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Susceptibility of Bush Stone-curlews (*Burhinus grallarius*) to sodium fluoroacetate (1080) poisoning

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Abstract. Although baiting for feral predators with sodium fluoroacetate (1080) benefit native fauna over much of Australia by reducing the abundance of those predators, there is a potential risk of poisoning to non-target species. Bush Stone-curlews (*Burhinus grallarius*) have declined over much of mainland southern Australia owing to predation by Red Foxes (*Vulpes vulpes*), but they have the potential to eat and be affected by 1080 baits. This paper explores the extent to which 1080-baiting programs may have an adverse effect on extant or reintroduced populations of Bush Stone-curlews. Our study used Bush Stone-curlews from a population that was not adapted to naturally occurring 1080, so our results are likely to be relevant throughout the range of the species. We determined the approximate lethal dose (ALD) of 1080 for Bush Stone-curlews to be 10–15 mg kg^{-1}. Thus a Bush Stone-curlew weighing 700 g would need to eat between three and four baits, each containing 3 mg of 1080, to receive an ALD. In unforced trials, the Bush Stone-curlews in our study did not eat (undosed) meat or grain baits. Dying food blue did not deter Bush Stone-curlews from eating it. Thus, sole reliance on blue dyes to deter non-target species from taking baits seems unwise. Our results indicate that reintroduction programs for Bush Stone-curlew are unlikely to be affected by concurrent 1080-baiting for feral animal control.

Introduction

Sodium fluoroacetate (1080) baits have long been used to control feral vertebrates over much of Australia. Reduction of populations of Red Foxes (*Vulpes vulpes*) using meat baits, and of European Rabbits (*Oryctolagus cuniculus*) using grain baits (Saunders *et al*. 1995; Williams *et al*. 1995) has been critical to the recovery of populations of many native animals (Short and Smith 1994). Foxes and Rabbits are very sensitive to 1080 (Meldrum and Bignall 1957; McIlroy and King 1990), whereas some native animals have developed tolerance to the toxin, through their coexistence with fluoroacetate-bearing plants (Twigg and King 1991). Several animal species are also known to be able to detect fluoroacetate and consequently reduce their intake of food treated with 1080 (Sinclair and Bird 1984; Calver *et al*. 1989*a*; Twigg and King 1991; Körtner and Watson 2005).

The Bush Stone-curlew (*Burhinus grallarius*) is a charadriiform bird, and of conservation concern in southern Australia (Gates and Paton 2005). The species has declined or disappeared over much of mainland southern Australia and predation by introduced predators, including Red Foxes, has been implicated as an important contributor to this decline (Marchant and Higgins 1993; Garnett and Crowley 2000). Bush Stone-curlews persist, or are the subject of reintroduction programs, at locations where 1080 baits (both meat and grain) are being used to control Rabbits and introduced vertebrate predators (feral and domestic Cats (*Felis catus*), and Red Foxes) for conservation purposes. The toxicity of 1080 to several Australian bird species has been documented (Twigg and King 1989, 1991; King *et al*. 1996). However, no data appear to be available on the susceptibility of any charadriiform birds to 1080-poisoning. The extent to which toxic baits, in their various forms, may pose a threat to Bush Stone-curlews is not known.

Bush Stone-curlews eat mainly arthropods and small vertebrates, but also some vegetation and seeds (Marchant and Higgins 1993). This diet suggests they may eat some Fox baits and therefore potentially be exposed to 1080. Despite the low proportion of seeds reported in the diet, Bush Stone-curlews may also eat 1080 from treated grain (mainly oats) used to control Rabbits. In the past, Stone-curlews have died after eating poisoned pollard when it was used as bait to control Rabbits (Froggatt 1921). It has been suggested that birds may be discouraged from eating poisoned grain-baits by dying the treated grain a colour that birds do not normally associate with food (Batcheler 1978; Bryant *et al*. 1984). However, recent evidence suggests that some birds quickly learn to ignore the colour, and readily eat dyed baits (Jongman *et al*. 2000). Thus, the effectiveness of this approach is not known.

This paper explores the extent to which 1080-baiting programs are likely to have an adverse effect on extant or reintroduced populations of Bush Stone-curlews. Specifically, we provide information on (1) the toxicity of 1080 to Bush Stone-curlews, (2) the likelihood that they will eat meat and grain baits, and (3) the degree to which blue dye discourages Bush Stone-curlews from eating a food item.

Materials and methods

All birds used in this study were captive bred and part of the collection of Adelaide Zoo. The ancestors of these birds were collected in the Adelaide area, before the species became locally extinct there (C. McKechnie, personal communication, 2004).

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A total of eight individual Bush Stone-curlews were used for all trials in this study.

Toxicity determination

The combination of plasma citrate responses and approximate lethal dose (ALD) were used to determine the toxicity of 1080 (sodium fluoroacetate) to Bush Stone-curlews. The plasma citrate method uses changes in plasma citrate concentration after administration of a dose to measure sensitivity to 1080 (Twigg and King 1991). Elevations in plasma citrate indicate the extent to which the tricarboxylic acid (Krebs) cycle has been disrupted, the major metabolic effect of fluoroacetate (Twigg and King 1989, 1991).

For the toxicity study, birds were housed in individual cages in a room at 25°C, and exposed to a natural photoperiod at the Animal Health and Research Centre, Adelaide Zoo. They were placed in these cages the day before the trial, and allowed to acclimate, before being dosed at 0900 hours the next day. Doses of 1080 (Tull Chemical Co., Oxford, AL, USA) were given in aqueous solution, intra-peritoneally, immediately caudal to the sternum to avoid the air-sacs. Solutions of 1080 were corrected for purity to ensure an accurate dose. Birds were anaesthetised with isoflurane (Abbott Australasia Pty Ltd, Kurnell, NSW, Australia) while being dosed to avoid movement during application of 1080 treatments and thus ensure accurate doses were given at the correct site. Dose volumes given to individual birds varied with their weight and with the concentration of the 1080 solution in each treatment, but all birds were given less than the equivalent of 1% in volume for body-weight (700–800 g for Bush Stone-curlew). Treatment doses followed a geometric progression (0 mg kg^{-1}, 2.5 mg kg^{-1}, 5 mg kg^{-1}, 10 mg kg^{-1}, 20 mg kg^{-1}). These values were chosen based on published data for other similar Australian birds (Twigg and King 1989; King *et al*. 1996) and because they produce maximum information on sensitivity to 1080, while minimising deaths among experimental birds.

Blood samples were collected just before administration of a 1080 dose, then 3 h, 6 h, 12 h and 24 h after the dose. This allowed determination of peak plasma citrate concentrations after each dose. Blood samples (1 mL) were collected with a heparinised syringe from the jugular or brachial veins. Four individual Stone-curlews were subjected to each dose treatment, except 20 mg kg^{-1}, where $n = 3$. Individuals were randomly assigned to treatments, with the constraint that all eight individuals were used once before any birds were re-dosed. At least 8 weeks elapsed between different dose treatments for individual birds, following Twigg and King (1991).

Plasma citrate was measured using an enzyme test-kit for the determination of citric acid (catalogue no. 10 139 076 035, from Roche r-biopharm, Mannheim, Germany). In this assay, citrate is converted to oxalo-acetate, and acetate in the reaction catalysed by citrate lyase. Oxalo-acetate and its decarboxylation product, pyruvate, are reduced to L-malate and L-lactate, respectively by reduced nicotinamide-adenine dinucleotide (NADH) in the presence of the enzymes L-malate dehydrogenase and L-lactate dehydrogenase. The amount of NADH oxidised in these reactions is stoichiometric to the amount of citrate present. NADH was measured by means of its light absorbance at 340 nm on a COBAS BIO centrifugal analyser (Roche Analytical Instruments, Nutley, NJ, USA). Any free pyruvate present in the sample is not measured because of the order of pipetting the reagents. Samples were run against known standards (0–80 μg).

We determined an approximate lethal dose (ALD, the lowest dose likely to cause death) from a small number of deaths, in combination with peak plasma citrate concentrations following a graded series of increasing doses (see Calver *et al*. 1989*b*; Twigg and King 1991; King *et al*.1996). The steep dose–response curve for 1080 suggests that deaths will occur at doses only slightly higher than those at which severe symptoms of toxicity are first observed, and allows approximation of what a lethal dose would be from an increasing progression of doses (Calver *et al*. 1989*b*).

Bait consumption by Bush Stone-curlews

To determine the acceptability of baits to Bush Stone-curlews, birds were exposed to meat and grain 'baits', with no 1080 added. One bird was placed in each of two adjoining 2 m wide × 4.5 m long × 3 m high enclosures for 12 days. The two birds were in visual contact with each other through wire mesh separating the enclosures. Water was available *ad libitum*. During the first 4 days birds were fed only the diet usually given to Bush Stone-curlews in the Adelaide Zoo's collection. This consisted of beef mincemeat, chopped fruit and Wombaroo insectivore-mix (Wombaroo Food Products, Mt Barker, SA). This is referred to as the normal diet. A bowl of normal diet was randomly placed at one of three predetermined feeding stations in each enclosure every day.

During the next 8 days of the trial, dried horsemeat and grain 'baits' were placed at two of the feeding stations in each cage. Normal diet was placed at the other feeding stations. The three types of food were allocated to each feeding station in each enclosure randomly on each day, so that the location of food changed daily. The meat and grain 'baits' were *not* treated with 1080 for the purposes of these trials. In all other respects they were the same as baits used in field programs. The meat 'bait' consisted of 50–100-g pieces of sun-dried horsemeat. The grain 'bait' was dried oats. These treatments allowed birds to acclimatise to the enclosure during the first 4 days, and tested whether birds will voluntarily take meat or grain 'baits' when adequate alternative food is available during the next 8 days. We wanted to determine whether Bush Stone-curlews would eat baits in the field following translocation for conservation management purposes. Therefore we did not do a no-choice bait-acceptance trial because translocations are unlikely to be considered in conditions of drought, when alternative foods are in short supply. At the end of these trials, birds were returned to a stock enclosure, where food and water were available *ad libitum*. Eight different individual birds were used in these trials.

Normal diet and meat and grain 'baits' were weighed before being placed in the cage each afternoon. Any remaining food, meat or grain was then weighed at the end of 24 h. The difference in weights was used as a measure of the amount of each diet eaten. Experience with these foods under the experimental conditions used suggested that the moisture content of the food would not change appreciably, so hydration controls were not included in these trials for logistical reasons. The enclosure was made bird-proof before beginning the trials to avoid wild

birds stealing the grain. A rodent control program was undertaken to ensure that consumption by rodents would not compromise the trial. The treatments were compared with a two-way analysis of variance (ANOVA), with food treatment and individual birds as factors.

Consumption of dyed food

The acceptability of blue-dyed food to Bush Stone-curlews (n = 6) was tested by comparing their responses to un-dyed normal food (see above) and normal diet mixed with non-toxic blue food colour.

One bird was placed in each of the two adjoining enclosures (used in the previous experiment) for 10 days. The two birds were in visual contact with each other through wire mesh separating the enclosures. Water was available *ad libitum*. Food was placed daily at two predetermined locations in each of the two enclosures. Each food container was randomly allocated to one of two treatments (blue-coloured or natural-coloured), so that birds could respond to each bowl of food independently each day.

Food in each bowl was weighed before being placed in the cage each afternoon. Any remaining food in the bowl was then weighed at the end of 24 h. The difference in weights was used as a measure of the amount of food eaten by each Stone-curlew. The treatments were compared with a two-way ANOVA, with dye treatment and individual birds as factors.

Results

Toxicity determination

All birds dosed at levels from 0 to 10 mg kg^{-1} survived (Fig. 1). None of the birds dosed at levels up to 5 mg kg^{-1} showed any obvious symptoms of 1080 poisoning. One of the four birds dosed at 10 mg kg^{-1} showed a slight tremor and unsteadiness on its feet before recovering. All three birds dosed at 20 mg kg^{-1} exhibited tremors and an inability to stand before they died. Birds that died did so within 24 h of being dosed. These findings indicate that the approximate lethal dose (ALD) for Bush Stone-curlews is 10–15 mg kg^{-1}.

For all birds dosed at levels from 0 to 10 mg kg^{-1} of 1080, plasma citrate levels peaked 3 or 6 h after the dose and then declined. In the three birds given 20 mg kg^{-1}, plasma citrate continued to rise until 12 h after the dose. This was the last sample obtained from these birds before they died.

Bait consumption by Bush Stone-curlews

Bush Stone-curlews consumed the normal diet provided (86.5 ± 32.57 g day^{-1}), whereas they did not consume sun-dried horsemeat meat (–1.6 ± 4.99 g day^{-1}) or grain (–4.3 ± 16.88 g day^{-1}; Fig. 2). These differences were significant ($F_{2,66}$ = 137.5, P < 0.001). The negative values represent measurement error associated with weight gained by dried meat and grain owing to moisture following rain. The interaction between diet and individual birds was not significant ($F_{10,66}$ = 0.579, P = 0.825). There were no differences in the amount of food consumed by different individual Bush Stone-curlews ($F_{5,66}$ = 0.799, P = 0.554).

Consumption of dyed food

Bush Stone-curlews consumed equal amounts of uncoloured (95.5 ± 6.70 g day^{-1}) and blue-dyed food (93.6 ± 7.51 g day^{-1}; $F_{1,5}$ = 0.056, P = 0.815; Fig. 3). The interaction between colour treatment and individual birds was not significant ($F_{1,5}$ = 0.198, P = 0.961). There was a difference in the amount of food consumed by different individual Bush Stone-curlews ($F_{5,32}$ = 8.040, P < 0.001). This resulted from one individual that consumed very little food during the first 2 days of the trial.

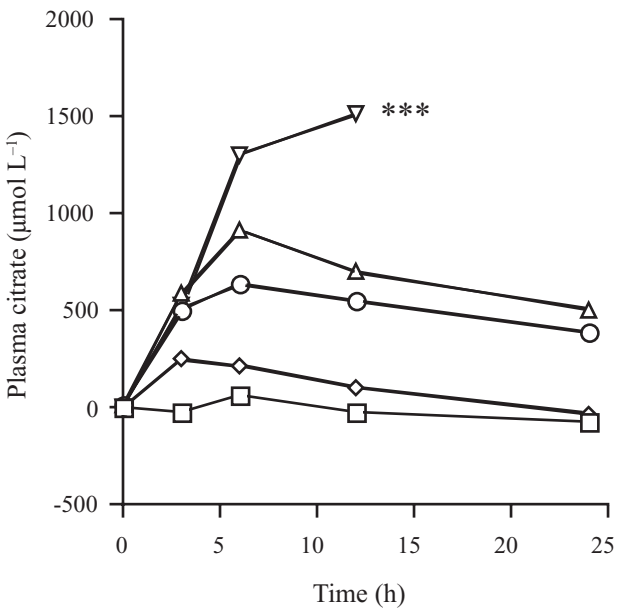

Fig. 1. Mean changes in mean plasma citrate (µmol L^{-1}) over 24 h following various doses of sodium fluoroacetate (1080) to Bush Stone-curlews. Doses: □ = 0 mg kg^{-1}, ◇ = 2.5 mg kg^{-1}, ○ = 5 mg kg^{-1}, △ = 10 mg kg^{-1}, ▽ = 20 mg kg^{-1} (n = 3). * indicates a death.

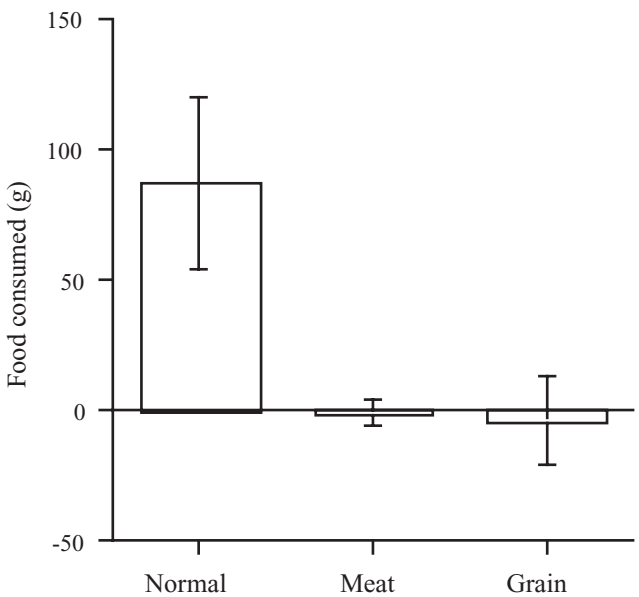

Fig. 2. Mean mass (g) (± s.e.) of normal captive diet and two kinds of bait consumed by Bush Stone-curlews (n = 6) over 24 h.

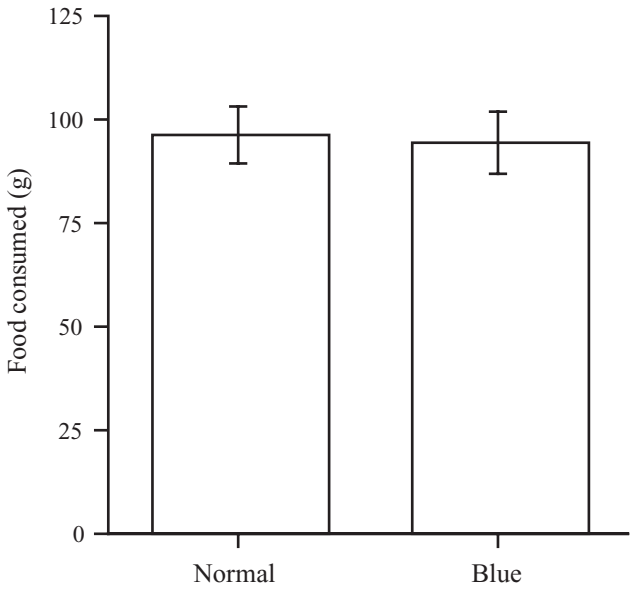

Fig. 3. Mean mass (g) (± s.e.) of un-dyed and blue-dyed food eaten by Bush Stone-curlews (n = 6) over 24 h.

This bird consumed non-dyed and dyed food in approximately equal quantities.

Discussion

Our results show that the ALD of 1080 for Bush Stone-curlews is around 10–15 mg kg^{-1}. Thus a Bush Stone-curlew weighing 700 g would need to eat 7.0–10.5 mg of 1080 in a short time (< 6 h) to receive an ALD. This would equate to consumption of three to four predator baits each containing 3 mg of 1080. The Australian standards for 1080 baits for Fox, and Dingo and wild Dogs (*Canis lupus*) are between 3 mg and 6 mg, respectively (VPC 2002), although 10-mg baits are used in Queensland for Dogs (Fleming *et al*. 2001). Similarly, Stone-curlews would have to consume 375–750 grains of oat bait to receive an ALD from conventional oat baits where all grains contain a standard rate of 0.02–0.04 mg 1080 grain^{-1} (Twigg *et al*. 2003). These amounts would increase by ~1.5 times for LD_{50} dose levels to be ingested (Calver *et al*. 1989*b*), suggesting an LD50 of ~15–23 mg kg^{-1}. Thus, even if they ate baits, which was not the case in our study, 1080 poisoning of Bush Stone-curlews is unlikely to result from well conducted standard baiting programs for the control of Rabbits, Foxes or wild Dogs.

Because of their past coexistence with plants containing fluoroacetate, many animal species are known to show geographical variation in their sensitivity to 1080 (Twigg and King 1991). We are uncertain whether the birds used in our study had received a genetic contribution from ancestral populations that coexisted with fluoroacetate-bearing vegetation, as suggested for Malleefowl (*Leipoa ocellata*) and Emus (*Dromaius novaehollandiae*) (Twigg and King 1991). However, we believe this was unlikely as the Stone-curlews we used were from ancestors that were caught in South Australia. These birds have contributed to many of the captive breeding programs in southern Australian zoos. Our results therefore, are likely to represent the sensitivity of unadapted Stone-curlews to 1080 and are thus relevant across Australia where reintroduction and conservation programs are undertaken. It is possible that the tolerance of Stone-curlews from areas with fluoroacetate-bearing vegetation may be higher than that reported for the SA conspecifics. Our results also indicate that reintroduction programs, including those in South Australia, are unlikely to be affected by concurrent 1080-baiting for feral animal control in the wild.

There is much debate about the utility of dying bait to deter birds. Our data clearly show it doesn't work for Stone-curlews. The failure of the added blue dye to deter Stone-curlews from feeding on treated food provides further evidence that the deterrent effect of blue or green dyes varies considerably between bird species (e.g. Jongman *et al*. 2000). It also appears that any deterrent effect may be rather short-lived with some species learning to recognise treated food within a few days (e.g. corellas (*Cacatua*); Jongman *et al*. 2000). Thus, although the use of such dyes does not impinge upon efficacy, the sole reliance on them for deterring non-target species from taking bait seems unwise.

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