

# Bait colour and moisture do not affect bait acceptance by introduced Pacific rats (*Rattus exulans*) at Henderson Island, Pitcairn Islands

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**Abstract** Rodent eradications are a useful tool for the restoration of native biodiversity on islands, but occasionally these operations incur non-target mortality. Changes in cereal bait colour could potentially mitigate these impacts but must not compromise the eradication operation. Changing bait colour may reduce mortality of Henderson crakes (*Zapornia atra*), an endemic globally threatened flightless bird on Henderson Island, Pitcairn Islands, South Pacific Ocean. Crakes had high non-target mortality in a failed 2011 rat eradication operation and consumed fewer blue than green cereal pellets. We examined which cereal bait properties influenced its acceptance by captive Pacific rats (*Rattus exulans*) on Henderson Island. We held 82 Pacific rats from Henderson Island in captivity and provided them with non-toxic cereal bait pellets of varying properties (blue or green, moist or dry). We estimated the proportion of rats consuming bait using logistic generalised linear mixed models. We found no effect of sex, females' reproductive status, bait colour or bait moisture on rats' willingness to consume baits. Rats' bait consumption was unaffected by cereal bait properties (colour or moisture). The use of blue bait is unlikely to affect future eradication operational success but may reduce non-target mortality of Henderson crakes. Timing cereal bait distribution in relation to precipitation may also reduce crake mortality without compromising palatability to rats.

**Keywords:** baits, Henderson Island, island restoration, non-target safety, rat eradication

## INTRODUCTION

The eradication of introduced rodents is a common conservation intervention, especially on islands, and has been accomplished on > 580 islands worldwide (DIISE, 2016), with benefits to native biodiversity (Lavers, et al., 2010; Buxton, et al., 2014; Jones, et al., 2016). In some cases, eradication operations may result in non-target mortality, and mitigation can include housing captive populations of species likely to be affected, or by using cereal pellets that are less palatable to non-target species (Empson & Miskelly, 1999; Hoare & Hare, 2006; Pitt, et al., 2015; Oppel, et al., 2016a; Oppel, et al., 2016b). In such cases, it is crucial that the mitigation measures do not decrease the likelihood of a successful rodent eradication operation, and that the rodents are exposed to a sufficient quantity of cereal bait, are susceptible to the rodenticide used, and will consume a sufficient dose.

Rarely, though more so in the tropics and subtropics, these eradication operations fail to remove rodents for a variety of operational, biological, and environmental reasons (Holmes, et al., 2015). Eradication success in the tropics is generally lower than in temperate systems because there is less seasonal change in the environment, and consequently a less predictable period of food-resource limitation, which is the ideal time for an eradication operation (Holmes, et al., 2015; Russell & Holmes, 2015). Consequently, understanding which factors may influence rodents' acquisition of a lethal dose of bait are crucial for improving the probability of success in tropical systems (Lamoreux, et al., 2006).

Factors affecting bait acceptance by Pacific rats (*Rattus exulans*), a common introduced rodent in the Pacific Ocean tropics and subtropics (Atkinson, 1985; Varnham, 2010; Keitt, et al., 2015), are poorly known. A number of factors can influence rat food choice, including physical characteristics such as bait colour and hardness (Booth, et al., 1974; Clapperton, 2006; Hegab, et al., 2014). Murine rodents (including rats and mice) have colour vision, including sensitivity in the UV range (Jacobs, 1993; Jacobs, 2009), and there is evidence that cereal bait colour

does affect the likelihood of acceptance by rats (Hegab, et al., 2014).

Blue or green cereal pellets are the most effective at reducing avian non-target mortalities, but there is considerable variation in bait attraction among species. Kea (*Nestor notabilis*) and weka (*Gallirallus australis*) were less likely to eat green pellets than blue (Hartley, et al., 2000; Weser & Ross, 2013), whereas North Island robins (*Petroica longipes*) and Henderson crakes (*Zapornia atra*) were less likely to consume blue pellets than green (Hartley, et al., 1999; Oppel, et al., 2016b). Henderson Island, part of the Pitcairn Islands in the South Pacific Ocean, was the site of a failed eradication operation for Pacific rat in 2011, which also resulted in non-target mortality of Henderson crakes (Amos, et al., 2016; Oppel, et al., 2016a). Subsequent work found that Henderson crakes consumed less blue bait than green, and did not consume dry pellets (Oppel, et al., 2016b).

Here we report on the factors affecting Pacific rat bait acceptance on Henderson Island, Pitcairn Islands, South Pacific Ocean. Our goal was to compare Pacific rats' acceptance of both moist and dry cereal pellets of these two colours to determine whether measures to reduce the non-target mortality of Henderson crakes might affect success of future eradication operations on Henderson.

## METHODS

### Rat capture & acclimatisation

Rats were captured on Henderson Island's plateau using either Tomahawk (27 × 16 × 13 cm, Metal Rat Cage Trap, Key Industries, Auckland, New Zealand) or Sherman (22.9 × 8.9 × 7.6 cm, Sherman Traps Inc., Tallahassee, Florida, USA) live traps baited with a 2 × 2 cm piece of coconut (*Cocos nucifera*). Individual rats' knowledge of coconut prior to its presentation during the cage trial is presumed to be limited, because the areas where rats were captured were > 500 m away from the nearest coconut grove on

the island. We chose coconut as bait because it was easily available and highly effective in attracting rats, while alternative baits (*Pandanus tectorius* fruit, peanut butter, chocolate, semolina, soap, and mixtures thereof) largely failed to capture sufficient individuals. Although the choice of coconut as trap bait may have predisposed some trapped rats to exhibit universal acceptance of coconut in the trials (see Results), particularly given the large movements possible in this population (Oppel, et al., 2019), the use of a non-natural food source (e.g., peanut butter, chocolate) may have resulted in capturing only curious or bold rats who will readily accept new food items, which may have biased our assessment of acceptance rate of bait pellets, another novel food item (Booth, et al., 1974).

We held 81 rats in captivity for 12 days each during October–November 2015. Rats were weighed using a spring balance to the nearest 1 g, fitted with a uniquely numbered ear tag, and their sex was determined from external anatomy. They were first allowed to acclimatise for four days in sex-specific communal wire cages (70 × 60 × 30 cm) of up to four individuals, where they were fed commercial rodent food (Rabbit and Guinea Pig Muesli, Topflite, Oamaru, New Zealand) *ad libitum* in a single ceramic food dish and water was provided by both a commercial water dispenser (Critter Canteen, SuperPet, Walnut Creek, California, USA) and a large clamshell. Each cage contained four hollow *Pandanus tectorius* logs, with small, loose pieces of coconut bark providing cover and visual barriers. After four days, rats were weighed as before, and moved to individual wire cages (70 × 50 × 30 cm) with the same food and water regime, and environmental enrichment. Rats were considered to have acclimated if, after three days in individual cages, their mass differed by < 10% compared to their mass at capture, and we observed no anomalous behaviour. Rats that had lost > 10% of body mass were allowed two additional days to acclimate and were then reassessed using the same criteria. Any individuals that did not acclimate were not subjected to the experimental trial and were euthanised by cervical dislocation and used in other research (Lavers, et al., 2016).

### Bait acceptance trial

Individuals which acclimated were assigned randomly to one of four treatment groups based on combinations of bait colour (green or blue) and moisture (moist or dry); cereal bait pellets (nontoxic ~2 g Pestoff 20R, Animal Control Products, Whanganui, New Zealand) were otherwise identical. The pellets were surface coated with dye and did not lose their colour after soaking in water. The green dye was a composite of tartrazine powder (with the Chemical Abstracts Service (CAS) number 1934-21-0), Brilliant Blue powder (CAS number 3844-45-9) and sodium sulphate (CAS number 7757-82-6), and the blue dye was Hexacol Indigo Carmine Supra Blue R2613 (CAS number 860-22-0). Cereal pellets were presented to rats either dry, as manufactured, or moist, after being soaked in water for three hours. Rats received fresh cereal bait pellets and natural foods daily.

Between 06:00-08:00 (UTC-8) on Day 1 of the trial, rats were provided with sufficient natural food (coconut, *Myrsine hosakae* seeds, and whole *Pandanus tectorius* fruit) to ensure that they were sated but not consuming any single food item completely, and a single cereal bait pellet. Food consumption was assessed between 06:00-08:00 on Day 2: if the rat had consumed a significant portion of a pellet (~1 g), the individual's trial was ended and the rat was euthanised. If the bait was not eaten, the rat received a fresh batch of natural food of the same amount as on Day 1 while the number of pellets was increased to five in an attempt to overcome neophobia and increase the number

of trial rats consuming bait. This procedure was continued for two more days if needed, with rats receiving 10 and 20 pellets on Days 3 and 4, respectively. Rats that had not eaten bait after Day 4 were given five bait pellets and all natural foods, with the exception of coconut (which was accepted universally), on experimental Day 5. The trial ended the next day regardless of outcome.

Food consumption was monitored daily and all remaining food from the previous night removed and the cage inspected to ensure no natural food item was completely consumed, and any remaining bait pellets counted. The remains of any partially eaten pellets were inspected and the amount eaten estimated to the nearest 25%. Where a natural food item was completely consumed, the result was ignored and the test repeated with the same individual.

All captive rats were humanely euthanised by cervical dislocation at the end of the trial. Females were examined internally to determine reproductive status: breeding was indicated by the presence of foetal pups, a highly vascularised uterus, or lactation.

### Statistical analysis

We used logistic generalised linear mixed-effects models (GLMMs) with a logit link to test whether bait acceptance (yes/no) varied as a function of the following fixed factors: sex (female/male), bait colour (blue/green), and bait moisture (moist/dry), and trial day. We treated 'individual' as a random effect to account for potential serial autocorrelation (Bolker, et al., 2009). We included main effects only, as the effective sample size would reduce the statistical power to detect the effect of interactions in our dataset. We constructed a series of models with varying biologically meaningful combinations of the terms above, as well as an intercept-only model (Table 1) and evaluated

**Table 1** The ranked set of candidate models for examining captive Pacific rats' acceptance of bait on Henderson Island. Models with  $\Delta AIC_c < 2$  were considered competitive (i.e. the top 3).

Model	k	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	w <sub>i</sub>
Intercept only	2	214.80	0.00	0.33
Moisture	3	215.89	1.10	0.19
Sex	3	216.62	1.82	0.13
Colour	3	216.85	2.05	0.12
Sex + Moisture	4	217.72	2.92	0.08
Moisture + Colour	4	217.96	3.16	0.07
Sex + Colour	4	218.68	3.89	0.05
Day	5	219.15	4.35	0.03
Sex + Colour + Moisture	5	219.80	5.00	0.03
Day + Moisture	6	220.54	5.75	0.02
Day + Sex	6	221.11	6.31	0.01
Day + Colour	6	221.30	6.50	0.01
Day + Sex + Moisture	7	222.49	7.69	0.01
Day + Moisture + Colour	7	222.66	7.87	0.01
Day + Sex + Colour	7	223.23	8.43	<0.01
Day + Sex + Colour+Moisture	8	224.62	9.82	<0.01

k: number of parameters, AIC<sub>c</sub>: Akaike's Information Criterion adjusted for small sample size, ΔAIC<sub>c</sub>: difference between each model and the most parsimonious model, w<sub>i</sub>: Akaike model weight.

them in a multi-model selection framework using Akaike's Information Criterion adjusted for small sample size (AIC<sub>c</sub>) (Burnham & Anderson 2002). Models with  $\Delta\text{AIC}_c < 2$  were considered competitive. All models were fit using Laplace approximation in the package lme4 (Bates, et al., 2014) in R 3.3.0 (R Core Team, 2017), and we present mean parameter estimates ( $\beta$ )  $\pm$  standard error.

## RESULTS

We captured 82 rats of which 81 acclimated to the captive trial. Overall, 48% of captive rats ( $n = 39$ ) consumed the non-toxic cereal pellets. The intercept-only model, where bait consumption varied among individuals, but not with any other factors, received the most support, but models that included the single terms for sex, bait colour, and bait moisture had  $\Delta\text{AIC}_c < 2.0$  (Table 1). Using each of these single-factor models, there was no difference in bait acceptance between sexes (males:  $\beta = 0.532 \pm 0.012$ , females:  $\beta = 0.536 \pm 0.014$ ), and no effect of bait colour (blue:  $\beta = 0.533 \pm 0.018$ , green:  $\beta = 0.534 \pm 0.018$ ), or moisture (dry:  $\beta = 0.540 \pm 0.015$ , moist:  $\beta = 0.529 \pm 0.012$ ; Fig. 1). Bait acceptance did not differ with females' reproductive status (calculated parameter estimates for breeding:  $\beta = 0.539 \pm 0.033$ ,  $n = 17$ ; not breeding:  $\beta = 0.543 \pm 0.025$ ,  $n = 22$ ; one individual not of breeding age:  $\beta = 0.520$ ). All models that included trial day had  $\Delta\text{AIC}_c > 4$ , so were not considered further (Table 1).

## DISCUSSION

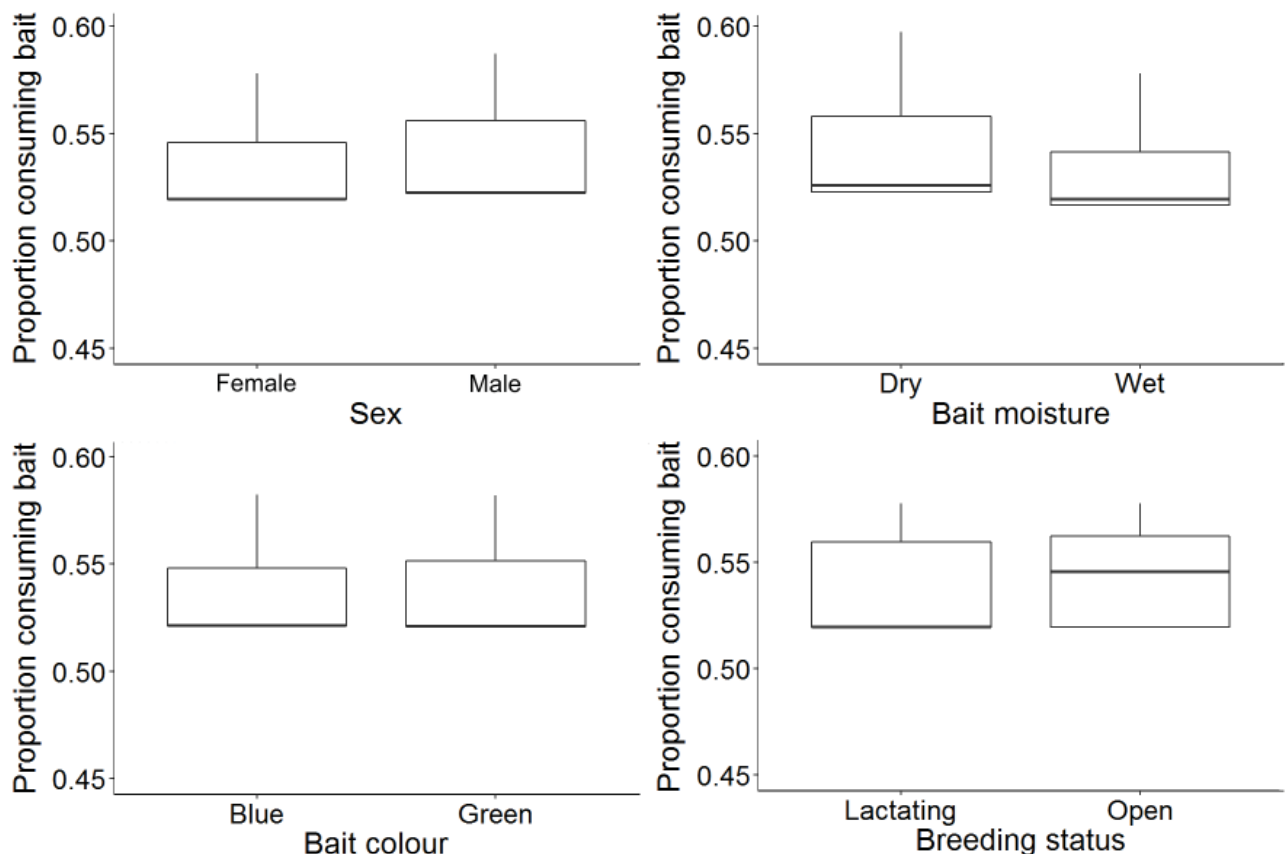
We found no evidence for bait colour, moisture, sex, or reproductive status affecting the consumption of bait pellets by captive Pacific rats. Blue and green bait pellets are frequently used in rodent eradication operations (Clapperton, et al., 2015), and the use of blue pellets may

therefore reduce the reported non-target mortality among Henderson crakes (Oppel, et al., 2016b) without affecting the efficacy of rat eradication operations.

Rats ingested dry and moist pellets equally, which is important operationally as Henderson crakes do not consume dry pellets (Oppel, et al., 2016b). While rainfall patterns on Henderson are unpredictable and aseasonal (Spencer, 1995), targeting any future eradication operation at periods of low rainfall is unlikely to affect the outcome for rodents, but may reduce the risk of non-target mortality. In the longer term (i.e. longer than the four days used in our captive trial), rainfall will increase the degradation rates of bait, thereby reducing rats' exposure to bait, regardless of their inherent preference to consume bait that is dry or wet (Berentsen, et al., 2014).

The aseasonal breeding often found on tropical islands means that baiting operations are more likely to include breeding females than operations in temperate regions. Concern has been expressed that pregnant and lactating rodents are less likely to eat bait if their nutritional needs are not met by the bait matrix (Keitt, et al., 2015), though this also assumes that rats could identify the nutritional content of bait pellets without consuming a lethal dose (i.e., one pellet; Amos, et al., 2016). Our results suggest that not only are female Pacific rats as likely to consume bait pellets as males, but that, at least for this particular bait formulation, females' reproductive status is unlikely to influence bait acceptance.

Importantly, while only 48% of trial rats consumed bait pellets, this general acceptance rate cannot be used to infer potential acceptance rates in free-ranging rats during an eradication operation. Evidence of higher or lower bait acceptance rates in the wild than in cage trials is equivocal (Clapperton, 2006) but several important limitations



**Fig. 1** There was no effect of rats' sex, breeding status, bait colour, or bait moisture on the proportion of Pacific rats consuming bait. Values are from generalised linear mixed models. Dark bars are the median, boxes are the interquartile range, and whiskers the range.



of cage trials have the potential to lower acceptance below what would be typical in the wild. For example, the provisioning of commercial rodent food during the acclimatisation phase ensures an unbiased test of food preference because test subjects are not food stressed (which results in selection of food items based on dietary deficiencies rather than food palatability). However, the chemical composition of food plays an important role in diet selection in free-ranging rats, with individuals self-selecting food based on physiological need (Rozin, 1976). Another major limitation of cage trials, specifically with regard to acceptance of novel food items such as bait, is the absence of social learning. While rats are predominantly solitary foragers, the identification and adoption of novel food items is heavily influenced by social interactions with conspecifics (Galef Jr, 1996). Cage trials should therefore only be considered as useful tools for identifying potential problems that can be explored further by field trials. On Palmyra Atoll, for example, rats were found to prefer coconut over cereal bait pellets in cage trials, but later field trials found adequate bait uptake, and a toxic cereal bait eradication was successful (Buckelew, et al., 2006; Alifano & Wegmann, 2011).

## CONCLUSIONS

Our findings suggest Pacific rats have no preference between green or blue bait pellets, nor if bait is moist or dry. This suggests that individual variation is a significant driver of bait acceptance, regardless of other demographic parameters (Nathan, 2016). While a baiting operation timed when rats are breeding carries increased risks and is preferably avoided, pregnant or lactating females are as likely to accept bait as non-pregnant females. Any future operation on Henderson Island should use blue bait pellets, and time the operation for dry conditions, in order to reduce non-target mortality of Henderson crakes without affecting rat bait acceptance.

## ACKNOWLEDGEMENTS

The authors declare no conflicts of interest. We thank N. Duffield, S. Havery, and A. Skinner, for assistance in the field, J. Kelly, A. Schofield, C. Stringer, and J. Vickery for logistical support, and the Government of the Pitcairn Islands for permission to work on Henderson. Methods were approved by the RSPB Ethical Advisory Committee (protocol EAC 2015/01). The David and Lucile Packard Foundation, Darwin Plus: Overseas Territories Environment and Climate Fund, British Birds and generous donors helped to fund this expedition. Comments from J. Lavers, S. Opper, I. Robinson, A. Schofield, and three anonymous reviewers improved earlier drafts.

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