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SHORT COMMUNICATION

# Assessing kea perception of cereal baits using modelling of spectral reflectance

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**Abstract:** Kea (*Nestor notabilis*) are highly inquisitive parrots endemic to Aotearoa/New Zealand that often interact with novel items in their environment. To help reduce the risk of by-kill of kea during aerial 1080 pest-control operations, we investigated how kea perceive the different types of cereal baits typically used in such pest control. We measured the spectral reflectance of a range of baits including baits that incorporated different levels of a bird-repellent (anthraquinone) or a UV-reflecting biomarker (pyranine, used in conservation to determine whether a bird has interacted with bait), non-toxic prefeed baits, and green-dyed, toxic bait. In the absence of information about kea vision, we constructed a model of parrot vision by averaging spectral sensitivities of three parrot species. We found that kea are unlikely to visually distinguish between two baits that contain different concentrations of bird-repellent when they are dyed similarly (both green-dyed or both undyed). Additionally, kea are likely to visually distinguish between green-dyed and undyed baits, and baits with or without the biomarker pyranine. Our findings support the addition of bird repellent to pre-feed baits intended to establish learned avoidance behaviour. However, the addition of pyranine might inadvertently influence kea perception and interactions with bait. This research highlights the importance of considering the visual perception of a vulnerable non-target species to reduce risk during pest control efforts.

**Keywords:** 1080, anthraquinone, colour discrimination, colour vision, kea, spectrophotometry, visual ecology

## Introduction

New Zealand has recently embraced the ambitious goal of eradicating mammalian predator populations of brushtail possums (*Trichosurus vulpecula*) and ship rats (*Rattus rattus*) by 2050 (Russell et al. 2015). This programme aims to protect native biodiversity and reduce the incidence of bovine tuberculosis in livestock. One of the most effective tools for achieving this goal is to eliminate or reduce predator populations over large spatial extents using aerial baiting with sodium fluoroacetate, 1080 toxicant (Spurr & Anderson 2004).

However, predators can develop an aversion to toxic bait as 1080 is a fast-acting toxin and quickly causes loss of appetite and illness (Henderson et al. 1999). Animals may also survive 1080 baiting if they consume a sublethal dose (Ross et al. 2000; Ogilvie et al. 2000). Baiting programmes, therefore, aim to ensure baits contain lethal doses of 1080 for the target species, that baits do not fragment during sowing, and that the level of bait density ensures high encounter rates (Nugent et al. 2011. In addition, previous exposure to non-toxic pre-feed baits prior to toxic baits increases consumption of toxic bait. Pre-feeding non-toxic baits allows neophobic predators to sample baits with no-adverse effects, and increases the chance of predators consuming a lethal dose when exposed to toxic bait (Nugent et al. 2011).

Removal of predators through aerial poisoning operations benefits native species, although there is some risk of by-kill. For instance, kea (*Nestor notabilis*) are highly intelligent parrots that are omnivorous and feed primarily on the forest floor (Diamond & Bond 1999; Greer et al. 2015). Kea readily investigate novel food objects (Kemp et al. 2019) and thus may be at risk of direct poisoning by feeding on 1080 baits during aerial operations, particularly if they have learnt that the non-toxic prefeed bait is an acceptable food source (Orr-Walker & Roberts 2009). Where some kea are killed during 1080 poisoning operations the subsequent increased productivity of the local kea population generally outweighs any losses because of reduced predation by possums and rodents (Kemp et al. 2018). However, by-kill of kea is a concern as they are a long-lived species that suffer from many threats (for instance, climate change, lead poisoning and vehicle strike; McLelland et al. 2010; Tennyson et al. 2014; Kennedy 2017). Kea are currently classified as endangered by the IUCN (c. 4000 mature individuals; BirdLife International 2017) and Nationally endangered by the NZTCS (Robertson et al. 2016).

One strategy for reducing consumption of toxic cereal bait by wild kea is to add a bird repellent such as anthraquinone to non-toxic prefeed baits. If kea are exposed to non-toxic repellent baits prior to exposure to toxic baits, they may develop aversion. Typically, these baits otherwise resemble (at least to the human eye) the green-dyed appearance, texture, and orange or cinnamon scent (used to mask the odour and taste of 1080) of 1080 baits (Cowan et al. 2016). Anthraquinone is a secondary repellent for birds by causing some gastro-intestinal

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irritation on consumption. This promotes learned avoidance in birds, based on the sensory cues (colour, texture and smell) associated with the bait. Crucially, for birds, colour (including UV) tends to be a more valid cue for avoidance than taste or odour (Werner et al. 2008; Werner et al. 2014); although scent may play a greater role for discrimination in birds than previously thought (Rossi et al. 2017). In experiments with domestic chicken (*Gallus gallus domesticus*) responses to aposematic prey, colour is more important than pattern or shape for learned avoidance (Aronsson & Gamberale-Stille 2008). Furthermore, some colours (e.g. red) may be more effective cues than others (Svádová et al. 2009), and parrots exhibit a preference for bi-coloured fruit (i.e. fruit that contrasts against its background; Boyes & Perrin 2010). We note that the conclusions of these studies should be applied to kea with caution, as kea evolved in an environment without aposematic prey and are not closely related to the animals studied in these examples. However, kea do exhibit preferences for certain colours (Weser & Ross 2013), which suggests colour is a good candidate for aversion cues in kea. Other potential bait additives include non-toxic biomarkers such as pyranine, which reflects in the UV spectrum (Barrash-Shiftan et al. 1998). When kea interact with bait containing pyranine, their feet, bill and faeces will fluoresce bright green under a blacklight (Fairweather et al. 2014), so it is a useful tool for measuring potential bait uptake.

Colour perception depends on the visual system of the animal, and their photoreceptor sensitivities (Kelber 2016; Renoult et al. 2017). Parrots such as kea are tetrachromats, i.e. the retina contains four classes of single cones, each with a different visual pigment typically peaking in the UV, blue, green and red wavelengths (Hart & Vorobyev 2005). This system is different from human vision, which is trichromatic, with only blue, green and red photoreceptors (Rowe 2002). Pest mammals targeted by 1080 baits, such as possums, rats, stoats and mice are dichromats with visual spectrums focussed on blue and green wavelengths (Jacobs 2009; Ebeling et al. 2010). Hence, it is likely parrots such as kea, but not humans or possums, can see in the UV spectrum (Oglivie et al. 2006; Carvalho et al. 2011; Aidala et al. 2012). Target rodents, such as mice and rats, however, can likely see under UV-illumination (Leinonen & Tanila 2018).

Depending on the wavelengths reflected by baits, kea, humans and pest mammals may perceive them quite differently. Pyranine biomarker, for instance, reflects in the UV spectrum (Barrash-Shiftan et al. 1998). Using UV-reflective additives may be useful for encouraging deterrence in birds (Ballinger & Werner 2016). Blackbirds and woodpeckers, for example, may be able to learn to avoid UV-reflective food as they can be trained with UV cues (Werner et al. 2012; O'Daniels et al. 2017).

If the appearance of toxic bait is sufficiently different to non-toxic pre-feed bait, this may pose a problem for 1080 drops, as kea readily interact with novel food and items. Hence, it is important to know whether any visual differences are detectable to kea. Here, as a first step to determine this, we analysed the spectral reflectance of two non-toxic baits (with and without green dye) with no additives or combinations of three additives. We used a visual model based on parrot spectral sensitivities to determine whether kea might discriminate visually between different types of non-toxic cereal baits. Baits containing 1080 were not tested in our study, as we relied on baits used for behavioural experiments with kea.

As few studies characterise avian retinal properties, we have used data from the only studies (to date) that quantify the specific spectral sensitivities of birds from the order of long-lived parrots (Psittaciformes) to create an 'average parrot vision' as a proxy for kea, compared to other orders that have different spectral sensitivities (Table 1; Hart 2001). Species used were the crimson rosella (*Platycerus elegans*; Knott et al. 2013); the amazon parrot (*Amazona ochrocephala panamensis*; Tinbergen et al. 2013), and budgerigar (*Melopsittacus undulates*; Goldsmith & Butler 2003). These species and kea have Cys90 genes that confer UV sensitivity (Carvalho et al. 2011), and their diets are mainly seeds and fruit (Long 1984).

Human vision is not a suitable proxy for bird vision (Eaton 2005; Håstad & Ödeen 2008). Among birds, photoreceptors and spectral sensitivities are phylogenetically conserved (Hart 2001). Therefore, a proxy that is more closely related to kea than humans is useful for modelling their vision. Nevertheless, the lack of spectral sensitivity data or retinal characterisation for birds in the family to which kea belong (Nestoridae) means that our findings should be applied with caution (Bitton et al. 2017).

**Table 1.** Reported and median spectral sensitivities across several bird orders selected from Hart (2001) and reported and median spectral sensitivities across the order of Psittaciformes - the crimson rosella (*Platycerus elegans*; Knott et al. 2013); the Amazon parrot (*Amazona ochrocephala panamensis*, Tinbergen et al. 2013), and budgerigar (*Melopsittacus undulates*; Goldsmith & Butler 2003).

| Species (order) | UVS/VS | SWS | MWS | LWS |
|---|---|---|---|---|
| *Anas platyrhynchos* (Anseriformes) | 415 | 452 | 506 | 567 |
| *Spheniscus humboldti* (Ciconiiformes) | 403 | 450 | | 543 |
| *Coturnix coturnix japonica* (Galliformes) | 418 | 450 | 505 | 567 |
| *Chloebia gouldiae* (Passeriformes) | 371 | 440 | 500 | 563 |
| *Strix aluco* (Strigiformes) | | 463 | 503 | 555 |
| *Struthio camelus* (Struthioniformes) | 405 | 444 | 505 | 570 |
| *Nothoprocta perdicaria sanborni* (Tinamiformes) | - | - | 566 | - |
| Median (across all reported orders): | 415 | 452 | 506 | 567 |
| *Amazona ochrocephala panamensis* (Psittaciformes) | 371 | 440 | 499 | 566 |
| *Platycerus elegans* (Psittaciformes) | 365 | 440 | 509 | 567 |
| *Melopsittacus undulates* (Psittaciformes) | 370 | 445 | 508 | 565 |
| Median (across Psittaciformes): | 370 | 440 | 499 | 566 |

## Methods

### Bait manufacture

All cereal bait types used in the study were made by Orillion Ltd (Wanganui, New Zealand). The bait matrices (Wanganui #7 and RS5, with and without anthraquinone, with and without pyranine, with and without cinnamon and orange scent, with and without green dye) are representative of baits used in standard aerial 1080 poisoning operations against possums and rodents.

We tested ten different bait types that had been used in kea bait response behaviour trials (Nichols et al. 2020). Samples of each bait type were closely inspected for consistency in texture and colour and five different samples of each type (from one 10-kg bag per bait type) were assayed at Manaaki Whenua Landcare Research (Lincoln, New Zealand) for quality control and to confirm the concentration of anthraquinone. Anthraquinone sometimes dissipates during the process of bait manufacture (Cowan et al. 2016).

### Spectral measurements

We measured the spectral reflectance of each of the ten different bait types ($n = 12$ per bait type, each pellet of roughly uniform size and shape; Fig. 1) using a fibre optics spectrometer (Ocean Optics ST2000). As a white light reference, we used a WS-1 reflectance standard. The probe was 5 mm in diameter.

We also measured the spectra of five natural backgrounds matching those where operational baiting in kea areas is likely to occur (Fig. 2). These included red tussock ("tussock", *Chionochloa rubra*), tall fescue grass ("grass", *Festuca arundinacea*) , greywacke rock ("rock"), fresh kāmahi leaves ("leaves", *Weinmannia racemosa*) and brown soil ("dirt"). We measured the spectral reflectances of bait and backgrounds in a dark room.

*Spectral analysis: are samples perceptually different?*

We assessed whether bait colours were discernible against each background, and whether bait colours were discernible against each other (while against varying backgrounds), using the receptor noise-limited vision model (Vorobyev & Osorio 1998). This model estimates the chromatic contrast between a given pair of spectra based on a weber fraction (a measure of signal to noise ratio). We present contrasts as just noticeable differences (JNDs), where a value of 1 implies that differences can be observed by the modelled observer when objects are stationary under bright conditions. In more natural

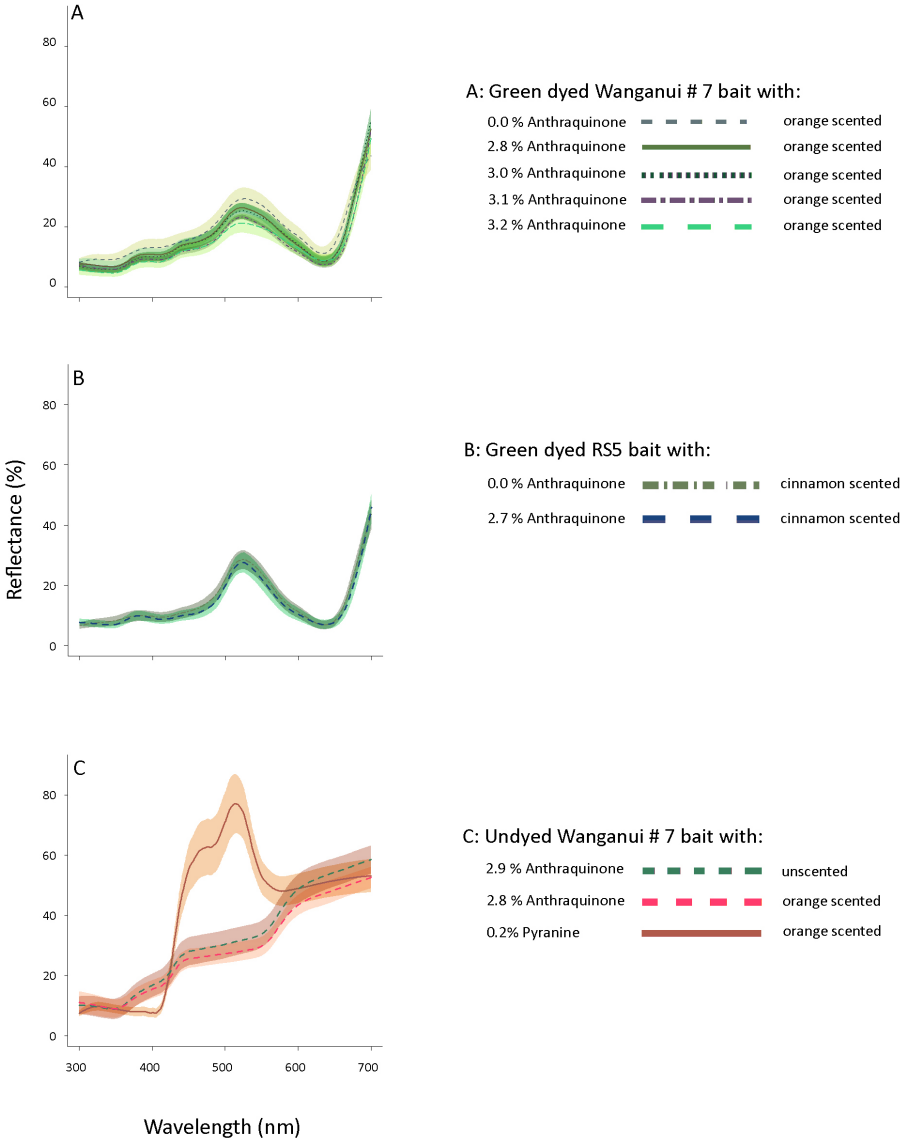


**Figure 1.** Spectral measurements of green-dyed Wanganui baits (top left); green-dyed RS5 baits (middle left) and undyed Wanganui baits (bottom left) with varying levels of anthraquinone and pyranine. Baits are scented either with cinnamon or orange. Shaded area indicates the standard deviation of the mean spectral reflectance for all measured bait.

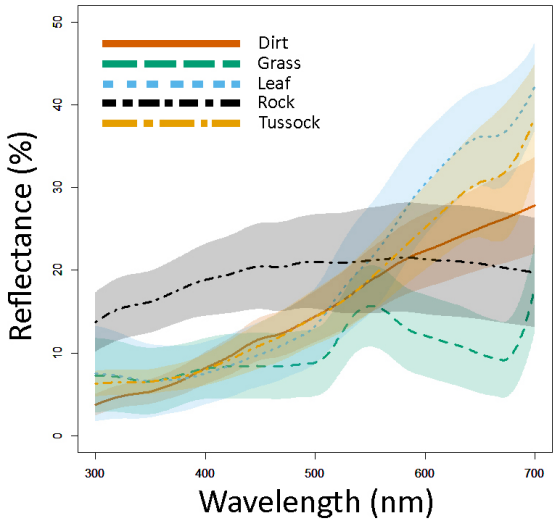


**Figure 2.** Spectral measurements of backgrounds Kea are likely to encounter: red tussock ("tussock", Chionochloa rubra), tall fescue grass ("grass", Festuca arundinacea), greywacke rock ("rock"), fresh kāmahi leaves ("leaves", *Weinmannia racemosa*) and brown soil ("dirt"). Shaded area indicates standard deviation of mean spectral reflectance for each background.

conditions, however, any objects that contrast by less than 3 JNDs are unlikely to be able to be visually distinguished from one another (Thurman & Seymoure 2016).

We calculated chromatic differences and generated the quantum catches of all reflectance measures with the von Kries transformation (Vorobyev & Osorio 1998) to allow background colour to inform perceptibility. We constructed a visual system for an 'average parrot' by averaging the spectral sensitivities of the amazon parrot (*Amazona ochrocephala panamensis* spectral sensitivities: 371, 440, 499, 566; Tinbergen et al. 2013), crimson rosella (*Platycerus elegans*, spectral sensitivities: 365, 440, 509, 567; Knott et al. 2013) and budgerigar (*Melopsittacus undulates*, spectral sensitivities: 370, 445, 508, 565; Goldsmith & Butler 2003). This yielded a visual model with spectral sensitivities: 368.7, 441.7,505.3 and 566. We used cone abundances from the crimson rosella (1, 1.24, 3.96, 4.18) and used neural noise, rather than quantum noise. We used a Weber fraction of 0.1. We implemented this in R 3.5.3 (R Core Team 2018) using the PAVO package (Maia et al. 2018).

We calculated all possible pairwise JNDs between spectra (7200 contrasts for each background). To determine if JNDs were greater than 1 (and therefore perceptibly different) we used a bootstrap procedure to generate geometric means and 95% confidence intervals (Maia & White 2018).

*Spectral analysis: are samples statistically different?*

To investigate whether bait colours differed statistically from each other, and from background samples, we used the PERMANOVA procedure (Maia & White 2018). This procedure involves creating a colour distance matrix in Mahalanobis space between samples (using the chromatic contrasts discussed above), and then analysing with PERMANOVAs to determine if samples are statistically different. We used the adonis function in the vegan package in R (Oskansen et al. 2019), followed by ANOVA to determine differences between groups using F values. A multivariate analogue of Levene's test for homogeneity of variance (betadisper function in vegan package) indicated that our data met the assumption of homogeneity of variance ($p$ value > 0.05 for all backgrounds), so no transformation was required.

Samples were considered distinct and likely to be discriminable if the results from the PERMANOVA were statistically significant, and if the bootstrapped confidence intervals did not include the threshold for discrimination (JND = 1).

## Results

Colour contrast differences between the backgrounds and the baits were statistically different for all combinations ($p < 0.05$), except for undyed baits which did not contrast significantly against tussock, dirt and leaf (F = 10.3, 10.2 and 11.4 respectively, relative to mean F value of 94.0). These findings corresponded with the likely perceptible differences to a parrot (Fig. 3): all JNDs were greater than 1 for all combinations except undyed bait vs tussock, and undyed bait vs dirt comparisons.

JNDs for all bait-bait comparisons were statistically different ($p = 0.01$ for all comparisons). This outcome largely aligned with measured perceptible differences; many baits were detectably different (Fig. 4; see Appendix S1 in Supplementary Materials for imperceptible differences). The background against which the bait-bait comparison was made did not impact discernibility. Only the contrasts of dyed and undyed baits against baits with pyranine had JNDs greater than 3.

## Discussion

To our knowledge, this study is the first to model the appearance of different predator control baits from a non-human perspective.

### Comparison of baits to background

Kea are likely to be able to discern all baits (dyed and undyed with varying levels of anthraquinone or pyranine) from the backgrounds we tested here, except for undyed baits on a tussock or dirt background. Hence, on some substrates, kea interactions with undyed prefeed baits may be less than on other substrates. Fewer interactions with undyed prefeed baits may reduce the efficacy of training kea to avoid these baits in all environments.

Green dye is routinely used in toxic bait for its supposed camouflage effect and green is the colour least preferred by kea in choice tests (Weser & Ross 2013; Cowan & Crowell 2017). Here, we report that green-dyed baits were only just discernible against all backgrounds (JNDs confidence interval < 4). Green dying baits is therefore likely to have some camouflage effect, as the JND values are low, and baits are probably only detectable to a parrot if they are in bright light (Thurman & Seymoure 2016). Hence, kea may not interact with the bait once it is dyed green, depending on light intensity in their environment. In addition, bait laced with pyranine is likely to be quite discernible against any background to kea in nature (all JNDs confidence interval > 4; $p < 0.05$). Therefore, if increased discernibility does increase interaction, pyranine should never be added to toxic baits, unless it is strongly justified. Bait laced with pyranine is likely quite discernible against any background to kea in nature (all JNDs confidence interval > 4; $p$ value <0.05). Choice tests that explore kea

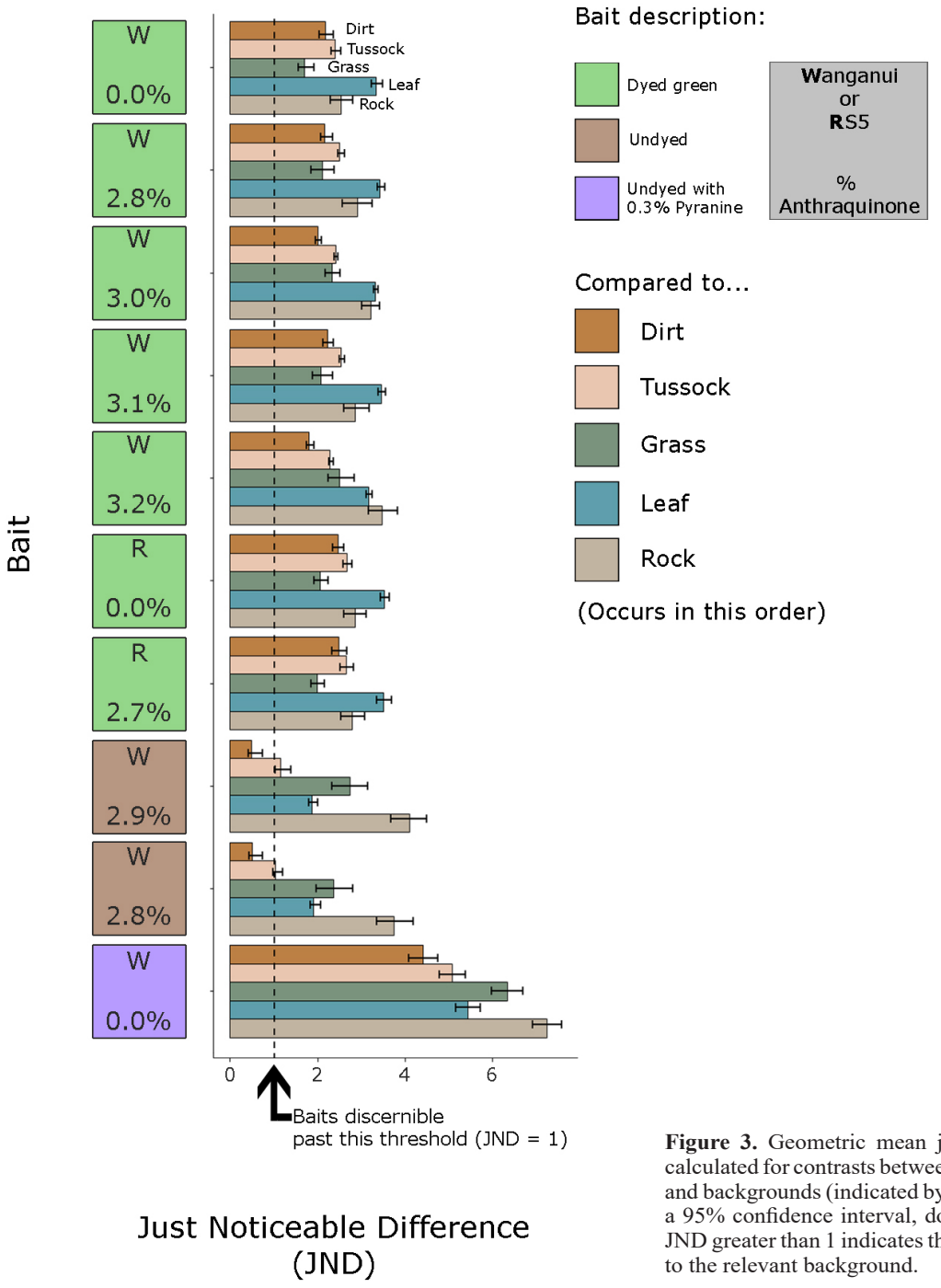


**Figure 3.** Geometric mean just noticeable differences (JND) calculated for contrasts between bait (indicated with key on right) and backgrounds (indicated by colour of bar). Error bars indicate a 95% confidence interval, dotted line indicates a JND of 1. A JND greater than 1 indicates that the bait is perceptually different to the relevant background.

behaviour when presented with green-dyed (non-toxic) baits or pyranine at varying light intensities could help discern whether kea do, in reality, detect these baits or interact more with detectable bait.

### Comparison of baits with different levels of Anthraquinone

Importantly, kea appear unlikely to to visually distinguish between green-dyed cereal bait with or without repellent/anthraquinone (JNDs confidence intervals < 1; $p > 0.05$). Anthraquinone reduces kea consumption of cereal baits (Nichols et al. 2020), and consumption of rodenticide bait and crops in other birds (Mastrota & Mench 1995; Werner et al. 2011; Carlson et al. 2013). Because kea are unlikely to discriminate between cereal baits with or without anthraquinone, it appears to be a useful tool for future aversion training.

### Comparison of green-dyed and undyed bait

Kea likely can visually distinguish between undyed vs. green-dyed bait, in well-lit environments (1 < JNDs confidence interval < 3, $p$ value = 0.01). This result is in line with behavioural studies that indicate kea have colour preferences for food (yellow most preferred, green least preferred; Weser & Ross 2013). In nature, it may be difficult for kea to discern between undyed and green-dyed bait (we report JNDs < 3, below the threshold for discernability in nature; Thurman & Seymoure 2016).

Colour is an important factor for learned avoidance in

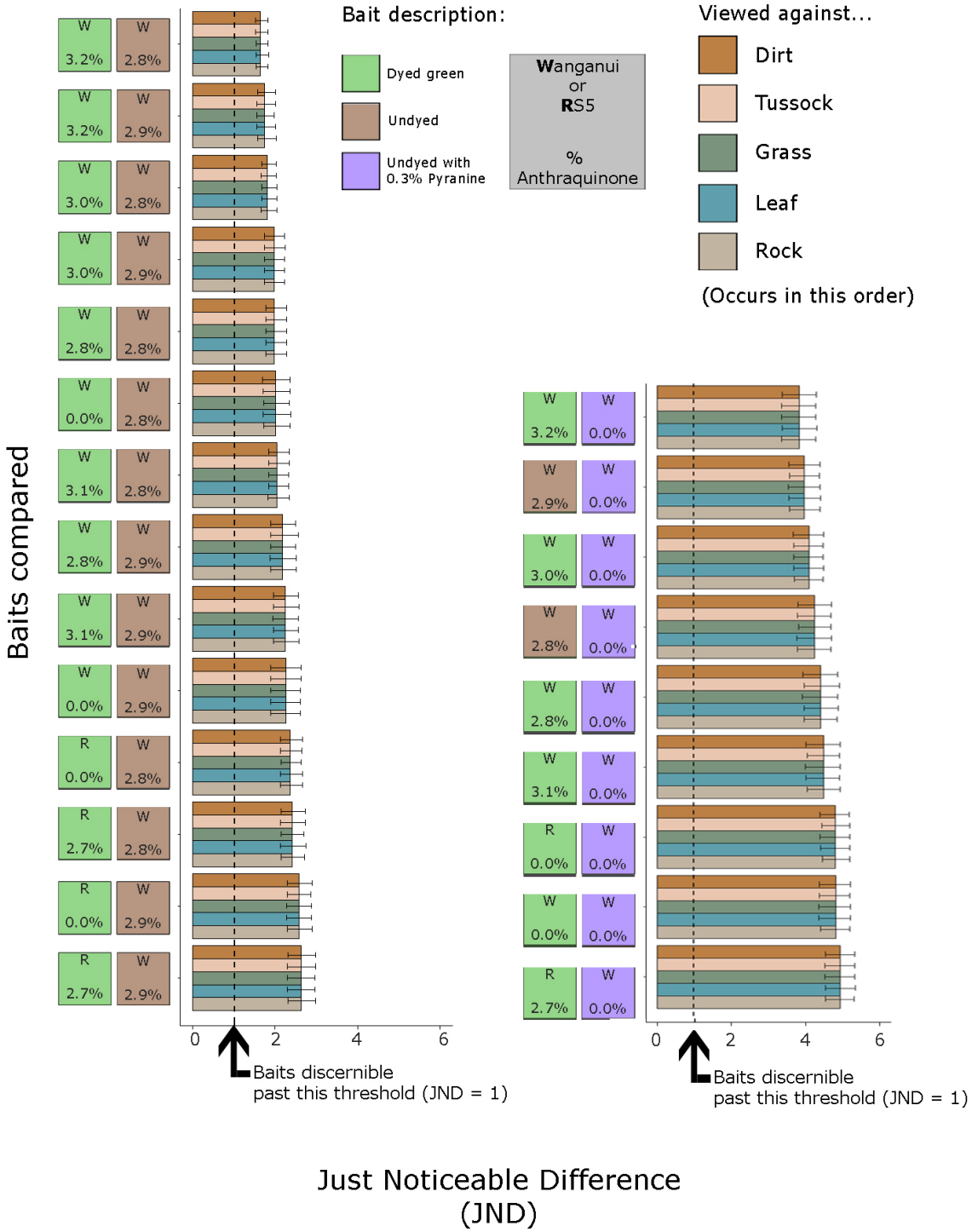


**Figure 4.** Geometric mean just noticeable differences (JND) calculated for contrasts between two baits (indicated with key) viewed against different backgrounds (indicated by colour of bar). Error bars indicate a 95% confidence interval, dotted line indicates a JND of 1. We show only those comparisons that were discernible (i.e. lower bound for confidence interval was above 1).

birds (Werner et al. 2008; Aronsson & Gamberale-Stille 2008; Svádová et al. 2009). Toxic baits are typically dyed green but non-toxic baits are not (Cowan & Crowell 2017). If undyed, non-toxic prefeed baits are used in an attempt to teach kea aversion, and these are then followed by sowing green-dyed toxic baits, these may look visually novel to kea depending on light levels in nature (as suggested by our findings, JNDs > 1). This approach may result in the opposite of the desired management outcome, with kea interacting even more with the green-dyed toxic bait because they exhibit opportunistic feeding behaviours, learn to investigate food, and have a highly inquisitive nature (Diamond & Bond 1999; Huber & Gajdon 2006; Young et al. 2012; Kemp et al. 2019). If the aim of sowing repellent-laced prefeed baits is to teach kea learned avoidance, they must resemble toxic baits as closely as possible, including the colour, texture and smell.

### Comparison of pyranine-laced baits

Kea are likely able to detect the UV-reflectance of pyranine. All comparisons with pyranine, including against the backgrounds, had JNDs > 3, i.e. the threshold for detectability in nature (Thurman & Seymoure 2016). Our PERMANOVA suggested these differences were also statistically different ($p = 0.01$).

A detectable change in the colour of bait through the presence/absence of pyranine could possibly create a novelty effect, with consequent increases in interactions between kea and bait. A small sample of wild kea in the Perth River valley, South Westland, had higher than expected interaction rates with non-toxic cereal baits laced with pyranine (Nichols et al. 2020). Further research with behavioural trials that directly compare kea responses to various combinations of bait colour and additives would clarify the extent to which colour perception drives novelty responses in kea, and would be valuable knowledge for future aversion training.

### Conclusions and recommendations

Our model results are based on an 'average' parrot vision, so some caution is needed when extrapolated to kea. As this study only investigates non-toxic bait, future work should determine whether the addition of 1080 itself might also change visual perception of bait by kea. Our results suggest that anthraquinone likely does not impact on the visual discernibility of baits. However, our model demonstrates that there are detectable differences between green-dyed and undyed baits, though small. This highlights potential problems with the current practice of using undyed pre-feed and green toxic baits. The current Code of Practice for use of 1080 bait in kea habitat is to use RS5 baits with a cinnamon lure, and that all toxic baits must be dyed green (DOC, Threats Manager Southern 2020). Hence, it is imperative that future research determine whether kea exhibit a novelty reaction to green-dyed non-toxic bait, after being pre-fed un-dyed non-toxic bait. Importantly, we highlight that pyranine (or other UV-reflective additives) should never be used in toxic baits as this is visually discernible to kea and may promote interaction with it as a novel food.

When dealing with species that are innately curious, it is important to consider how they view their environment. As New Zealand increases its efforts to become predator-free, we must consider how our native birds, rather than humans, perceive the tools we are using as this could influence how they interact with them. This is a particularly important consideration as many predator control operations occur in the habitats of vulnerable non-target bird species.

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## Supplementary material

Additional supporting information may be found in the supplementary material file for this article:

**Appendix S1.** Geometric mean just noticeable differences (JND) calculated for contrasts between two baits (described with key on right) against different backgrounds (indicated by colour of bar).

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