23	19

There was variation in response to colour evident between birds and, to a lesser extent, between test-days. Despite this variation, significant overall food colour preferences were found. Robins pecked at yellow and green cake more than at mid blue, light blue or brown cake. Red cake was pecked at less than yellow cake but more frequently than light blue or brown cake ( $\chi^2 = 29.4$ , d.f. = 5,  $P < 0.001$ ; Table 2; Fig. 3). There was no evidence that the amount of pecking or colour preferences differed between populations ( $\chi^2 = 2.9$ , d.f. = 1,  $P = 0.09$ , and  $\chi^2 = 8.9$ , d.f. = 5,  $P = 0.11$  respectively).

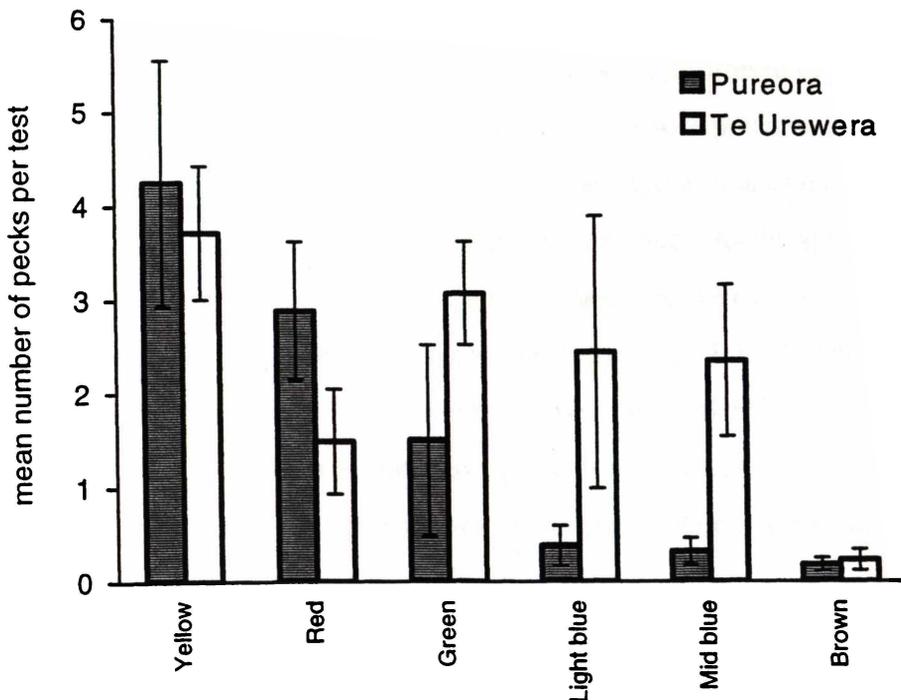


Figure 3: Mean number of pecks ( $\pm$  s.e.) directed at coloured cake per test by two separate populations of robins, Pureora (n=22) and Te Urewera (n=19).

Table 2: *Scaled means ( $\pm$  s.e.) resulting from the GLMM analysis for number of pecks directed at coloured novel food per test for each of the six colours offered to robins. The Least Significant Ratio is 3.5. Means not sharing any letter are significantly different at  $P < 0.05$ .*

	Scaled mean	s.e.
Yellow	5.76 <sup>a</sup>	2.7
Green	2.58 <sup>ab</sup>	1.1
Red	1.57 <sup>bc</sup>	0.8
Mid blue	0.64 <sup>cd</sup>	0.4
Light blue	0.50 <sup>cd</sup>	0.3
Brown	0.38 <sup>d</sup>	0.2

There was no evidence to suggest that individuals within a single territory influenced one another's colour preferences although only a pronounced effect could have been detected with the number of pairs tested. The number of pecks directed at cake during each 30 min test did not change systematically from test to test ( $\chi^2 = 2.9$ , d.f. = 5,  $P < 0.07$ ).

## Discussion

Robins showed differing preferences for the novel food offered depending on the colour of the food. There was some variability in colour preference between individual robins. We did not use the same green dye in this study as that currently specified for poisonous baits in New Zealand. As the spectral reflectance of poisonous baits have not been measured we can only say that the baits appeared similar in colour to those currently used. It is of concern that such a high proportion of robins (38%) pecked at the green cake (Pureora 29%, Te Urewera 42%). Judging from our tests, green is not the optimum colour for preventing bird deaths. Further studies are needed to determine whether the shade of a colour affects robin preference for that colour.

Robins showed the least interest in pecking the two blue and brown cakes (Table 1), suggesting that these colours may be better deterrents for birds. Not only did robins, overall, direct fewer pecks at blue and brown cake, but the cakes

were pecked at by fewer individual robins (Table 1). Preference trials, such as this study however, do not tell us what the robins would have done if confronted by a single colour rather than a choice of colours. Brown was the colour that was closest to the colour of the background soil, at least for human viewers, suggesting contrast may be a factor worth investigating in food colour studies with birds.

No significant differences in colour preferences were detected between the two populations studied. This raises the possibility that the colour preferences recorded may be general among robins. Care was taken in this study to test robins in very similar habitats, over the same time period. Further studies are needed to determine whether colour preference for a novel food varies with season or site.

Our previous study tested preferences in a group of 14 wild-caught weka for the same colours offered in this study (Chapter 3). The robin preferences differed from those found on Day 1 only in that the robins ranked green among their most preferred colours while weka ranked it among their least preferred.

Robins tried an average of only 2 - 3 colours with very few robins trying all six colours. This behaviour contrasted to that of weka where all but one tried every colour ( $n = 14$ ). Robins also showed no tendency to increase the number of pecks directed at coloured cake over time whereas weka consumption built up rapidly. By the end of the trial weka were eating over 70% of the food of every colour. These differences in behaviour toward coloured novel food suggest that robins, unlike weka, may avoid eating baits dyed some colours for the duration of a poisoning operation.

Robins became familiar with the test arrangements very quickly and spent long periods of time foraging on the test areas. Despite this thirty-seven percent of the robins did not try the coloured novel food, eating only natural foods within the test area. This pattern of behaviour differs markedly from that observed in weka (Chapter 3) where 100% of the birds that explored the test area ate the coloured

food. A parallel exists between the extent to which each species consumed the novel foods in the trials and the situation observed in the field during poisoning operations. That is, a much lower proportion of robins die in such operations than weka. For example robin mortalities of between 43 and 55% have been recorded following poisoning operations with carrot 1080 baits (Powlesland et al, 1998) and 30 to 64% with brodifacoum baits (Empson, 1999; Brown, 1997). This compares to weka mortalities of between 80 and 100% with Talon baits (Eason and Spurr, 1995).

In conclusion robins were found to have colour preferences and these preferences did last for the duration of the trial suggesting colour may work as a deterrent for this species. Preference was found to differ between individual robins making it unlikely any colour will deter all individuals from baits. The similarities in colour preferences between robins and weka, two very different species with different food sources, raises the possibility that preferences may be similar in other native bird species as well.

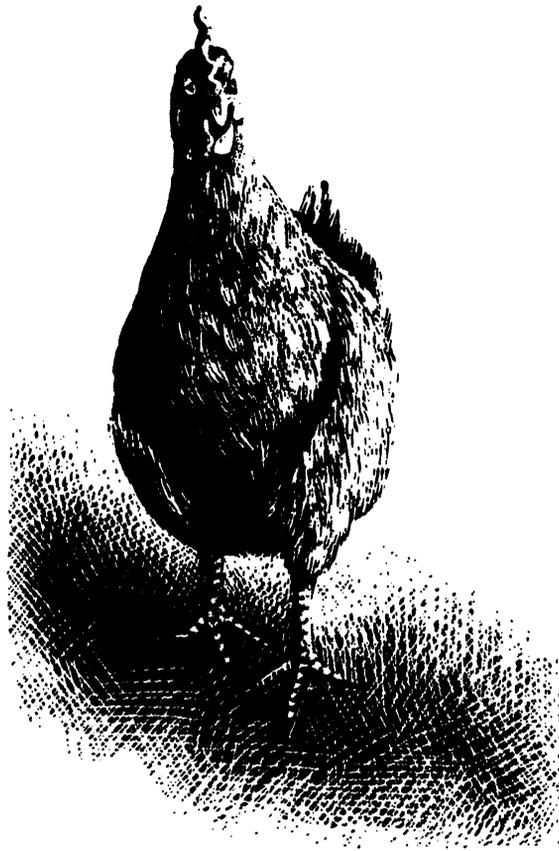
## **Acknowledgements**

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## CHAPTER 5

### **The effect of an insect defensive odour on the consumption of a familiar food by New Zealand robins (*Petroica australis*) and novel food by domestic chickens (*Gallus domesticus*)**

Lynette Hartley, Joseph Waas, Cheryl O'Connor

#### **Abstract**

There has been considerable research in New Zealand seeking to identify a non-lethal repellent that will deter birds from eating poisonous baits intended for mammalian pests. We investigated whether methyl pyrazine, an insect defensive odour, would be useful in deterring birds from baits. The odour is believed to deter birds when combined with novel, but not familiar, food and our first experiment supported this conclusion. Wild New Zealand robins (*Petroica australis*) were not deterred from mealworms, a familiar food, by the addition of methyl pyrazine. Previous researchers have suggested that methyl pyrazine may be more effective as a deterrent in combination with some colours (such as red and yellow) than other colours. In a second experiment we found that the addition of methyl pyrazine to coloured novel food did not alter the colour preferences of free-range chickens. Methyl pyrazine did not deter chickens from the coloured novel food and we speculate that the distinctiveness or conspicuousness of a novel food, rather than novelty per se, may play a role in whether pyrazine is effective as a deterrent. More work is needed to see whether methyl pyrazine could be useful in deterring native birds from poisonous baits. We also found that a chicken's previous experience of another coloured food affected its colour preferences in subsequent tests. The implications of these results are discussed in terms of poisonous bait design.

**Keywords:** aposematic, bird foraging behaviour, colour, methyl pyrazine, odour, poisonous baits, preferences, previous experience, warning signals.

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## Introduction

There has been considerable research in New Zealand seeking to identify a non-lethal bird repellent that will deter native birds from poisonous baits (Chapter 1). Avery (1997) suggested that repellent researchers should investigate repellents based on the signals used in animal communication, such as predator-prey signals. Many toxic insects have conspicuous colours, patterns, smells and other characteristics that may communicate the toxicity of the insects to predators (Schuler and Roper, 1992). Seven spot ladybirds (*Coccinella septempunctata*), for example, are toxic to blue tit nestlings (*Parus caeruleus*; Marples, Brakefield and Cowie, 1989) and have: 1) a distinctive red and black colour pattern, 2) a distinctive odour and 3) a bitter taste (Marples, Van Veelen and Brakefield, 1994). Because the displays used by toxic insects often confront several of a predator's senses at the same time Avery (1997) suggested that multiple cues presented together may be more effective than repellents presented on their own. In support of this proposal methyl anthranilate combined with a visual stimuli (calcium carbonate) was found to enhance the effectiveness of the repellent methoicarb in promoting food avoidance by red winged blackbirds (*Agelaius phoeniceus*; Mason, 1989).

When a bird encounters a poisonous bait in the wild, the situation is analogous to a bird encountering a novel prey. Some prey will be toxic, or otherwise unpleasant for birds, while other prey will be edible. A bird must evaluate a possible food item from the cues available. We have already investigated the influence of one possible cue that birds may use to evaluate novel food, colour. We offered three species of birds; chickens (*Gallus domesticus*, Chapter 2), weka (*Gallirallus australis*, Chapter 3) and New Zealand robins (*Petroica australis*, Chapter 4), novel food in six different colours. All three species showed colour preferences, preferring red and yellow novel food to either of the blues offered. In the present study we investigated whether the inclusion of additional cues would be more effective in deterring birds from novel food than colour alone.

A large number of unrelated, toxic and unpalatable insect species have independently adopted the same characteristic odours, methyl pyrazines, as defensive signals (Rothschild, 1961; Rothschild and Moore, 1987). We investigated whether the addition of one of the methyl pyrazines to food would reduce the amount of food eaten. The pyrazine we tested is an approved additive to foods in the USA (Flavour and Extract Manufacturers Association No. 3433) which suggested it may not be deterrent to the target mammals. In addition, it is a very powerful and stable odour (Guilford *et al.*, 1987; Rothschild and Moore, 1987) and therefore may be effective at low concentration and long lasting when incorporated into baits.

This is the first time that the use of methyl pyrazine had been investigated for protecting birds from poisons. Methyl pyrazine has two unusual features that necessitated preliminary trials to determine what combination of cues were likely to produce the most deterrent effects. The first characteristic was that methyl pyrazine was not believed to be aversive to birds on its own (Rowe and Guilford, 1996) but appeared to interact with other aspects of novelty to enhance any deterrent effect associated with a novel food (Guilford *et al.*, 1987; Marples and Roper, 1996; Rowe and Guilford, 1996). Methyl pyrazine odour, for example, had been shown to deter chicks from food but only when the food was dyed to make it novel (Marples and Roper, 1996). In order to test whether methyl pyrazine was aversive on its own in a New Zealand context, we offered robins a familiar food accompanied by methyl pyrazine odour (Experiment 1).

The second unusual characteristic ascribed to methyl pyrazine by some researchers was that it may interact with some colours, such as red and yellow, to deter birds but not with other colours, such as green (Rowe and Guilford, 1996). Rowe and Guilford (1996) suggested that red and yellow are common among toxic insects because they are 'warning' colours for birds and that green is not a 'warning' colour. The status of other colours, such as blue, was not clear and the toxicity of insects to birds has not been systematically surveyed and examined for colour

associations. We wanted to know which colours reduced consumption by birds the most in combination with methyl pyrazine, so we offered free-range domestic chickens novel food in six colours with methyl pyrazine (Experiment 2).

We used free range chickens as a model species in the second experiment because we have already shown that they have detectable colour preferences for novel food and that these colour preferences were similar to those of, at least some, native New Zealand birds. We investigated whether the colour preferences observed differed between birds receiving the novel coloured food with pyrazine odour and those receiving it without odour. We also examined the colour preferences of chickens that received the same coloured food and pyrazine odour in combination with a bitter taste to see whether the addition of a third cue affected colour preferences or food consumption. From our previous studies we predicted that chickens offered plain novel food would prefer red and yellow food to the other colours. If pyrazine odour interacts with red and yellow to facilitate a strong avoidance of these colours, then birds receiving odour with the food should rank red and yellow among their least preferred colours rather than their most preferred. Similarly, the birds receiving a bitter taste in addition to the other two components would also avoid the red and yellow but the avoidance may be even stronger because the birds were receiving an additional deterrent cue, bitterness.

This study had two main objectives. The first was to investigate the effect on consumption by robins of adding pyrazine odour to a familiar food to see whether pyrazine itself was aversive to a native New Zealand bird. Because pyrazine was believed to interact with novelty our second objective was to investigate whether the addition of pyrazine, or pyrazine and bitterness, to novel food altered the colour preferences of free-range chickens or deterred them from eating.

## **EXPERIMENT 1: IS PYRAZINE ODOUR AVERSIVE TO WILD ROBINS IN ASSOCIATION WITH A FAMILIAR FOOD?**

### **Methods**

#### *Test birds*

Robins are small, predominantly insectivorous, passerines that generally feed on or near the ground. They are territorial year round (Heather and Robertson, 1996). The robins we tested were wild birds at Tahae, Pureora Forest Park, Central North Island, New Zealand (38°25'S, 35°10'E). These robins were chosen as they present a unique opportunity to test wild birds in a natural environment. They had been trained to come to tapping noises made by humans and were regularly fed up to 12 mealworm larvae (*Tenebrio* sp) once or twice a week as part of an ongoing unrelated study (Powlesland, Knegtman and Marshall, 1998). The individuals in the experiment were thus very familiar with mealworms but otherwise foraged normally in a natural forest environment.

#### *Test procedure*

The experiment was conducted in June 1998 (austral winter) between 0800 and 1330 h. Seventeen robins received two training sessions in the two days prior to testing. We located individual robins by standing in the middle of each territory and tapping the lid of a jar. When a robin appeared, a test area (1m<sup>2</sup>) was selected that was flat and clear of vegetation. The same test area within each territory was used for the entire experiment. During training robins were offered six live mealworms. Each mealworm was presented on a square of grey towelling measuring 5 x 5 cm. The squares of towelling were arranged an equal distance from one another, in a circle with a radius of 40 cm. Initially robins were cautious about approaching the towelling squares but, after two training sessions, all robins readily ate mealworms off the squares.

On the evening prior to the test day either 0.2 mL of pyrazine solution or 0.2 mL of water was applied to fresh grey towelling squares with a pipette. The towelling

squares were then sealed in plastic bags overnight. Pyrazine was obtained in a purified form from a commercial supplier (Pyrazine Specialties Inc., Atlanta, USA) and was diluted in water to form a 0.0003% solution. This was the same concentration which was found to increase the latency with which chicks ate and drank in a study by Marples and Roper (1996). Bags containing pyrazine and non-pyrazine towelling squares were stored separately throughout the trial to prevent cross contamination. Similarly, when in the field, one researcher always carried and handled pyrazine squares while another always carried and handled the non-pyrazine squares.

All robins were tested on the same day which was clear and calm. One mealworm was presented on each of six squares of grey towelling as in the training sessions. Pyrazine and non-pyrazine towelling squares alternated around the circle with half the robins receiving a pyrazine square in the furthest left position and half the robins receiving a non-pyrazine square in that position, to control for any position biases the robins might have expressed.

Once the squares and mealworms had been set up in a robin's territory on the test day, the resident bird or birds were attracted by tapping. Most robin territories were occupied by a pair of birds so either or both individuals were likely to appear. Robins could be identified by coloured bands on their legs except one unbanded individual who was identified by location and behaviour. The identity of the first robin to arrive at each test area and the number and type of mealworms which it ate were recorded. As the second robin approaching a test area was not offered a balanced presentation of odour and non-odour mealworms, the test was stopped if a second bird arrived and started eating.

### *Analysis*

A Chi-square test was used to compare the total number of mealworms eaten of each type (i.e. odour or non odour). Whether the robins ate mealworms in runs of a particular type, perhaps by eating all the non-odour mealworms first, was

examined for those robins that ate five or more mealworms, with a series of runs tests (Walpole and Myers, 1972).

## Results

There was no evidence that robins discriminated between odour and non-odour mealworms ( $\chi^2 = 2.3$ , d.f. = 16, N.S.). The seventeen robins we tested ate an average of 4-5 mealworms each, from the six offered, and consumed an average of 2 ( $\pm 0.2$  S.E) odour and 2 ( $\pm 0.2$  S.E) non-odour mealworms (Fig. 1). All birds ate both types of mealworm and there was no evidence robins ate in runs of one type of mealworm (runs test mean  $P = 0.71$ , N.S.)

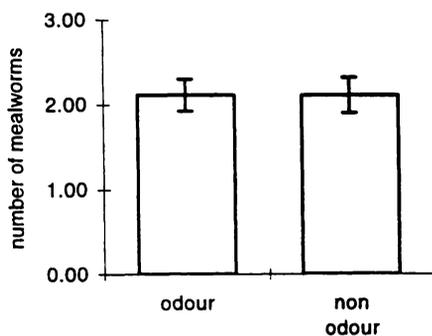


Figure 1: Mean ( $\pm$  S.E.) number of pyrazine odour and non-odour mealworms eaten by robins ( $n = 17$ ).

## Discussion

Robins ate mealworms associated with pyrazine odour just as readily as those that were not associated with the odour. Because mealworms were a familiar food for the robins this finding supports the conclusions of Guilford *et al.* (1987), Marples and Roper (1996) and Rowe and Guilford (1996) who found that pyrazine did not deter domestic chicks from familiar food. These authors found that pyrazine did deter chicks from food that had been dyed to make it novel. We were unable to dye mealworms different colours because they were impermeable to all the food-safe dyes we tried. As a result we were unable to test whether robins were deterred from coloured, and hence novel, mealworms by pyrazine odour.

It is possible that the robins did not respond to the pyrazine odour because they could not detect it. Domestic chicks, however, have been shown to respond to pyrazine at this concentration (0.0003%; Marples and Roper, 1996) and at a lower concentration (0.0000025%; Guilford *et al.*, 1987). Both the above experiments on chicks were conducted in a laboratory and it is possible that the concentration we used was approaching the lower limit of detection in an outdoor situation.

The first experiment showed that pyrazine was not aversive to robins on its own, that is without novelty. The next step was to investigate the effect that the addition of two cues, colour and pyrazine, or three cues, colour, pyrazine and bitterness had on consumption of a novel food.

## **EXPERIMENT 2: DOES THE ADDITION OF PYRAZINE, OR PYRAZINE AND BITTERNESS, TO NOVEL COLOURED FOOD ALTER THE COLOUR PREFERENCES OF CHICKENS?**

### **Methods**

#### *Test birds*

The chickens (Shaver brown strain) we tested were 15 - 18 months old and lived in a group of approximately 100 birds on Benzies Free-Range Egg Farm (Pearce Rd, RD 5, West Melton, Canterbury, New Zealand). They had been raised since hatching on the farm and were fed indoors daily at 0800 h on poultry pellets supplemented with garlic and vitamins. They normally consumed all the feed provided by 1000 h and foraged out of doors throughout the rest of the day for natural foods. In addition to their regular food, the chickens received a wide range of fruit and vegetables fortnightly, outdoors, depending on season. Coloured leg bands allowed us to identify individuals. There was no artificial lighting in the housing area or outdoors.

Of the 36 chickens used in this experiment, most had taken part in an earlier experiment several months previously where they were exposed to flour-based

coloured pellets (Chapter 2). Twenty one of the chickens had experienced pellets of six colours in the previous trial (red, yellow, green, two shades of blue, and brown) while a further 11 had experienced blue pellets in four different shades but no other colours. Four of the chickens had not taken part in any previous trial but had been trained to the test procedure with black and white flour-based pellets (Chapter 2).

### *The test food*

As there is evidence that methyl pyrazine is only deterrent when it is in association with novelty we offered chickens artificial fruit, a food which they had not experienced previously. The artificial fruits were agar-based cubes (3 x 4 x 3 mm) containing fructose and glucose made to a recipe supplied by M. Stanley and A. Lill (Monash University, Victoria, Australia). The artificial fruit differed from the flour pellets used in the previous chicken trial (Chapter 2) in that they were shiny (not matt), were cubes (not cylinders), and had a different composition and taste (at least to humans). The artificial fruit were dyed six colours, red, yellow, green, mid blue, light blue, and brown using food colours supplied by Bush Boake Allen, New Zealand Ltd. The spectral reflectance curves of the colours are shown in Fig. 2 and were measured by Justin Marshall (University of Queensland, Australia).

### *Test procedure*

The chickens were tested between 1400 and 1900 h during December. They were placed, one at a time, in an outdoor test box (0.9 x 0.9 x 0.5 m high) set on bare soil with open mesh walls. The procedure was the same as that established in a previous colour preference experiment with free range chickens (Chapter 2). The artificial fruits were presented in piles of each colour, 10 cm apart, laid out in an arc. Each pile contained 20 artificial fruit. Chickens were placed in the test box facing the arc of colours and were free to move around the coloured food and thus view it as they would when foraging normally. Tests lasted two minutes each day per bird for six days. All chickens received the same six colours each day but the

position of colours in the line was changed daily between birds and days using a Latin square design to ensure that: all colours were offered to each bird each day; all colours appeared in every position for each bird during the course of the trial; and each colour appeared next to each of the other colours twice and only twice for each bird. A movable panel ensured the colours were always offered in shadow. The dampness, and therefore colour, of the ground was kept constant using water applied with a mist sprayer.

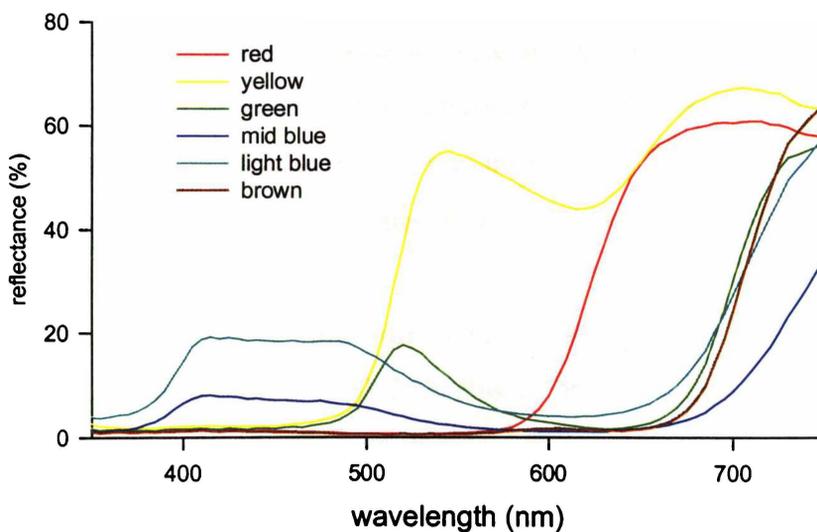


Figure 2: *Spectral reflectance curves of the coloured artificial fruit offered to free-range domestic chickens. Spectral reflectance curves show the relative proportion of light reflected off a coloured surface at each wavelength.*

The chickens were tested in three groups of 12 balanced for previous experience. The first group of chickens was offered coloured artificial fruit without any additives. The second group was offered coloured artificial fruit into which pyrazine had been incorporated and the third group was offered coloured artificial

fruit into which both pyrazine and Bitrex (denatonium benzoate) had been incorporated.

The artificial fruit were made by dissolving the agar and sugars in sufficient boiling water to make up 300 mL at a time. As the agar solution cooled 15 mL of ethanol solution, pyrazine solution, and/or Bitrex solution were added depending on the treatment. Delaying the addition of the pyrazine solution until the agar had partially cooled ensured that the evaporative loss of pyrazine was kept to a minimum. The ethanol solution was composed of 30 mL of ethanol made up to 250 mL with water. The pyrazine solution was composed of 0.5 mL of pyrazine dissolved in 30 mL of ethanol and made up to 250 mL with water. Once the pyrazine solution was incorporated into the agar solution the resulting artificial fruit had a pyrazine concentration of 0.01% which was higher than that used in the previous robin experiment because we were concerned that a 0.0003% solution may not have been sufficiently concentrated to be effective in an outdoor situation. The concentration in this experiment was the same as that used effectively by other researchers with day old domestic chicks (Rowe and Guilford, 1996).

The bitter solution was 50 ppm Bitrex and this concentration resulted in artificial fruit with a concentration of 0.00025% Bitrex. We chose Bitrex because it is exceedingly bitter (Kaukeinen and Buckle, 1992) and has no detectable odour for humans. Domestic chicks cannot identify it by odour at a concentration of 0.005% but find it aversive at this concentration (Marples and Roper, 1997). We assumed, therefore, that the artificial fruit containing bitrix would taste bitter to chickens but smell the same as the fruit offered to the pyrazine group. We wanted the fruit to be noticeably, but not unbearably, bitter for chickens as we wanted the chickens to consume at least some of them. We, therefore, used Bitrex at a concentration of 2.5 ppm.

Care was taken to avoid cross-contamination between non-odour, odour, and odour with Bitrex treatments by moving the test box to a new location and

washing our hands between treatments. All the pellets presented to any one bird were from the same treatment so cross contamination was not a problem within any one test. At the end of each two minute test we counted the number of pellets remaining of each colour and calculated the number of pellets consumed.

### *Analysis*

Nine chickens ate sporadically during the experiment and were omitted from the analysis of pellet consumption. Consumption data were analysed as the percentage of pellets consumed of those available and data from all six test days were used in the analysis. The data were logit transformed (empirical logit transformation; Sokal and Rohlf, 1995) prior to analysis to reduce the built in non-normality and heteroscedasticity. Because the data were of a repeat measures type observations from day to day were expected to be non-independent due to consumption values being strongly correlated with consumption values on previous days. This was examined by testing various models using the residual maximum likelihood procedure (REML) in the Genstat 5.3 statistical package (Patterson and Thompson, 1974).

We took account of the pattern of increasing consumption observed from day to day by fitting a straight line to the logit transformed data. Fitting a linear timetrend to the data allowed us to analyse differences in consumption on Day 1 (i.e. y intercept) and differences in the rate at which consumption increased over the six days of the experiment (linear day). The fixed effects considered in the model were a chicken's previous experience (history), the treatment (i.e. colour, colour with odour, or colour with odour and bitter taste), colour on Day 1, linear timetrend and the position of a colour in the layout. The interactions between previous experience, treatment, colour and linear timetrend were also retained as fixed effects in the model because of their significance ( $P < 0.05$ ).

The non-independent nature of the observations was taken into account by the terms considered in the random effects model. The random effects comprised the

individual chicken, the variability in consumption between chickens each day, between chickens in the amounts they ate of different colours (chicken colour) and between chickens in which positions in the layout they tended to eat from (chicken position). The interactions between chicken colour and linear timetrend, and chicken and linear timetrend were also tested.

A spline curve was also fitted to the logit transformed data and analysed as a random term (Verbyla *et al.*, 1999). The spline term allowed for serial correlation between consecutive observations on the same bird or bird-colour combination. This term allowed us to account for variation such as one bird eating more or less than expected for several days and was consequently another way of removing variability in the data that was not related to the effects we were interested in. The interaction between the spline term and individual chicken was also analysed as a random term.

The REML procedure provided likelihood ratio tests (LRT) and Wald tests for the components of the random and fixed models, respectively, both of which are asymptotically distributed as Chi-square with appropriate degrees of freedom.

## Results

We found no evidence that chickens consumed different amounts of artificial coloured fruit depending on whether it was simply coloured, accompanied by pyrazine odour, or accompanied by pyrazine and a Bitrex (treatment, Table 1). Likewise, there was no evidence that the chickens' colour preferences differed depending on the type of artificial fruit they received (treatment  $\times$  colour, Table 1). This was not because chickens did not show colour preferences, they ate different amounts of the artificial fruit on Day 1 depending on the colour of the fruit (colour, Table 1).

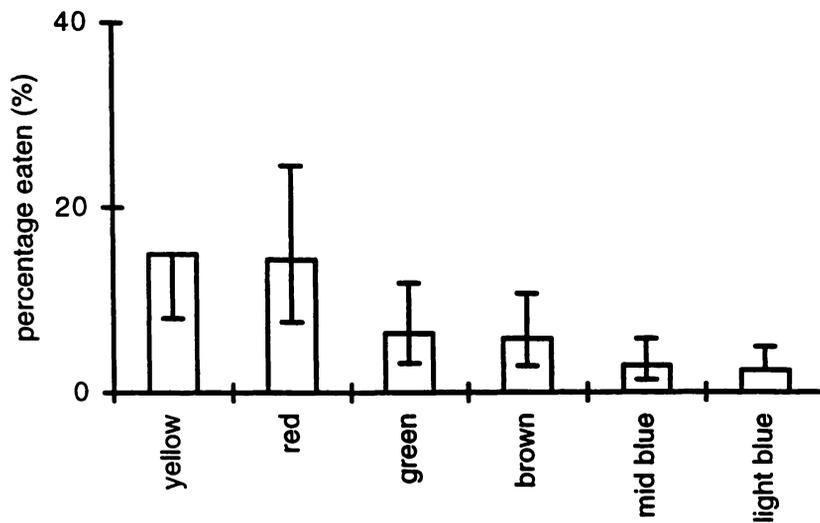
Table 1: *Statistical results for fixed and random effects generated by likelihood ratio tests (LRT) from the REML analysis.*

<i>Source</i>	<i>df</i>		<i>p</i>
Fixed effects		Wald	
history	3	2.3	0.51
treatment	2	1.8	0.41
colour	5	19.1	0.002
linear timetrend	1	144.0	< 0.001
position	5	15.0	0.01
treatment × history	5	2.2	0.82
treatment × colour	10	7.7	0.66
treatment × linear timetrend	2	5.4	0.07
history × colour	15	68.4	< 0.001
history × linear timetrend	3	10.4	0.02
colour × linear timetrend	5	15.2	0.01
Random effects <sup>a</sup>		LRT <sup>a</sup>	
spline	1	6.1	0.007
chicken × spline	1	4.3	0.02
chicken	1	41.2	< 0.001
chicken × linear timetrend	1	10.1	< 0.001
chicken day	1	25.6	< 0.001
chicken colour	1	36.5	< 0.001
chickens position	1	18.5	< 0.001
chicken colour × linear timetrend	1	6.2	0.006

<sup>a</sup> The LRT statistic is an equally weighted mixture of  $\chi^2$  variates with zero and one degree of freedom. The *p* value is thus 0.5 times the probability for a  $\chi^2$  with one degree of freedom. (Stram and Lee, 1994).

The colour preferences chickens showed in this experiment were strongly affected by the colour of pellets they had received in the past (history × colour, Table 1). Chickens that had experienced pellets of all six colours previously ate more red, yellow and brown artificial fruit than mid blue or light blue fruit on the first day, while their consumption of green artificial fruit appeared intermediate (Fig. 3a). Chickens that had previously experienced pellets in four shades of blue preferred mid blue, light blue and brown artificial fruit to red and yellow fruit on Day 1 with green artificial fruit again intermediate (Fig. 3b). Of the chickens that had not previously experienced pellets two failed to eat and the preferences of the remaining two chickens were unclear.

a)



b)

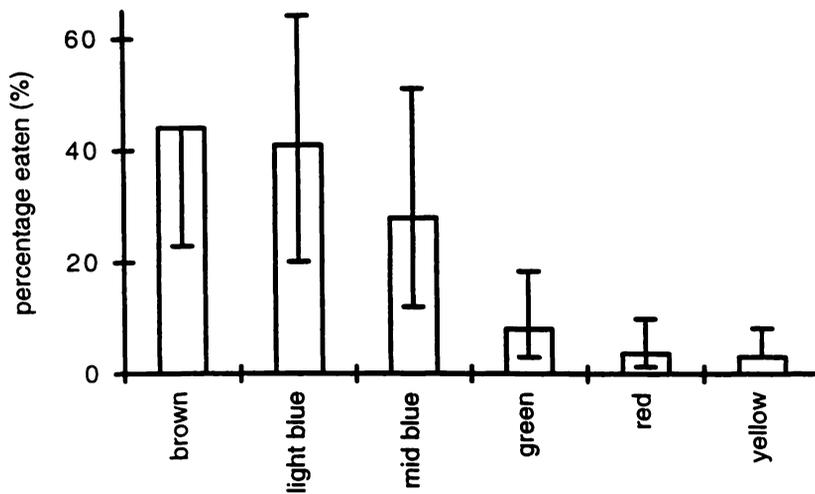


Figure 3: *The adjusted mean consumption of coloured artificial fruit with both pyrazine and Bitrex on Day 1 by chickens that had previously encountered a) pellets of all 6 colours and b) pellets of 4 shades of blue. Colours are ranked from the most preferred to the least preferred. The error bars represent half least significant differences. Means are different at  $P = 0.05$  if the error bars do not overlap.*

As in previous experiments (Chapter 2) the consumption of fruit started at a low level but increased over time (linear timetrend, Table 1, Fig. 4). Not only did chickens consume different amounts of the different coloured artificial fruit on the first day but they increased their consumption of fruit during the course of the trial at different rates depending on the colour of the fruit (colour  $\times$  linear timetrend, Table 1, Fig 4b). Consumption of the artificial fruit of colours that were less preferred on Day 1, generally increased at a greater rate than the consumption of fruit of colours that were more preferred on Day 1, although different chickens differed in the rate at which they increased their consumption of the different colours (chicken  $\times$  linear timetrend, Table 1).

Chickens also increased their consumption at different rates depending on their previous experience (history  $\times$  linear timetrend, Table 1). Chickens that had not taken part in a previous experiment (Chapter 2) and had therefore not experienced coloured pellets prior to this experiment appeared to eat less artificial fruit initially but subsequently increased their consumption faster than the other chickens. This pattern resulted in chickens eating the same amount overall regardless of their previous experience (history, Table 1) and there was no interaction between the amount eaten of each fruit type and previous experience (treatment  $\times$  history, Table 1).

The chickens did not increase their consumption at different rates depending on which type of fruit they received although this approached significance (treatment  $\times$  linear timetrend, Table 1). Over half the chickens in the analysis that received Bitrex artificial fruit failed to eat all the fruit offered on Day 6. The situation was reversed for the chickens that received the fruit that did not contain Bitrex with well over half of these chickens eating all the pellets offered on Day 6. This difference resulted in a slower rate of increase for the chickens receiving Bitrex fruit than the groups receiving either of the two types of non-Bitrex fruit.

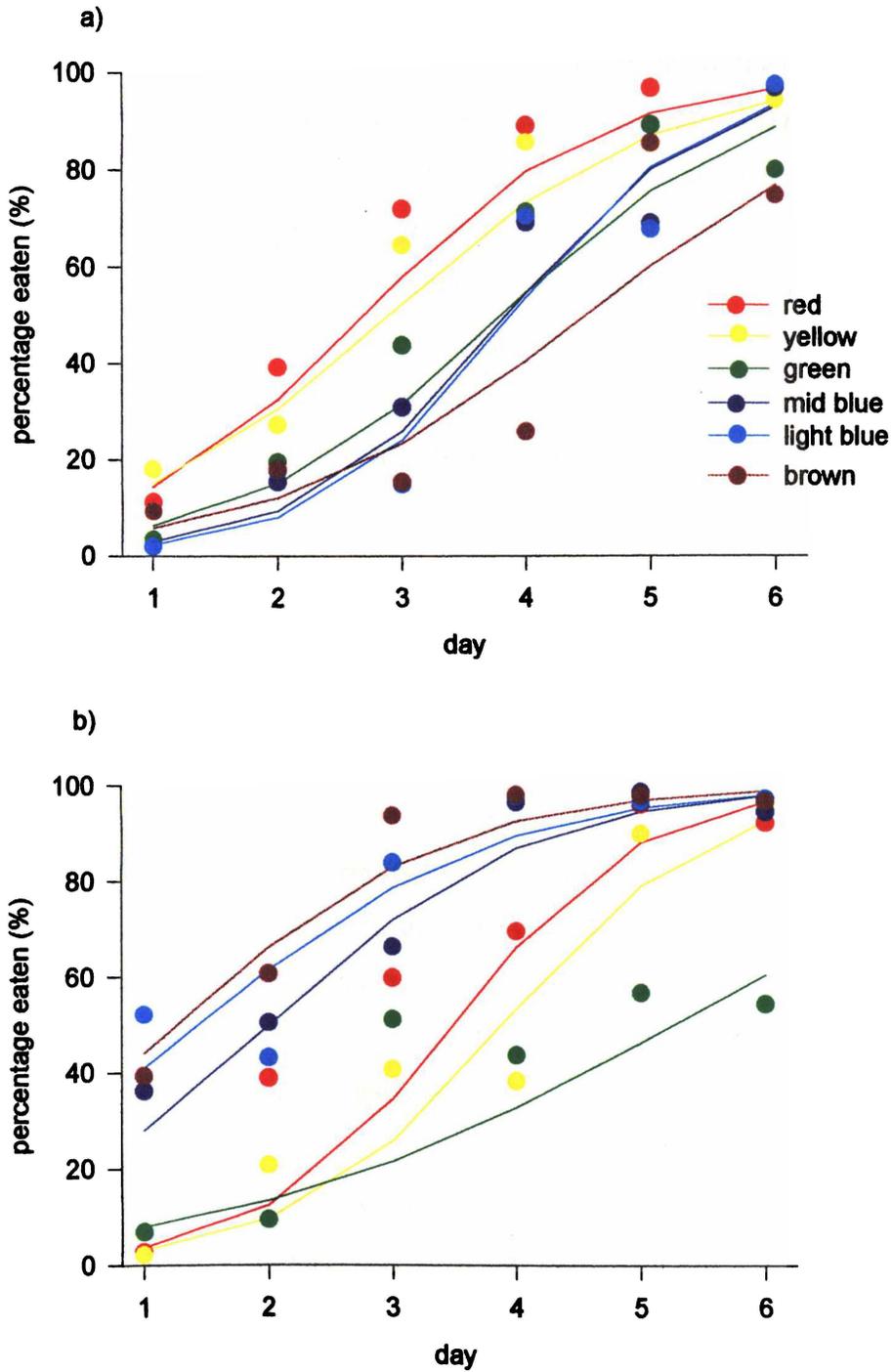


Figure 4: The percentage of artificial fruit containing pyrazine and bitrex eaten per day by chickens. The lines present the predicted values backtransformed from the original logit model and the symbols present the back transformed observed means. Chickens had previously encountered pellets of a) all six colours and b) four shades of blue.

There was considerable variability amongst chickens. Individual chickens differed from other chickens in how much they ate overall (Table 1), how much they ate each day (chicken day, Table 1), how much they ate of the different colours (chicken colour, Table 1), and how much they ate from different positions in the layout (chicken position, Table 1). Overall chickens ate less from the positions at either end of the layout (position, Table 1). Chickens also differed in the rate at which their consumption increased from day to day (chicken  $\times$  linear timetrend, Table 1). The spline term was significant (Table 1) as was the interaction between chicken and the spline term (Table 1) suggesting some of the variability in consumption was due to chickens eating a bit more or less than expected for several days.

## Discussion

The colour preferences shown by chickens were not altered by the addition of pyrazine or pyrazine and Bitrex to the artificial fruit. The chickens that had been offered all six colours in the past showed the same colour preferences for novel food as previously (Chapter 2) irrespective of whether the artificial fruit contained pyrazine. These chickens preferred red, yellow and brown artificial fruit to mid blue, light blue, and green fruit on Day 1 (Fig. 3a). The chickens that had only experienced blue pellets previously chose the blue and brown artificial fruit in preference to fruit of the other colours (Fig. 3b).

In contrast to previous research, not only did we find that pyrazine had no effect on colour preference it also had no effect on the amount of artificial fruit eaten. It could be argued that the chickens did not regard the artificial fruit as novel since they had experienced pellets of similar colours previously. If this was the case then the chickens may have ignored the pyrazine odour because it accompanied a familiar food. However, the amount eaten on the first day (Fig. 3) and the patterns of increasing consumption shown by the chickens (Fig. 4) were similar to those shown by chickens in earlier experiments (Chapter 2) when they had not

experienced coloured food in the test box previously suggesting that the chickens did regard the food as novel.

Several differences existed between our study and previous studies in which researchers found that pyrazine combined with novelty to deter birds from eating. Firstly, previous experiments (Marples and Roper, 1996; Rowe and Guilford, 1996, Rowe and Guilford, 1999) tested young chicks with limited past experiences while we tested adult free range chickens. Adult red-winged blackbirds (*Agelaius phoeniceus*) have been shown to detect and respond to pyrazine in food (Avery and Nelms, 1990). These blackbirds were wild caught and it is possible that the captive reared chickens were less experienced with unpleasant foods and less cautious as a consequence. Birds that are more cautious may be more responsive to methyl pyrazine.

A second difference between the present and previous studies was that in previous studies researchers have made the food offered to chicks (chickcrumbs) novel by dyeing it different colours (Marples and Roper, 1996; Rowe and Guilford, 1996). In our experiment the chickens were offered similar colours to those they had already experienced but the food itself was novel. This raises the possibility that only some types of novelty interact with pyrazine i.e. only foods that are novel in colour not foods that are novel in composition. Pyrazine may, therefore, combine with only a subset of novel foods, those that are distinctively different to the foods a particular bird has experienced in the past. Several authors have discussed the importance of the distinctiveness or conspicuousness of the signals used by brightly coloured toxic insects (Gittleman and Harvey, 1980; Marples and Roper, 1996; Roper and Redston, 1987). The difficulty in evaluating this idea is that, as humans we cannot be sure which characteristics would make a novel food distinctively different for a particular bird species. Nevertheless it would be worthwhile testing whether pyrazine combined with a novel coloured food reduced consumption by a native bird species.

Increasing the number of components presented in association with a novel food did not increase the magnitude or duration of the preferences associated with food of different colours. The chickens may not have increased their consumption of the Bitrex fruit as quickly as the other fruit types but we found no support for the proposal that a multicomponent approach may be more effective in pest control, at least for the combination of components we tested. The chickens that ate very few fruits in this experiment were excluded from the analysis but there was no evidence that a disproportionate number of these chickens were from any one of the treatment groups.

Several researchers have commented on the apparent dual role observed in nature for colours such as red (Guilford *et al.*, 1987; Jones and Roper, 1997, Marples, Roper and Harper, 1998). Red is a common colour in toxic insects and authors have described colours such as red as 'warning' colours for birds (Schuler and Roper, 1992; Marples and Roper, 1996; Rowe and Guilford, 1996) implying that it carries the message 'this food item is dangerous watch out'. However in other contexts, birds seem to prefer red. For example a disproportionate number of plants dependent on birds to disperse their seeds have fruit that are red or black in: southern Africa (Knight and Siegfried, 1983), Europe (Turcek, 1963), Australia (Willson, Irvine and Walsh, 1989), Japan (Nakanishi, 1996), Illinois USA (Willson and Thompson, 1982) and New Zealand (Lee, Wilson and Johnson, 1988; Willson *et al.*, 1989; Williams and Karl, 1996). Likewise in our studies we have consistently found red to be one of the most preferred colours for chickens (Chapter 2), weka (Chapter 3) and robins (Chapter 4). We suggest, therefore, that if red does carry a message for birds, that message is context dependent. It may be best to view red as an 'alerting' colour for birds (Guilford *et al.*, 1987; Rothschild and Moore, 1987) rather than a warning colour. We suggest that if red carries a message for birds it is 'take notice'. Red, for example, may be conspicuous for birds but, we suggest, they rely on other associated cues to decide whether to eat the red object or leave it well alone. This apparent conflict over the role of red in bird foraging decisions warrants more study.

The consumption patterns shown by chickens in this experiment were remarkably similar to the patterns shown by the chickens in previous experiments (Chapter 2). Chickens steadily increased their consumption from test to test until they were eating all the fruit available of every colour. There was considerable variability in consumption between chickens and this showed up in the analysis as differences in the interactions between individual chickens and the time related terms, linear day and spline. This variability between individuals was also observed in a colour preference trial with a native bird species, New Zealand weka (Chapter 3) and in a previous experiment with chickens (Chapter 2). The similarity observed between weka and chickens suggests that the overall pattern of consumption observed i.e. that of increasing consumption from test to test, is a common behaviour pattern among several bird species and that within this behaviour pattern there is some individual variability.

## **General Discussion**

We found no evidence that pyrazine was effective in deterring robins from a familiar food and no evidence that pyrazine was effective as a bird deterrent in combination with novelty, colour or bitterness for adult chickens. Poisonous baits are novel to native birds when they encounter them in the wild so this second result is disappointing.

In our first experiment, with robins, the birds were offered mealworms accompanied by airborne pyrazine odour. In the second experiment the chickens came into direct contact with the pyrazine because the pyrazine solution was incorporated into the artificial fruit which the chickens ate. The second method of presentation has the most relevance for a pest control situation in that the pyrazine would have to be incorporated into baits and birds eating them would be exposed to both airborne and taste cues. Our arrangement, although appropriate in a poisonous bait context, differs from some previous pyrazine experiments in which researchers have allowed only airborne cues to reach birds. Avery and Nelms (1990), however, found pyrazine was effective in combination with other cues in

reducing consumption of food by red winged blackbirds when it was added to food at a 0.0001% concentration which was more dilute than that used our experiment. It seems unlikely that adding the pyrazine solution to the baits themselves would explain the lack of effect we observed.

The colour preferences shown in this experiment, by chickens that had previously experienced all six colours, were the same as those shown by chickens in the past (Chapter 2) and the same as those shown by the two native species tested, weka and robins (Chapter 3 and 4). All three species preferred red and yellow to blue novel food. It appears that colour preferences in birds may be more generalised than previously recognised. It also appears that when a bird encounters a novel food it bases its initial foraging decisions, at least partly, on these pre-existing colour preferences.

We found that the colour preferences of chickens were, however, also affected by their previous experiences of coloured food. Previous studies assessing whether it is possible to alter a bird's colour preferences for food by pre-feeding with particular colours have obtained mixed results. For example, Puckey, Lill and O'Dowd (1996) failed to alter the collective preferences of silvereyes (*Zosterops lateralis*) by manipulating their diet prior to testing and Willson and Comet (1993) found that the colour of food that captive Northwestern crows (*Corvus caurinus*) were fed as chicks had no detectable effect on their food-colour choices later on. Brunner and Coman (1983), however, did influence the preferences of wild crimson rosellas (*Platycercus elegans*) by pre-feeding them with blue grain for two weeks. Colour preference in birds appears to be modified more readily by altering the rewards associated with particular colours than by pre-feeding. Slaby and Slaby (1977), for example, found that the colour preferences of Stella's jays (*Cyanocitta stelleri*) for peanuts in their shells could be modified by removing the nuts from some of the shells and hence changing the food reward associated with each colour. Melendez-Ackerman, Campbell and Waser (1997) also found that the preferences of hummingbirds for red and white flowers could be reversed by

manipulating the amount of nectar available at each flower type. It appears that pre-existing colour preferences are not fixed but can be modified by experience. In the present study we found that birds' colour preferences changed over time. As our experiment progressed chickens tried the colours that they initially avoided. Eventually most of the chickens ate all the fruit of all the colours offered. It appears that birds may have colour preferences which they base their initial foraging decisions on but that these preferences can be modified by experience.

If colour preferences in birds are not fixed but can be modified the implications for poisonous bait design are unclear. Does the modification, for example, imply that a bird that ate purple berries seasonally would be more inclined to try purple-dyed poisonous baits during the purple berry season? Likewise would a bird be more inclined to try a poisonous bait the longer the bait was lying around? Poisonous baits should be field tested prior to use with wild birds in one-choice tests at the appropriate time of the year.

This study has reconfirmed that chickens have colour preferences but failed to identify a combination of cues that would deter chickens from eating a novel food for more than a day or two. Mid blue still appears to be the most suitable colour for dyeing poisonous baits but chickens and weka, over time, sample foods of colours that are less preferred and incorporate those foods into their diet.

We found that pyrazine did not deter robins from a familiar food and did not deter chickens from a novel food of familiar colours. It is possible the pyrazine failed to deter the chickens from the novel food in this experiment because the novel food was not sufficiently distinctive or conspicuously novel and we suggest the combination of pyrazine and a distinctively coloured food should be investigated with a native bird species. It remains unclear, however, which colour is the most promising to test in combination with pyrazine.

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## CHAPTER 6

### Can colour and an insect defensive odour deter native New Zealand birds from eating baits?

Lynette Hartley, Joseph Waas, Cheryl O'Connor

#### Abstract

This study is one of a series investigating ways of deterring birds from poisonous baits that are intended for introduced mammalian pest species in New Zealand. We examined whether the addition of pyrazine, an insect defensive odour, to coloured, novel food, reduced consumption by birds. Experiments were conducted both in captivity and in the wild. We chose pyrazine as many distinctively coloured insects, that are toxic to birds, release this odour when attacked. Captive weka (*Gallirallus australis*), a native New Zealand rail, were offered blue and red novel food to which pyrazine had been added and the same coloured food without pyrazine. Blue was selected as previous experiments found that blue was one of the least preferred colours for weka. Red was selected as it is a common colour among brightly coloured toxic insects. Weka ate significantly less of both the red and blue food with pyrazine odour than the red and blue food without the odour. Consumption of the food without odour increased over each of the six consecutive test days but consumption of the food with odour remained low and static throughout the experiment.

We next compared the consumption by birds in the wild of non-toxic cereal baits that had been dyed blue and smelt of pyrazine with the same baits that had been dyed green and flavoured with cinnamon. The green-cinnamon baits were those commonly used in poisoning operations in New Zealand. Twenty forest sites had trays of either blue-pyrazine baits or green-cinnamon baits and the weight of bait eaten from each was recorded over six days. Ground-level feeders were visited by weka. Fewer blue-pyrazine baits were consumed on the first day from these

feeders and the difference in overall consumption approached significance. Weka are a particularly difficult species to deter from food so this is a very positive outcome. Pyrazine may be even more effective in deterring other native birds from poisonous baits.

**Keywords:** Alerting, colour, feeding deterrent, *Gallirallus australis*, odour, methyl pyrazine, weka.

## Introduction

New Zealand native birds sometimes eat poisonous baits intended for introduced mammalian pest species. Several bird species seem vulnerable to eating baits including weka (*Gallirallus australis*) and New Zealand robins (*Petroica australis*). Weka are chicken sized, flightless rails whose vulnerability is possibly due to their omnivorous feeding style and inquisitive nature (Chapter 3). They have been seen eating poisonous baits on numerous occasions (Brown, 1993; Eason and Spurr, 1995; Spurr and Powlesland, 1997) and deaths of between 90 and 100% have been recorded in some poisoning operations (Eason and Spurr, 1995; Empson and Miskelly, 1999). Robins are small, insectivorous passerines that occasionally supplement their diet with fruit. Deaths of between 30 and 50% of robins have been recorded in some poisoning operations (Brown, 1997; Walker and Elliott, 1997; Powlesland, Knegtman and Marshall, 1998; Empson and Miskelly, 1999). Both species are endemic to New Zealand and our studies to date have concentrated on investigating bait characteristics that may deter these two species from baits.

Poisonous baits are novel to birds when they encounter them and we have investigated two possible cues, colour and odour, that may be important in influencing whether a bird will eat a novel food. We found that all three bird species tested; weka, robins, and domestic chickens (*Gallus domesticus*) had colour preferences when offered coloured novel foods (Chapters 2, 3 and 4). It appeared that, for all three species tested, colour was one of the factors that influenced whether a bird would eat a novel food. Although the birds showed colour preferences when they first encountered novel foods, the weka and chickens soon increased their consumption until they eventually ate all the food that was available of all the colours.

We have also investigated whether the addition of the odour, methyl pyrazine, affected a bird's consumption of a novel food. Toxic insects are often brightly coloured (Schuler and Roper, 1992) and have conspicuous odours (Marples and

Roper, 1996), such as methyl pyrazine, which are released when they are attacked. We found that methyl pyrazine did not deter wild robins from a familiar food or adult free range domestic chickens from a coloured food of a novel formulation (Chapter 5). The chickens tested had experienced a food of a different formulation in similar colours previously. Several researchers have questioned whether a bird's consumption of a novel food may be related to the conspicuousness of the food (Gittleman and Harvey, 1980; Roper and Redston, 1987; Guilford, 1990; Marples and Roper, 1996) and it is possible the pyrazine failed to deter the chickens from the novel food offered because the food was not conspicuously novel. In this study, therefore, we wanted to investigate the effect on consumption of combining pyrazine with a conspicuous novel food which the test birds had not encountered previously.

The difficulty with this objective was that we cannot know which cues will make a food conspicuously novel to birds. We used a test food of a novel formulation that was dyed red. We chose red as many toxic insects have pyrazine as a defensive odour and have red in their colour patterns. Researchers have speculated that red is common among such insects because red is distinctive to birds (Rothschild and Moore, 1987). In addition Rowe and Guilford (1996) found that pyrazine deterred birds from eating novel food when that food was red.

We chose weka as an indicator species because weka are known to eat baits and die in poisoning operations and have similar colour preferences to the other birds we have tested. Since weka appear to be more difficult to deter from baits than the other native species tested, robins (Chapter 4), we suggest that a deterrent that is effective on weka may also be effective on other native bird species.

The weka we tested had not been involved in colour preference trials in the past and were naive to the coloured pellets we used. We compared a weka's preference for red novel food with pyrazine to their preference for blue novel food without pyrazine. We could not offer individual weka both red food with, and

without, odour because the consumption of one may have interfered with the consumption of the other because they had a cue in common (red). Blue was one of the least preferred colours for the group of weka tested previously (Chapter 3) and therefore the most effective cue that we have identified to date for reducing weka consumption of a novel food. By comparing weka consumption of blue to that of red plus pyrazine we were able to evaluate whether red plus pyrazine was more effective in reducing consumption than blue and therefore whether it may be more effective in deterring birds from baits. We do not know whether blue is conspicuous for weka. We, therefore, offered other individual weka a choice between blue novel food with pyrazine and red novel food to see whether blue and pyrazine was a more effective combination than red and pyrazine in reducing consumption.

In a second experiment we tested the most effective combination identified in the first experiment on wild birds in the field. In our studies to date we have generally offered birds a choice between several options to determine their preference. In a poisoning operation, however, birds are exposed to only one type of bait and must evaluate a bait, if they encounter it, on its merits alone. We used baits of the formulation generally used in poisonous bait operations in New Zealand and the same baits dyed blue with pyrazine added. We wanted to see how much of each type of bait birds would eat if they encountered the bait in the wild. Individual wild birds only encountered one bait type and this mimicked the situation occurring in a poisoning operation. A variety of bird species had access to the baits.

The aim of these experiments were 1) to determine whether the addition of pyrazine to red or blue novel food reduced consumption by weka and 2) to see whether baits treated with the most effective combination of cues identified in the first experiment were less attractive to birds in the wild than the baits generally used in poisoning operations.

## EXPERIMENT 1: CAPTIVE EXPERIMENT

### Methods

#### *Test birds*

Thirteen wild caught weka were housed individually in pens measuring 4 m x 4 m x 2 m at Landcare Research's animal facility, Lincoln, New Zealand. The weka were wild birds from Solander Island, near Stewart Island, New Zealand, and had been in captivity for three months when the experiment started. Solander Island is uninhabited and relatively inaccessible so it is likely the weka had little experience of humans prior to capture.

Weka were fed a mixed diet of grated cheese, cat biscuits, rolled oats, canned spaghetti and canned cat food, and water was permanently available. They had access to food for 24 hours a day but for two weeks immediately before and during the trial, food was removed between 2100 and 1300 hr so that weka were hungry when they were tested. Weka did not eat all the food available under either feeding regime. They were occasionally observed to take natural food in the form of insects and seeds (L Hartley, *pers. obs.*).

#### *The test food*

Non-toxic pellets were hand made from flour, water, sugar, lard and food colouring (powdered dyes from Bush Boake Allen, New Zealand Ltd.) to the same recipe used previously with weka (Chapter 3). Pellets of two colours were presented, red and blue. The spectral reflectance curves of these two colours are shown in Fig. 1 and were measured by Justin Marshall (University of Queensland, Australia). It was assumed that the food colouring had no significant taste to weka although we did not specifically test this. No attempt was made to simulate existing poisonous baits in size, shape, colour or texture in this experiment. Rather, weka were presented with pellets of different colours that were completely novel to them. Pellets measured 7 x 7 x 3 mm and were small enough for weka, after initial investigation, to eat entire pellets.

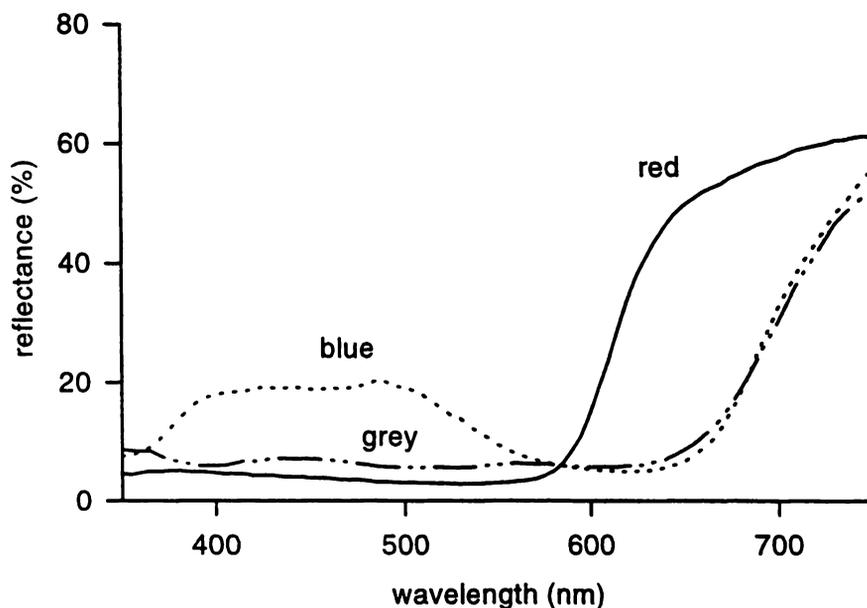


Figure 1: *Spectral reflectance curves of the red and blue pellets and the grey background towelling presented to captive weka in Experiment 1. Spectral reflectance curves show the relative proportion of light reflected off a coloured surface at each wavelength.*

#### *The test procedure*

A test area was set up in each pen by clearing a 1.0 x 0.5 m rectangle of vegetation (grasses) back to bare soil. The pellets were presented to each weka in two identical small, circular, clear plastic containers which were 8 cm in diameter and had a 4 cm lip. Each container had a grey circular towelling insert stuck to the bottom which allowed the odour to be presented. Twelve hours before each test 1 mL of either pyrazine solution or ethanol solution was applied to the towelling with a syringe. The pyrazine solution was made by dissolving 0.1 mL of pyrazine (Pyrazine Specialists, Atlanta, Georgia, USA) in 10 mL of pure ethanol and adding this to 990 mL of water. This process resulted in a 0.01% pyrazine solution. This was the same concentration we used previously with chickens (Chapter 5). The control ethanol solution was made up in the same way, without the pyrazine. Twenty pellets, were placed on the damp towelling and the plastic container was sealed overnight. In a previous trial with pyrazine (Chapter 5) we

incorporated the solution into the food we offered to chickens and this is the preferred test method since it mimics the situation that would occur if pyrazine was incorporated into poisonous baits (i.e. birds would be exposed to both the smell and any taste of the pyrazine). In this trial, however, we were unable to incorporate the pyrazine into the pellets since they were baked and the pyrazine odour would have evaporated during baking so instead we allowed the smell of the pyrazine solution to permeate the pellets for 12 hours immediately prior to each test. One hour prior to testing the pellets were removed, a further 1 mL of the appropriate solution was applied to the towelling and the pellets were returned to the plastic container which was then resealed. Lids were removed finally as the pellets were presented to the weka.

All weka were offered a choice between a plastic container holding twenty red pellets and another containing twenty blue pellets. Containers were presented 25 cm apart. For seven of the weka the blue pellets had been treated with the pyrazine solution while the red pellets had been treated with the ethanol solution. For the remaining six weka this combination was reversed. Weka were randomly assigned to treatment groups and within each treatment group half the weka received the red pellets on the left and half received the red pellets on the right on the first day. The position of colours alternated on subsequent days.

Weka were tested individually between 0730 and 0900 h for 15 minutes on six consecutive days in February. Containers and towelling were re-used but were dried out of doors for at least eight hours before being re-filled for the following day. This amount of time was not sufficient to completely clear the smell of pyrazine from the containers but the containers were used for the same treatment and bird each day so there was no risk of cross-contamination.

The weka had been familiarised with the plastic containers by offering them a choice of familiar foods (grated cheese and cat biscuits) presented in the containers on the two days before testing. All weka were eating at least half the

food presented in this way before testing started. Black cloth was pegged to the wire mesh walls between the weka pens to prevent the weka from observing one another during the tests. Pellets were presented in shade for consistency.

### *Analysis*

The percentage of pellets consumed during the trial by each weka were analysed to take into account the incomplete block design and the nature of the response data. Analysis of variance of the percentages subjected to an angular transformation gave approximate results which were checked by fitting a generalised linear (logit) model, and by the REML (residual maximum likelihood) method. These analyses allowed simultaneous testing of both colour and odour main effects, together with the interaction between the two, however the measure of interaction was relatively imprecise being based on comparisons between birds.

## **Results**

The addition of pyrazine odour to the pellets significantly reduced consumption (F test ratio = 34.5, d.f. = 11,  $P < 0.001$ , Fig. 2) of both red and blue pellets. In the case of blue pellets, consumption was reduced to near zero with only one out of seven weka eating any blue when it was associated with pyrazine. This weka only ate one pellet on one occasion. Consumption of red and blue pellets without pyrazine increased daily (Fig. 3), while the consumption of red and blue pellets with pyrazine remained at or near zero throughout the trial. Although it appeared that weka ate less of the blue than the red pellets the difference in consumption was significant only at the 10% level (F test ratio = 3.28, d.f. = 11,  $P = 0.1$ ). The small sample size and very low consumption of pyrazine treated pellets in both treatment groups made an analysis of the interaction between colour and odour ( $P = 0.9$ ) relatively uninformative.

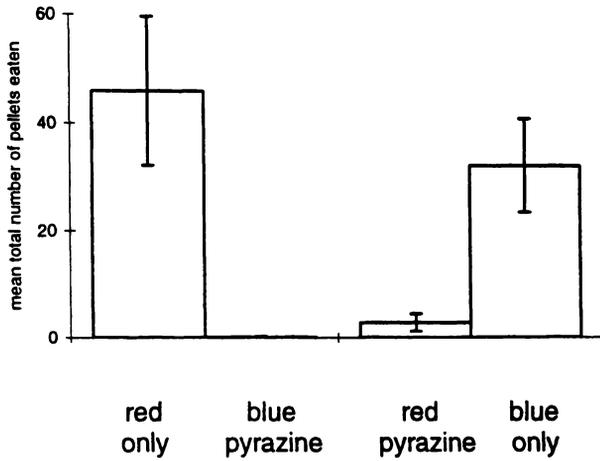


Figure 2: Average ( $\pm$  S.E.) total consumption by captive weka offered a choice of either blue-pyrazine and red non-pyrazine baits or red-pyrazine baits and blue non-pyrazine baits.

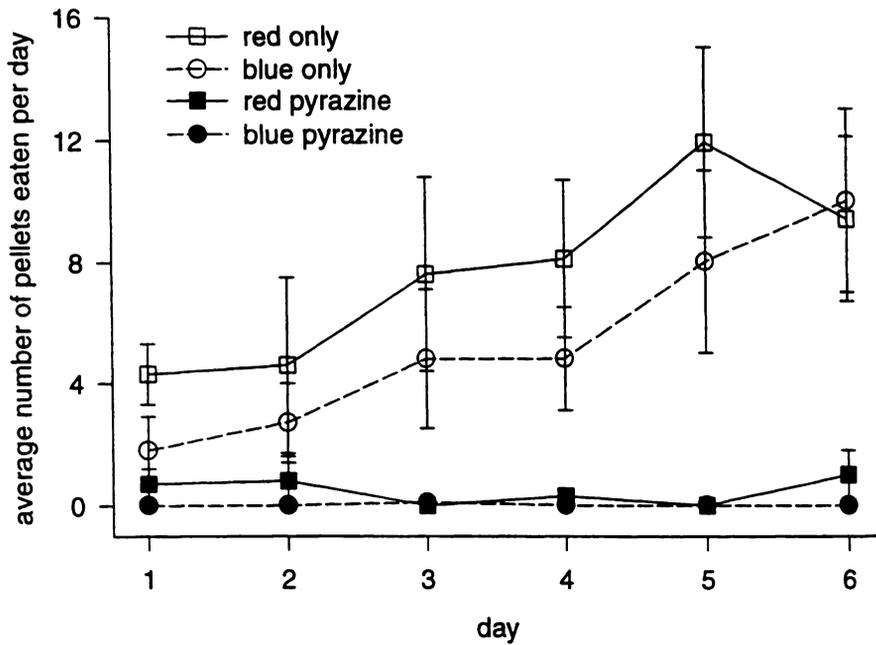


Figure 3: Average ( $\pm$  S.E.) number of pellets eaten per day out of the 20 offered to captive weka ( $n=13$ ).

## Discussion

Pyrazine was effective in deterring the weka in combination with both red and blue novel food. This result suggested that weka could detect the pyrazine and that the addition of pyrazine to poisonous baits may be very effective in deterring birds from eating them. Not only were the pellets with pyrazine eaten less on the first day but consumption remained low for the full six days of the experiment. This suggested that pyrazine may remain effective for an extended period, an important consideration as baits may remain toxic in the field for many weeks.

There was no evidence that red was more effective in combination with pyrazine than blue despite red with pyrazine odour being a commonly observed combination of cues in toxic insects. There has been no systematic survey of the toxicity of insects and their respective colour schemes, in New Zealand or elsewhere, so we cannot comment on the prevalence of blue relative to red toxic insects despite the perception that red toxic insects are more common (Schuler and Roper, 1992). Likewise, we do not know which of the two colours tested was the most distinctive or conspicuous for birds. The result of this experiment does, however, support the hypothesis that the distinctiveness of a novel food may influence how effective pyrazine is in reducing consumption since we have already found that chickens consumption of a novel food was not reduced by the addition of pyrazine when the novel food was presented in familiar colours (Chapter 5).

Weka consumed fewer blue pellets than red pellets and this is the expected result, as we have already shown that weka prefer red to blue novel food (Chapter 3). Consumption of both colours without odour increased daily and this also was the expected result judging by the pattern of increasing consumption observed previously with weka offered coloured novel food (Chapter 3). The weka in this experiment, unlike the weka in the previous study did not eat all the pellets without odour by the sixth day although they may have done so if the experiment had continued for longer.

One alternative explanation for the observation that weka were deterred from eating by pyrazine, while chickens were not, is that weka, as a species, may be more sensitive to pyrazine than the chickens tested. Another possible explanation is that wild caught birds, such as these weka, may be more sensitive to pyrazine than domestic individuals. It is important to test the effect of adding pyrazine to baits on a variety of native bird species in the wild to see whether the deterrent effect observed with these weka occurs with other wild native species as well.

We cannot be sure whether the deterrent effect observed in this experiment was because the pyrazine itself was aversive or because it interacted with the colours of the novel food. Several researchers have concluded that pyrazine itself is not aversive (Guilford *et al.*, 1987; Marples and Roper, 1996; Rowe and Guilford, 1996) and our studies to date support this conclusion (Chapter 5). In this experiment we did not offer weka a familiar food with pyrazine and do not know, therefore, whether the weka would have avoided this combination. If they had avoided a familiar food with pyrazine this would have shown that they found the pyrazine itself aversive.

While the mechanism by which pyrazine functioned in this experiment may not be clear, the addition of pyrazine to blue novel food effectively deterred weka, almost completely, from the food for an extended period. The next experiment investigated the effect on consumption of baits in the wild when they contained the two cues, blue and pyrazine.

## **EXPERIMENT 2: FIELD EXPERIMENT**

### **Methods**

#### *Study site*

Kapiti Island (1965 ha) is a nature reserve 40 km north of Wellington, New Zealand and 5.2 km off shore. This island was chosen as a study site as there is abundant native birdlife including weka and robins and no terrestrial mammals to

interfere with the baits. Flying birds on the island included robins, kaka (*Nestor meridionalis*), saddleback (tieke, *Philesturnus carunculatus*), kakariki (*Cyanoramphus* sp), wood pigeon (kereru, *Hemiphaga novaeseelandiae*), whitehead (*Mohoua albicilla*), tuis (*Prothemadera novaeseelandiae*) and bellbirds (*Anthornis melanura*). Flightless birds included weka, kiwis (*Apteryx australis*) and little blue penguins (*Eudyptula minor*).

### *Test sites*

Twenty test sites were set up along two tracks, a coastal track which follows the eastern side of the island from south of the slipway to Okupe lagoon at the northern end, and the Okupe valley track which runs inland from the lagoon. We judged that weka and robins were the most likely birds to visit the test sites since these were the species that have been observed to eat poisonous baits (Lloyd and Hackwell, 1993; Spurr and Powlesland, 1997). We did not want individual birds to visit more than one site and hence more than one type of bait. Both weka and robins are territorial so, by placing one test site in each territory, we hoped individual birds would not visit more than one site. Weka were believed to have larger territories than robins because they are larger birds but unfortunately the size and location of both weka and robin territories on Kapiti Island was unknown. Territory size for weka on Kapiti Island has been recorded as between 0.7 and 4.5 ha which equates to roughly 220 m by 220 m (Taylor, 1998). Weka numbers, however, were severely reduced over the entire island in 1996, three years before this experiment, in a poisoning operation for rats (Empson and Miskelly, 1999). Weka numbers had recovered rapidly but weka may not have reached their pre-poisoning density and thus may have had larger territories than previously (C. M. Miskelly, Department of Conservation, Wellington, *pers. comm.*). Weka had been observed, before the poisoning, to follow researchers for up to 200 m (C. M. Miskelly, *pers. comm.*). Test sites were set up 300 m apart and this distance was determined by pacing.

Sites were placed a sufficient distance off the tracks to be inconspicuous to human visitors to the island. They were placed in forest with trees at least 3 m high with canopy covering at least 60% of the sky above the site. Baits were presented in two 'feeders' at each site. Both feeders were black plastic trays 25 cm in diameter with a 5.5 cm high lip around the edge. Each site had one feeder tray resting directly on the ground allowing access to flightless birds and the other mounted on a pole 1.2 m high which prevented access by such birds, particularly weka. Both feeders had a circle of absorbent grey towelling 17 cm in diameter stuck to their base. Because feeders were exposed to the weather and the baits disintegrate when they get wet we did not want the baits to rest in the towelling in case they absorbed water. We, therefore, cut a circle 5.5 cm in diameter out of the center of the towelling to reveal the black plastic underneath. Baits were presented within this ring of towelling.

#### *Test procedure*

The experiment took place in winter mimicking the timing of many poisoning operations in New Zealand. The baits were RS5 cereal baits obtained from Animal Control Products, Waimate, New Zealand. These baits were identical to those used in many brushtail possum (*Trichosurus vulpecula*) control operations but were supplied without the poison or dye. Half the baits had cinnamon incorporated (0.1% volume/weight) by the manufacturer. We applied green dye to the surface of the cinnamon baits by plunging the baits into a dye solution (5 g of green dye per litre of water). Baits were plunged into the dye bath twice for 2 second periods and shaken using a large sieve to ensure all baits had contact with the dye on all surfaces. We used the green dye recommended for use in poisonous baits in New Zealand (Hexacol green V200A, Bayer NZ Ltd. Wellington, New Zealand). The other half of the baits did not have cinnamon incorporated and these were dyed blue using the same technique and the same dye used in Experiment 1 (8 g of blue dye per litre of water, dye from Bush Boake Allen New Zealand Ltd.). The spectral reflectance curves for both the blue and green baits, measured by Justin Marshall (University of Queensland, Australia), are presented

in Fig. 4. Neither the blue or the green were as saturated (intense) as the colours offered to weka in a previous experiment (Chapter 3) and the blue was slightly more green than that used in Experiment 1 of this study. Baits were oven-dried immediately after dyeing. The colour covered the surface of the baits to a depth of 2 mm. The centre of the baits remained the natural pale brown shade. It has been suggested that small birds are attracted to fragments of baits during poisoning operations (Harrison, 1978) so we cut some baits into 2 mm lengths and dyed these fragments as well.

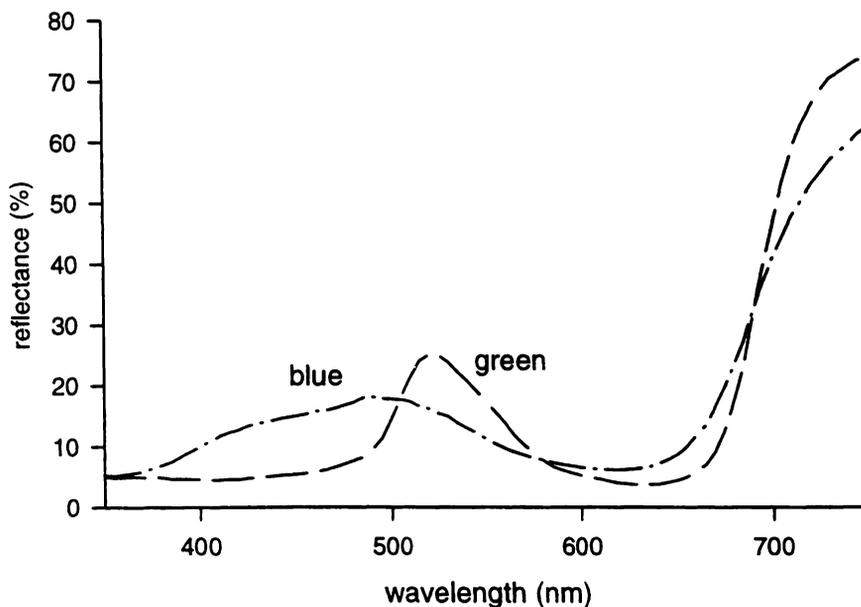


Figure 4: *Spectral reflectance curves of the blue and green baits presented to wild birds on Kapiti Island in Experiment 2. Spectral reflectance curves show the relative proportion of light reflected off a coloured surface at each wavelength.*

Twelve hours before presentation green baits (630 g) were placed in a glass bowl which contained towelling soaked in 80 mL of ethanol solution. A metal grid prevented the baits from contacting the damp towelling directly but the odour of the solution was free to circulate around the baits. Blue baits were treated

similarly in another bowl with a pyrazine solution. The ethanol and pyrazine solutions were the same concentrations and proportions per weight of pellets as those used in the captive experiment. The bowls were sealed overnight and the blue baits smelt of pyrazine to humans in the morning when they were removed from the bowls.

Approximately 30 g of baits were put out in the centre of the towelling ring in each feeder between 0730 and 1100 h. This amount was composed of approximately 20 whole baits and 0.5 g of fragments and was weighed to the nearest 0.01 g. An additional 6 mL of either pyrazine solution, in the case of blue baits, or ethanol solution, in the case of green baits, was added to the towelling ring after the baits were in place. This again was the same proportion of solution to pellets (by weight) used in the captive experiment. In order to sample the baits a bird had to reach over the towelling and was consequently exposed to the odour before trying the baits. Any baits or fragments remaining after 24 hours were removed from the feeder and weighed. When retrieving the baits each day we also noted their appearance (i.e. any peck marks or disturbance) and the presence of any birds near the sites. We were concerned that the addition of an insect odour may attract insects to eat the baits and hence increase the risk of secondary poisoning for birds so we recorded any insects we found on baits. Occasionally baits were found outside the feeder trays and these were collected so birds no longer had access to them. These baits were, however, considered eaten since the birds had removed them from the tray and were therefore not re-weighed.

We alternated blue-pyrazine and green-cinnamon sites along each track to ensure that sites were evenly spread among vegetation types. Both the high and low feeder at any one site received the same type of bait for the duration of the trial i.e. either green-cinnamon baits or blue-pyrazine baits. An additional three sites (ie. 6 feeders) were interspersed randomly between the other sites and treated similarly except that birds were prevented from touching or eating the baits by bird-netting raised on a wire frame above the feeders. The data gathered at these sites

provided information on how much moisture (and hence weight) the untouched baits absorbed during each 24 hour period.

On the third day of the experiment there was about 10 minutes of light rain which was insufficient to cause the baits to disintegrate and we were able to weigh the amount remaining at each feeder. Following this incident black polythene shelters were hung like small tents over the feeders. These shelters were 40 cm above the upper feeders and approx 60 cm above the lower feeders. The shelters were effective in preventing rain reaching the baits during short showers. Shelters were left for at least 24 hours before continuing the experiment to allow time for the birds to become familiar with them. The final three days of the trial were completed without further substantial rain.

To avoid odour contamination between the pyrazine and cinnamon solutions and baits they were stored in separate buildings and all weighing was done at these separate locations. All equipment was kept separate except the scales. To further avoid cross contamination one researcher carried the ethanol mix and put out and retrieved all the green cinnamon baits from the feeders while another researcher did the same with the pyrazine solution and blue baits.

### *Analysis*

The baits in the feeders that were not accessible to birds were heavier at the end of each 24 hours as they had absorbed moisture. The proportion of weight gained at each of the sites that were not accessible to birds was averaged separately for both high and low sites. These two averages were used to correct the weights of baits remaining at the test sites for moisture uptake. This was achieved by dividing the weight of the baits remaining at each feeder by the averaged value for the appropriate type of feeder (high or low).

For both high and low sites we calculated the average weight of baits eaten during the whole six day trial. This weight was compared for green-cinnamon and blue-

pyrazine baits by a Mann-Whitney test. For the low sites we also compared the amount eaten on the first day between treatments using the same test.

## Results

Robins were observed in the vicinity of seventeen of the twenty test sites and weka in the vicinity of twelve of the twenty sites. Both weka and robins were observed eating baits from the low feeders. Very few insects were observed on either type of bait (3 insects observed in total). Once the experiment had been running for several days some weka or pairs of weka were found waiting for the researchers at the feeders each morning. These weka would go straight to the feeders as soon as they were refilled and start eating baits. Eight of the weka seen near the feeders were colour-banded but only one of these weka was observed at two sites. The robins were not banded.

The pattern of consumption observed in this experiment differed from that observed in our previous experiments with consumption at any one feeder tending to sift very quickly from zero, or minimal consumption, to complete or nearly complete consumption. While the weka's consumption was observed to increase from day to day in a previous experiment (Chapter 3) the increase observed in the present experiment was more rapid. This meant that the type of analysis used in the previous experiments (Chapters 2 - 5), which involved fitting curves to the daily consumption and analysing the rate of increase, was not appropriate for these data.

The consumption at some feeders remained at or near zero for longer than at other feeders. On the first day 25 g or more was eaten, of the 30 g of baits available, from four of the green-cinnamon feeders while consumption at the remaining green-cinnamon feeders and all the blue-pyrazine feeders was below 10 g. At three of the green-cinnamon and eight of the blue-pyrazine feeders it was below 2 g. The difference in consumption was significant on the first day with more green-cinnamon bait eaten ( $14.4 \text{ g} \pm \text{S.E. } 4.2$ ) on average than blue-pyrazine bait

( $1.8 \text{ g} \pm 0.8$ ;  $H = 5.9$ , d.f. = 1,  $P = 0.016$ , Fig. 5). By the second day 25 g or more of the bait was eaten from five of the green-cinnamon feeders and only one of the blue-pyrazine feeders and less than 5 g was eaten from four green-cinnamon and five blue-pyrazine feeders. From Day 4 onward consumption was high at all but one of the feeders. Consumption remained at or near zero at this feeder (blue-pyrazine) throughout the experiment. Over the six days of the experiment the difference between the average amount eaten per day of the blue-pyrazine baits and the green-cinnamon baits was significant at the 10% level with less blue-pyrazine baits ( $17.4 \text{ g} \pm \text{S.E. } 4.8$ ) consumed than green-cinnamon baits ( $22.8 \text{ g} \pm \text{S.E. } 3.3$ ;  $H = 2.8$ , d.f. = 1,  $P = 0.096$ ).

Very little bait was consumed from the high feeders at any point in the experiment (Fig. 6) although occasionally we observed that baits had been disturbed. The difference in consumption was again significant at the 10% level with fewer baits consumed on average at the blue-pyrazine ( $0.18 \text{ g} \pm \text{S.E. } 0.05$ ) than the green-cinnamon sites ( $0.45 \text{ g} \pm \text{S.E. } 0.12$ ;  $H = 3.4$ , d.f. = 1,  $P = 0.064$ ).

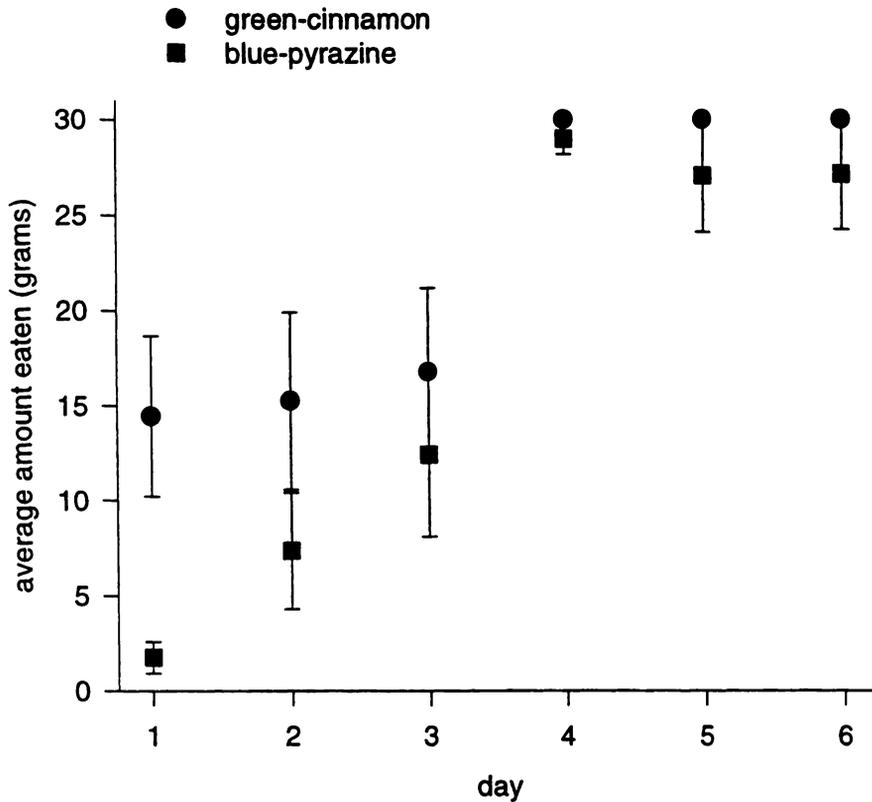


Figure 5: The average ( $\pm$  S.E) amount eaten per day by birds from the low feeders on Kapiti Island. The squares show the consumption of blue-pyrazine baits and the circles show the consumption of the green-cinnamom baits.

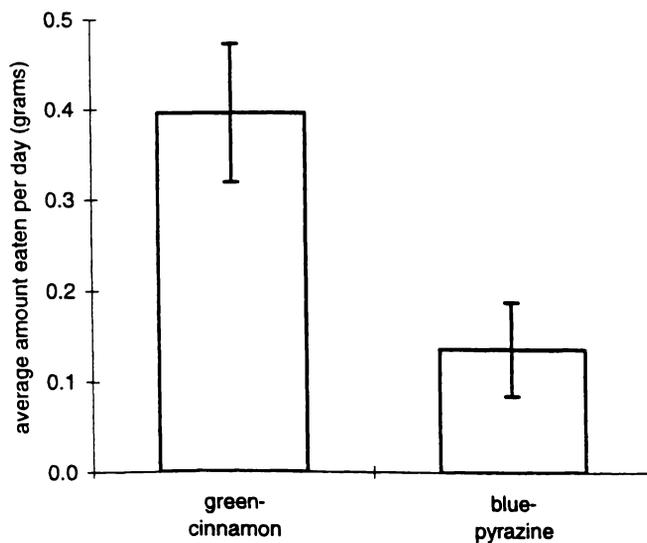


Figure 6: The average ( $\pm$  S.E.) amount eaten per day by birds from the high feeders on Kapiti Island over the six days the baits were offered.

## Discussion

During the course of this experiment both weka and robins were observed eating green-cinnamon baits resembling those used in poisoning operations in New Zealand. This confirms previous observations that these species eat baits directly during such operations (Brown, 1993; Eason and Spurr, 1995; Spurr and Powlesland, 1997) and emphasises the importance of finding an effective bird deterrent for native New Zealand birds.

The combination of blue and pyrazine reduced the amount of baits eaten relative to green-cinnamon baits. Consumption of blue-pyrazine pellets was lower on the first day and remained low for longer. This result suggests that pyrazine may be useful as a means of deterring birds from poisonous baits. While the overall difference in consumption was only significant at the 10% level and the birds did eventually eat the blue-pyrazine baits, at all sites except one, this was still a very positive result, because weka appear to be a particularly difficult species to deter from baits (Chapter 3). We suggest that the addition of pyrazine may result in even greater reductions in consumption by other native bird species.

Before Day 4 average consumption at the low feeders had been increasing steadily from day to day as all the baits were completely consumed at an increasing number of the feeders. From Day 4 onward consumption was at or near 100% at all but one site (Fig. 4). The jump in the proportion of sites at which all the baits were eaten followed the break in testing associated with the rain and the modification of the feeders to protect them from future rain. The feeders, therefore, had been empty for two days when baits were put out for Day 4. It is not possible to determine whether consumption would have reached nearly 100% on Day 4 anyway or whether the increase in consumption was associated with the break in testing.

While we have shown that blue and pyrazine are more effective than green and cinnamon, further testing is needed to establish the most effective combination of

cues. There are at least four components of the baits offered that may have influenced their relative consumption 1) the blue colour, 2) the green colour, 3) the cinnamon, and 4) the pyrazine. Green was always offered with cinnamon as this is the combination commonly used in poisonous baits and blue was always offered with pyrazine since this is one of the combinations we found to be the most effective in Experiment 1. Our previous studies on captive weka from the same population (Chapter 3) showed that the weka were more willing to eat green novel food than blue novel food. The initial differences in consumption observed between the blue-pyrazine and green-cinnamon baits offered in this experiment may, therefore, have been due to differences in colour preference alone. Because we did not offer birds a choice between pyrazine and cinnamon combined with a single colour (i.e. blue or green) we cannot comment on the relative effectiveness of pyrazine and cinnamon in reducing consumption. It may be that changing the colour of baits used in poisoning operations from green to blue will result in reduced consumption of the baits by birds. To check this it would be necessary to offer birds the blue and green baits with a) cinnamon, b) pyrazine and c) neither cinnamon or pyrazine. This would show whether merely changing the colour of the baits from green to blue would result in reduced consumption of baits by birds and whether pyrazine was more effective in reducing consumption than cinnamon. This knowledge would allow recommendations to be made on the optimum combination of cues (of those tested) for poisonous baits to discourage consumption by birds.

Weka were observed to show a great deal of interest in the feeders. Robins are ground-feeders and we observed one robin eating baits from one of the low feeders. Robins weigh around 3 g (Heather and Robertson, 1996) and we suggest that the amount consumed from the lower feeders by birds such as robins would have been small relative to the amount consumed by weka which weigh from 700 g (females) to 1000 g (males; Heather and Robertson, 1996). We did not observe any other birds near the feeders and we assume the consumption data at the low feeders was dominated by the weka. In retrospect in order to monitor the

consumption by birds other than weka, we would have needed to present baits in low feeders, protected from weka but still accessible to other types of bird.

Feeders would be required in habitats, other than forest, to attract the attention of other species.

The amount of water absorbed from the atmosphere by baits in 24 hours was considerable relative to the likely consumption of a small bird such as a robin. Some baits absorbed 10% of their original weight and absorption differed from feeder to feeder with, presumably, microhabitat. The amount of water absorbed by the baits was considerably greater than the weight of bait recorded as missing from the upper feeders making it difficult to confidently attribute such small changes to consumption. Future experiments should consider more accurate methods of measuring interest by small birds perhaps by recording visits to the feeders rather than consumption.

## **General Discussion**

The addition of pyrazine to the red and blue pellets was very effective in deterring captive weka from the pellets of both colours. When we offered blue-pyrazine baits to birds in the wild they also ate less of these baits than baits resembling those commonly used in poisoning operations in New Zealand.

We believe that further research on pyrazine as a bait deterrent for native birds would be worthwhile. Pyrazine is reasonably priced. Based on the 1997 prices used by Spurr and Porter (1998) the addition of 0.01% pyrazine to baits used for mammalian pest control in New Zealand would add about NZ \$580 per tonne to the price of baits (Pyrazine Specialists, Atlanta, Georgia). At a bait application rate of 5kg/ha this would increase the average cost of operations by about \$2.90 per ha from \$18 to \$21 for cereal based baits and from \$21 to \$24 for carrot baits.

While blue and pyrazine deterred the captive weka nearly completely the weka in the wild were more willing to eat the blue-pyrazine baits. There were several

differences between the captive and field experiment that may have contributed to the differences observed in consumption.

The tests on captive weka lasted for 15 minutes each day while the birds in the wild had access to the baits for 24 hours. Birds in the wild may have overcome the deterrent effect of pyrazine more quickly as they could re-visit the baits many more times during the course of twenty four hours.

We were unable to incorporate pyrazine into the baits during manufacture and the pyrazine odour may have evaporated off the baits and towelling in the wild trial during the 24 hour periods between replenishing visits thus reducing the pyrazine's effectiveness. We were also unable to incorporate the coloured dye throughout the baits and any baits that were broken while a weka fed would have revealed the core colour of the bait which may have appeared more attractive. We recommend that future researchers find a more satisfactory way of incorporating the pyrazine and colour into the test baits, such as during manufacture. Available technology from the food industry may provide a technique for maintaining the odour of food for an extended period of time.

The actual blue that we offered differed between the captive and field experiments (Fig. 1a and 1b). The same dye was used in both trials but the cereal based baits produced a greener blue because the base colour was beige rather than white. The qualities of the blue offered may affect how attractive it is for birds (Chapter 2).

A further difference between the captive and wild weka was in the considerable differences in background of the two groups of weka tested. Many of the Kapiti Island weka were held in captivity during the poisoning operation three years previously and had therefore experienced a range of novel foods provided by humans while in captivity. Few weka that were not in captivity survived the poisoning so many, if not all, of the birds we tested had either been in captivity for many months or were the offspring of such birds. The weka that were tested in

the captive experiment, in contrast, were from the remote Solander Island, off Stewart Island, in southern New Zealand. These weka would have rarely encountered people as the island is difficult to land on, has no buildings, and is rarely visited by humans. The Solander Island weka were observed to be shy, very cautious of people, and would not eat novel food readily (L. Hartley, *unpubl. data*). If, as has been proposed, pyrazine is not aversive on its own (Guilford *et al.*, 1987; Marples and Roper, 1996; Rowe and Guilford, 1996) but combines with distinctively novel food to reduce consumption, then the magnitude of any deterrent effect may be particularly sensitive to how familiar the test birds are with novelty. We suggest that the Kapiti Island weka were considerably more familiar with novel food than the Solander Island weka and, as a consequence, the pyrazine deterred them from the food we offered for a shorter period of time. We recommend further testing of pyrazine in the wild concentrating on a) native bird species other than weka, and b) wild weka that have had less extensive contact with people and whose behaviour may be more representative of other wild weka.

It remains to be seen whether pyrazine is deterrent for the target species, rats and possums, although there is evidence that it may not be deterrent to mammals since it is an approved additive to human foods in the USA (Flavour and Extract Manufacturers Association No. 3433). Weka, in general, are likely to be one of the most difficult species to deter from poisonous baits due to their inquisitive nature. Even though the deterrent effect observed in our field trial was small it may equate to a much larger deterrent effect in other native birds.

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## CHAPTER 7

### General Discussion

The aim of this Ph.D study was to investigate two characteristics of poisonous baits, colour and odour, that could be manipulated to make baits safer for native birds. Chickens (*Gallus domesticus*), weka (*Gallirallus australis*) and New Zealand robins (*Petroica australis*) were offered a choice of up to six types of food simultaneously and this method proved effective in identifying preferences. An understanding of which cues that birds show least preference for eating may allow the production of baits that birds avoid. The cues tested were not repellents because it had already been shown that bird repellents, while effective in deterring birds, also tended to repel the target species, particularly rats (Chapter 1).

Preference testing identified blue as the least preferred colour of those offered for all three species tested (Chapters 2-5) and the combination of blue plus pyrazine was effective in deterring captive weka from novel food (Chapter 6, Experiment 1). Blue plus pyrazine was more effective in deterring wild birds from realistic poisonous baits than the green and cinnamon combination currently in common use. Choice tests, then, were an effective technique for screening cues to identify a combination that made baits relatively unattractive for birds.

In addition to identifying a combination of bait cues that produced baits that were less likely to be eaten by birds several other important findings came out of the series of experiments that made up this study. The findings are reviewed below in the context of pest control.

This study found that all three species of bird tested had similar colour preferences. The most preferred colours were red and yellow and the least preferred were blue and, frequently, green (Chapters 2, 3, and 4). Two different

populations of the same species, robins, also showed the same colour preferences (Chapter 4). This consistency in preferences between species and populations was surprising as it was not previously thought that birds had generalised colour preferences. Marples and Roper (1997), for example, stated that it was “simplistic to think of birds as having generalised preferences for, or aversions to, food items of particular colours even within a single species or sex”. While colour preferences in birds have been tested in the past any similarities in preferences between species appear to have been obscured by researchers: 1) using birds with differing backgrounds, 2) using birds of vastly different ages and experiences, 3) having different objectives in the experiments, 4) presenting different colours and 5) using different ways of presenting the colours (Chapter 2). Kalmbach (1943) and Caithness and Williams (1971), however, observed that several bird species avoided green food. When I considered only those studies where adult birds were offered coloured food I found that half reported birds showing a preference for red and/or yellow food over blue and/or green food (Chapter 2) The consistency in colour preferences shown between the present and previous studies suggested that many species of birds may have similar preferences and that a colour that dissuaded one bird species from eating poisonous baits may be effective for many other species as well.

It is unclear why Kalmbach (1943) and Caithness and Williams (1971) found green to be the least preferred colour while the present study identified blue as the least preferred. Comparison between studies was hampered by the difficulty of not knowing exactly what colours were offered by previous researchers. The different results in this, Kalmbach’s (1943), and Caithness and Williams’s (1971) studies, for example, may have been related to differences in the specific blues and greens tested. The present study differed from previous colour preferences studies in that the spectral reflectance curves of the colours tested were presented. Future researchers of colour preferences should also present the spectral reflectance curves of the colours tested to allow comparison between the colours offered in different experiments.

In this study I wanted to offer all the different bird species the same colours but I found that the different species would not eat the same food. As a consequence a new formulation of coloured food was offered to each species and the colours were matched as closely as possible to the colours offered to the other species. Inspection of the spectral reflectance curves for the coloured foods offered in each experiment showed that the colours were very similar. The only curve that differed in shape or position between experiments was the one for the yellow artificial fruits offered in Experiment 2, Chapter 2. This curve, unlike the curves of the other yellow foods, shows a dip at around 600 nm. The yellow artificial fruit looked the same colour to humans as the other yellow foods offered and it is unclear if the fruit would have looked any different to chickens. The spectral reflectance curves of the green foods used throughout this study are very similar to the curve of the baits dyed with the green dye used to dye baits in New Zealand (Experiment 2, Chapter 6). This similarity suggested that the preferences shown and conclusions drawn in this study for green were valid for the green of poisonous baits.

This is one of the few studies that examined not only colour preferences of individual birds but also colour preferences when birds were offered the same colours repeatedly. These two measures are important for the following reasons.

A measure of the colour preferences of individual birds was important because it gave an indication of the variability in colour preference within a population and hence what proportion of individuals may avoid eating a particular colour. There was considerable individual variability and this has also been noted by other researchers looking at colour preference in birds (Willson and Comet, 1993) (Willson, Graff and Whelan, 1990; Puckey, Lill and O'Dowd, 1996). This finding suggested that it is likely that a proportion of individuals would eat baits no matter what colour they were dyed. It is probably unrealistic to seek a combination of

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cues that will deter all birds. Instead research should aim to deter as large a proportion of birds as possible from baits.

A measure of a bird's colour choices when it was offered the same colours repeatedly was important as it gave an indication of whether a bird was more likely to eat a bait of a particular colour as time passed. In the experiments described in this thesis the same pattern was observed repeatedly. Weka and chickens would start by eating a few of the coloured pellets, but by the last test (usually Day 6), these birds were generally eating all the food offered regardless of the colour. There were two aspects to this pattern. Firstly birds ate more and more pellets each day and secondly birds ate more and more colours each day. It was possible that the patterns observed indicated that wild birds would be more inclined to try novel food in the form of baits as time passed. Alternatively the pattern observed may have been related to the experimental design with birds only trying the less preferred colours after they had eaten the more preferred colours and learnt that they were safe. If the presence of more preferred colours encouraged birds to eat the other colours then wild birds may not try baits at all if only one bait type was used and this type was less preferred. Chapter 6, Experiment 2, provided support for the first scenario. When wild birds were offered only one type of food, not a choice, they ate more of the novel food as time passed. Recent research by Marples, Roper and Harper (1998) also supported this conclusion. When blackbirds and robins were offered novel coloured food repeatedly some of them ate it immediately while others took up to 87 exposures before they ate it. All birds did eventually adopt the novel food into their diets (Marples *et al.*, 1998). It appears from the weka and chicken studies that the longer baits are in the field the more likely birds are to try them.

The robins (Chapter 4) showed a different consumption pattern from the weka and chickens. Thirty seven percent of robins did not try the novel food offered during the six test days despite feeding on natural foods for extended periods on the test areas (Chapter 4). In addition, the robins that did eat the coloured food, only ate

two or three colours throughout the experiment. This latter difference may reflect differences in experimental method rather than species differences. The robins were offered more food of each colour relative to their size and appetite than the weka and chickens and were not, therefore, 'forced' to eat their less preferred colours as their more preferred colours ran out during each test. The robins may have been just as adaptable in their colour choices as the weka and chickens if they had been offered less food of each colour per test. Marples *et al.*'s (1998) study suggested that even the 'non-eating' individuals may have eaten the novel food eventually. It is important, therefore, to test birds' reactions to baits in the field for the length of time the baits will remain toxic and this process should be repeated for each species of concern.

This was the first study in which the same series of colours was offered to different species of birds. This consistency allowed comparison of preferences between individuals, populations and species but it did not allow consideration of the birds' preferences for shades or colours other than those offered. An analysis of the luminance of the colours offered to chickens in Chapter 2, showed that chickens may have preferred to eat light (luminant) colours over dark colours. The chickens, however, did not show a preference when they were offered four shades of blue. Two conclusion can be drawn from these results. Firstly it would be prudent to avoid using light coloured poisonous baits as birds may be attracted to eat them. Secondly birds, in this and previous studies, may have been choosing, at least partly, on lightness or a related attribute of the coloured food offered. Brown was the darkest of the colours offered but was not the least preferred so the relationship between lightness and preferences was not absolute.

In summary it appeared that although birds had pre-existing colour preferences which they based their initial foraging decisions on, at least some species, had behavioural mechanisms which allowed individuals to sample and eventually incorporate foods of less preferred colours into their diets. This finding is not surprising as there are numerous examples of birds adapting to novel food sources.

Silvereyes (*Zosterops lateralis*), for example, colonised New Zealand from Australia and now eat a range of foods not present in their native land (Heather and Robertson, 1996), as do other introduced bird species (Williams and Karl, 1996). There are also examples of birds changing their colour preferences when researchers have artificially manipulated the food reward associated with particular colours (Slaby and Slaby, 1977; Melendez-Ackerman, Campbell and Waser, 1997). Birds also readily adopt new food sources, such as fruit, into their diets as the fruit ripens in orchards (Porter, Rudge and Mc Lennan, 1994). The adaptability observed suggested that, even if a particular colour discouraged a bird from eating a bait the first time the bird encountered the bait, a bird may investigate the bait and eat it on subsequent encounters. While many species appear to have similar colour preferences and colour may be useful in deterring some birds from poisonous baits it will probably not be useful in deterring native birds with opportunistic feeding styles such as weka.

In Chapters 5 and 6 I investigated a second cue odour. Methyl pyrazine, an insect warning odour, was found to be an effective deterrent when combined with some cues but not others. It did not deter robins from familiar food or chickens from novel food of familiar colours (Chapter 5) but it did deter weka from food of a novel colour and formulation (Chapter 6, Experiment 1). Weka were deterred from both red and blue food when it was presented with pyrazine. It has been suggested that the conspicuousness or distinctiveness of cues may play a role in the deterrent effects associated with brightly coloured toxic insects (Chapter 5). My study supported this conclusion since pyrazine deterred weka in association with novel coloured food but not chickens in association with familiar coloured food.

This study was the first to attempt to adapt a natural signalling system to deter birds from baits. Research may also turn up other systems in nature which may be worthwhile testing. The defensive systems of New Zealand insects, for example, may be worth investigating. Some native New Zealand ants release methyl

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pyrazine (Ponerine subfamily; Fales *et al.*, 1992) and other New Zealand insects exude noticeable smells when attacked (Rowan Emberson, Lincoln University, Lincoln, *pers. comm.*; Greg Shirley, Department of Conservation, Wellington, *pers. comm.*).

A key problem in deterring birds from poisonous baits is the difficulty of identifying a deterrent that does not also deter the target pest species. The colour of a bait does not affect consumption by several mammalian species including possums but it remains to be seen whether the insect warning odour tested, methyl pyrazine, deters the target mammalian species in New Zealand. It is important that this is determined before further work is conducted on the addition of methyl pyrazine to baits to deter consumption by birds.

In summary the combination of blue plus pyrazine was more effective at deterring birds from realistic poisonous baits than the combination of green and cinnamon which is in general use. The majority of birds visiting the baits appeared to be weka and the small reductions in consumption observed for this species may equate to a considerably larger reduction in consumption by other native bird species with less adaptable feeding habits than weka. The combination of blue and pyrazine deserves more field testing. While it seems unlikely that all the individuals in a given population will be deterred by any particular combination of cues this study has shown that there may be better combinations than the one currently in general use in New Zealand.

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