Fox control and 1080 baiting conundrums: time to prepare for a CRISPR solution

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Abstract. For many years, managing rock-wallaby colonies (*Petrogale lateralis lateralis*) in the Western Australian Wheatbelt seemed to be a matter of routinely exposing foxes (*Vulpes vulpes*) to toxic baits (sodium fluoroacetate, 1080[®]) laid around their rocky outcrops. Recent research has revealed that 1080 baitings are no longer a viable management option. Baiting is flawed over the long term because it does not erase the wallabies' pervasive fear of being depredated by foxes, which can still make their menacing presence felt before succumbing to poison bait. Accordingly, a 'landscape of fear' exists on all rock-wallaby sites, creating a 'virtual boundary' beyond which they fear to forage. Severe overgrazing occurs, ultimately causing population crashes, leaving behind devastated outcrops greatly diminished in carrying capacity. The fallout from this scenario produces a management conundrum. Rock-wallaby populations are unstable in the absence of fox control, and conversely, they are also unstable under long-term fox control. Management is now left with few options, and the future of the colonies remains open. Other conundrums involving bait interference and mesopredator release are described. An alternative to 1080 baiting is clearly needed. Recent developments in gene engineering (CRISPR technology) offer a solution in the foreseeable future.

Additional keywords: 1080 predator control shortcomings, adaptive management, CRISPRCas9, gene-drives, predation, direct and indirect effects, rock-wallabies, top-down and bottom-up limiting factors.

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Introduction

In Australia toxic baits containing 1080[®] (sodium fluoroacetate) are widely used to protect native mammals from two introduced predators, the red fox (*Vulpes vulpes*) and the feral cat (*Felis catus*). Beginning in 1982, the toxin was first employed as a conservation research tool in a long-term predator (fox) removal experiment (The Wheatbelt Rock-wallaby Project) (Kinnear *et al*. 1988, 1998, 2010). In response to this research and other studies (Friend 1990; Kinnear *et al*. 2002), Western Australia adopted 1080 baiting as a management procedure to protect other endangered populations threatened by these predators (The Western Shield Program).

As the years have passed, some disturbing evidence has emerged revealing that the use of 1080 is creating some management conundrums that are proving difficult to resolve. This realisation is seriously complicating the management of many threatened mammals living in fragmented agricultural landscapes and remote areas of Western Australia (Dundas *et al*. 2014; Marlow *et al*. 2015a; Kinnear, unpubl. data). The Wheatbelt rock-wallaby (RW) populations present another conundrum. Pentland (2014) has shown that the long-term use of 1080 has indirectly resulted in catastrophic population declines of RWs accompanied by severe habitat damage. Herein lies the conundrum: without fox control, the RW populations are small, unstable, and prone to extinction (Kinnear *et al*. 1988, 1998, 2010); under fox control with 1080, they become more numerous, but over the long term the populations become unstable with devastating consequences demographically and environmentally. As an aid to help understand the causes of this conundrum and its ramifications, we briefly review some history and background to the RW project.

Historical: rationale of Wheatbelt predator-removal experiments

The design of the predator-removal experiments described by Kinnear *et al*. (1988, 1998) was influenced by observations made on the behaviour and abundance of RW populations living on two fox-free islands, and the population on a nearby island carrying foxes (Kinnear *et al*. 2002). On the fox-free islands it was observed that RWs foraged widely and were conspicuously abundant. In contrast, the converse was the case for both the island carrying foxes and the Wheatbelt populations (Kinnear

et al. 1988). With these contrasting scenarios in mind, we sought to experimentally mimic the island scenarios.

Accordingly, five Wheatbelt RW colony sites were viewed as islands carrying RW colonies in a 'sea' of wheat and pasture. Thus, we were able to partially exploit the classical experimental design of treatments versus controls (except for randomisation, which was not possible due to site proximities), that is, to make some sites 'fox-free' by poisoning foxes (Nangeen Hill and Mt Caroline Nature Reserves) and using other sites as unbaited controls (Kinnear *et al.* 1988). The working hypothesis predicted that RWs would eventually mimic the island scenarios, i.e. population increases in the fox-baited populations accompanied by an extension of their foraging ranges, and a null response by the unbaited controls.

Controlling foxes: 1080 experiments on making sites 'fox free'

The presence/absence of foxes on the experimental baited sites were detected by monitoring footprints on graded internal tracks and firebreaks as described in Kinnear *et al.* (1988; Fig. 1, herein). After extensive baiting trials (1979–82) (Kinnear *et al.* 1988), it became apparent that while foxes took palatable poison baits, it was not possible to keep a site footprint-free for long because, during summer and autumn, fox footprints would reappear in a matter of days and invariably within a month. This happened even when the frequency of baiting events was increased. Furthermore, whenever an invading fox was detected following a footprint-free period, it tended to explore the reserve presumably to inspect and establish a territory.

These observations cast doubts as to whether we were actually killing foxes, and so in an effort to answer this question, systematic searches for victims on Nangeen Hill Reserve and surrounding paddocks were implemented, with the following results: over a period of 43 months, 226 fox victims were recovered, 183 killed by poison baits and 43 by shooting (Kinnear *et al.* 1988, see fig. 3 therein). Given these numbers as a rough guide, then on average the invasion rate by foxes was four per month (183/43), with peak rates during summer–autumn when young foxes were dispersing (Coman *et al.* 1991; Carter *et al.* 2012). Despite this invasion rate, the RW populations increased and they occupied areas of their outcrops that had

not been previously utilised (see Figs 1 and 2) (Kinnear *et al.* 1988, 1998, 2010).

For years then, despite the inability to maintain a site fox-free for extended periods, managing Wheatbelt RW populations seemed to be a simple matter of routinely baiting for foxes, but this was to have unforeseen consequences. By the turn of the century, a looming crisis seemed inevitable; on Nangeen Hill weed invasion became conspicuous and later reached rampant proportions. Overgrazing was also conspicuous on the outcrops; trees and shrubs were stripped of bark, bare ground dug up and rhizomes eaten. This restricted foraging range was the rule despite the presence of food nearby on the meadow and surrounds that remained ungrazed. By 2011, however, population crashes occurred as predicted by Kinnear *et al.* (2010).

During this period, a study (Pentland 2014) focusing on RW behaviour and habitat utilisation led to the realisation that the baiting protocol was flawed despite the benefits of an extended

Fig. 1. The Nangeen Hill Nature Reserve outcrop. The hatched area includes the most deeply fragmented area of the outcrop, and it was this area that served as a predation refuge. Under fox control the colony increased ~4-fold and extended its occupation of the outcrop to areas less well fragmented (thicker white-outlined area). Grazing remained largely restricted to the rock itself and a portion of the meadow. Baits (3–4.5 mg sodium fluoroacetate, 1080®) consisting initially of meat and, later, prepared sausage-like materials ('Probaits': Marlow *et al.* 2015*b*), have been laid on all boundaries and internal tracks (narrow white lines) at monthly intervals since 1982. The reserve is now fenced principally to exclude foxes and cats.
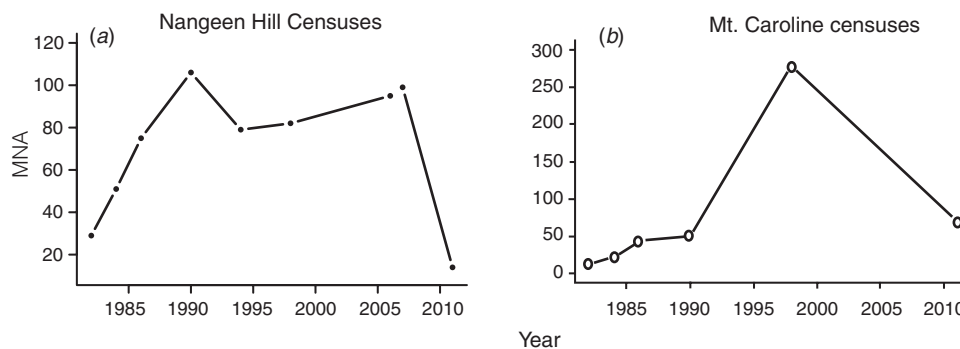

Fig. 2. Census histories for Nangeen Hill NR and Mt Caroline NR for the period 1982–2011 (for standard errors, see Table 1 and Kinnear *et al.* 2010).

stable period of population increases (4–15 fold: Fig. 2). It was flawed because it was never possible to maintain a site continuously fox-free, a situation caused by the unrelenting intrusions by foxes from the surrounding countryside. Consequently, foxes can therefore make their presence felt to the wallabies before succumbing to bait, and, in doing so, they are perceived as a threat (e.g. in addition to witnessing several threatening incidents, two RW kills were observed: Pentland 2014). Under this ever-present threat, a 'climate of fear exists within the colonies' with invading foxes reinforcing antipredator behaviour that causes the wallabies to restrict their foraging to the outcrop and nearby surrounds. This eventually led to overgrazing, severe habitat degradation and population collapses.

To summarise, after more than 30 years, this landmark project is now faced with a management conundrum that defies resolution in a manner one might like. It is a conundrum because even though the baited populations increased substantially, they eventually became unstable, crashing precipitously and leaving behind severely degraded habitats. On the other hand, without effective fox control, the colonies would exist as small, unstable, extinction-prone populations subject to bottle-necks with all the attendant genetic consequences, including extirpation (Kinnear *et al.* 1988; Eldridge *et al.* 1999, 2001; Frankham *et al.* 2013). Indeed, during the life of the project two unbaited populations became extinct (Kinnear *et al.* 1998, 2010).

Fox control and 1080: the need for an alternative

This paper emphasises the need to find an alternative to 1080 baiting. It describes some other conundrums that have arisen while using 1080 as a long-term management tool, and we list the numerous shortcomings that constrain and limit its use. Thus, there are many reasons to seek an alternative, and it is timely to provide reasons to do so, because of recent developments in cell biology – the CRISPR phenomenon – a technology that promises to revolutionise the control of invasive species in ecosystems. This will be discussed in due course, but first, we present data to inform the conservation community that while 1080 predator control may well provide transient benefits, in the long term it can be destructively counter-productive. Time for a change.

Materials and methods

The baited study sites: Nangeen Hill and Mt Caroline Nature Reserves

These outcrops are located south of the town of Kellerberrin, ~210 km east of Perth, Western Australia (31°50′08.6″S, 117°40′58.5″E) (Kinnear *et al.* 1988, figs 1 and 2 therein). Ongoing fox control has been in force since 1982.

Nangeen Hill Nature Reserve (NR) is a small rectangular reserve (~168 ha, 1400 × 1200 m) that encloses a semielliptical-shaped outcrop with a deeply fractured northern face (Fig. 1). A meadow stretches outward along the northern base bounded by thickets of dominant *Allocasuarina campestris*. Its southern face consists of smooth and lightly fractured rock that overlooks eucalypt woodland. The outcrop occupies ~15% of the reserve area.

Mt Caroline NR is a larger reserve (371 ha) consisting mostly of smooth rock with scattered rock-piles and a low, moderately fragmented escarpment that bisects the reserve. The vegetation consists mainly of open woodland with an understorey of grass and forbs. Paddocks of cereal crops and pasture abut the reserve boundaries. A track following the escarpment provided access that has been baited along with the firebreaks since 1982 (see Kinnear *et al.* 1988, 2010 for illustrations and further details).

Census methodologies and procedures

During the autumn of 2011, Thomas 'soft' traps were used to census both populations and included both refuge and non-refuge areas (Fig. 1) (Kinnear *et al.* 2010). For Nangeen Hill, the census replicated the methodology employed in 2006 and 2007 (Kinnear *et al.* 2010; Pentland 2014). On Mt Caroline reserve, traps were set at sites comparable with the 1998 census (Kinnear *et al.* 2010, see fig. 3 therein).

Captured individuals were transferred from the trap into a hessian bag, marked with ear tags or microchips, and the following details recorded: weight, sex, age class based on weight (adult, subadult, juvenile), female reproductive status, pes length, and general body condition. Pouched young were sexed and grouped according to size.

Condition indices (CI)

A tail-based index of body condition was obtained by palpating the base of the tail. For a wallaby typically in good condition, caudal vertebrae cannot be felt. For animals in poor condition due to malnutrition, the vertebrae can be felt to varying degrees. This index was therefore scaled as Ranks 1–3, with 1 being the poorest condition. Starving animals typically catabolise body fat initially, followed by muscle tissue, thus reducing body mass accordingly. However, declines in body weight may not always be evident as loss of mass (lipids, muscle) can be replaced by water (Bakker *et al.* 1982). This can confound indices based on establishing a relationship between weight and a fixed body part such as pes length.

Pathological tests

Blood samples from 34 individuals and fresh scat samples from 43 individuals captured on Mt Caroline were analysed by Vetpath Laboratory Services in Western Australia and Tasmania. Clinical analyses focused on haematology, biochemistry and faecal parasite loads.

Results

Census histories and population estimates

Population estimates for Nangeen Hill and Mt Caroline (Table 1, year 2011) were computed using three mark–recapture models: Program Capture, Schnabel, and Schumacher–Eschemeyer (Krebs 1999). After a period of high population numbers in the 1990s and middle 2000s, both populations collapsed by 2011 (Fig. 2; Table 1). Throughout this period there was a consistent poisoning program for foxes so that the population crash could not be linked to changes in baiting.

Table 1.   Nangeen Hill and Mt Caroline population estimates with confidence limits (CL, 95%)
MNA, minimum number alive. (In 1998, an additional 20 RWs were caught outside of Mt Caroline reserve at Dixon's Paddock: Kinnear *et al*. 2010.)

Site	Year	MNA	Capture (CL)	Schnabel (CL)	Schumacher–Eschemeyer (CL)
Nangeen NR	2007	99	124 (114–143)	99 (91–135)	108 (91–134)
Nangeen NR	2011	14	14 (14–14)	14 (12–18)	14 (13–16)
Mt Caroline NR	1998	276	330 (297–387)	283 (254–359)	313 (215–574)
Mt Caroline NR	2011	70	81 (75–88)	67 (58–81)	70 (58–85)

Pathology results

Scats: No evidence of toxoplasmosis was found in any wallabies tested in 2011 (22 individuals) and parasite levels were very low: only *Nematodirus* sp. eggs and *Coccidian* oocysts were found. There was no evidence of leucocytes, *Campylobacter*, yeast, protozoa, cysts or ova. Some evidence of *Salmonella* sp. Muechen was detected, but at non-significant levels.

Overall, the pathology report interpreted the results as typical of malnourished animals in poor body condition (Table 2). The haematology and biochemistry results were consistent with these observations. It was noteworthy that a significant portion of individuals tested had elevated levels of creatine kinase (signifying muscle break down) and low white blood cell counts. A full report is available by contacting coauthor N. Moore.

Condition indices

Overall, the 14 individuals trapped at Nangeen Hill in 2011 were in better condition compared with most of those captured on Mt Caroline. All individuals at Mt Caroline were in poor, or very poor, condition, as judged by their visual appearances (e.g. exposed ribs) and condition indices (Table 2). Historically, palpable caudal vertebrae were most uncommon in all previous trapping sessions (Mt Caroline, 1979–98; Nangeen, 1979–2006). There was no difference between sexes in regard to condition.

Reproductive status

Most females at both sites were carrying very small pouch young regardless of condition (Table 3). The physiological costs of carrying small pouch young are minimal, but rise sharply with the onset of thermoregulation by pouched young, i.e. milk volumes and energetic demands increase exponentially, thus limiting the ability of females in poor condition to carry pouch young to independence (Miller *et al*. 2010). Recruitment was nil and the surviving populations consisted of adults only (weights ≥3.0 kg: Pentland 2014).

Recent rainfall statistics: preceding and during the crash

Rainfall data (2006–11) based on *Malarga* property records, a farming property adjacent to Nangeen Hill. The data are restricted to the cooler productive growing seasons (April–October) as rain falling otherwise is unproductive due to high evaporation rates; in addition, it promotes weed growth in disturbed areas, e.g. paddocks and overgrazed RW sites (see Fig. 3).

Table 2.   The condition indices of individuals obtained by palpating the caudal vertebrae in 2011
The condition index (CI) was based on a scale of 1–3 with outcomes listed as population percentages. *n*, number of individuals

Site	CI = 1 (Very poor)	CI = 2 (Poor)	CI = 3 (Good)
Nangeen Hill NR	0% (*n* = 0)	79% (*n* = 11)	21% (*n* = 3)
Mt Caroline NR	44% (*n* = 30)	56% (*n* = 39)	0% (*n* = 0)

Table 3.   The reproductive status of *P. l. lateralis* females caught during 2011 based on presence of pouched young or evidence of lactation
There was no recruitment

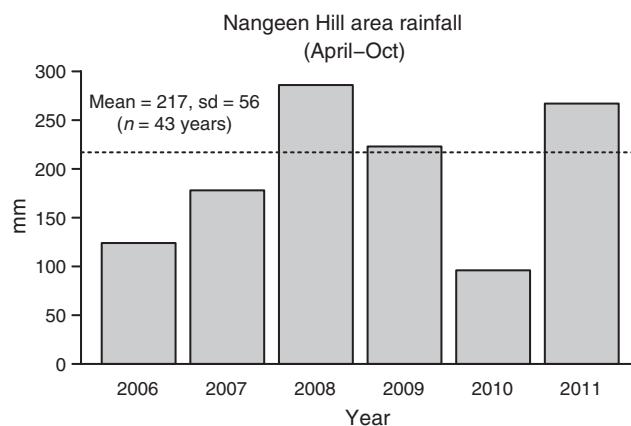| Site	Teats distended, not lactating	Teats lactating	Carrying pouch young
Nangeen Hill NR (*n* = 5)	0	0	5 (100%)
Mt Caroline NR (*n* = 33)	9 (27%)	1 (3%)	23 (69%)

Fig. 3.   Nangeen Hill area rainfall data 2006–11. The low rainfall of 2010 would have been a major contributing factor in precipitating the predicted crashes (Fig. 2; Table 1). Crop failures occurred in 2010.

Discussion

The Wheatbelt conundrum: the fear factor

The period spanning 1982–98 lent credence to the working hypothesis that the fox was an apex predator exercising top-down control, limiting RW colony densities and distributions (Kinnear *et al*. 1998, 2010). The numbers increased ~4-fold under baiting on Nangeen and on Mt Caroline by ~15-fold,

the latter creating a costly management problem due to crop incursions. By 2000 there were increasing concerns about habitat degradation caused by over-grazing and weed invasion on the outcrops, which ultimately culminated in catastrophic population declines due to starvation and possibly dispersal by RWs, a perilous step given the high fox densities in the surrounding countryside.

The conundrum facing management is that 1080 baiting as currently practiced is not a viable procedure for managing the RW colonies because of two intractable constraints: first, baiting is restricted to the sites *per se*, thus permitting the entry of foxes, and therefore providing opportunities for foxes to confront the wallabies menacingly; second, in the absence of control in the surrounding countryside, fox densities are high. This creates an ever-present pool of invaders, which, by their presence, however transitory, inculcates fear of being depredated, causing the wallabies to restrict their foraging range (Pentland 2014).

The recognition that fear of predators *per se* can indirectly affect prey demographics is a relatively new concept, and, as far as we are aware, this is the first case to come to light in Australia as a result of 1080 baiting. Its prevalence remains an open question and should be borne in mind by the conservation community. It has added another layer of complexity to predator–prey systems for it is now evident that predator–prey dynamics can be driven by two processes – mortality-driven direct effects (top-down), and by indirect (bottom-up) fear-driven effects. In the predation literature, two aphorisms have gained currency that capture the essence of the fear phenomenon: prey are visualised as living in a 'climate of fear' or, more popularly, in a 'landscape of fear' (Laundré *et al.* 2014; Suraci *et al.* 2015). In a classic test case of snowshoe hare cycles in North America, population declines are caused by direct predation, and indirectly, by the stress caused by predator chases of prey (Ginzburg and Krebs 2015; Sheriff *et al.* 2015).

Fearful behaviour can have profound outcomes demographically and ecologically. Berger-Tal and Kotler (2010), and McCauley *et al.* (2011) have shown that the presence of predators can affect prey survival, vigilance levels, reproduction, habitat usage and foraging activities (see also Creel and Christianson 2008; Sheriff *et al.* 2011; and *Functional Ecology* **27** (2013) for a collection of articles). Prey are known to starve rather than risk exposure to attacks by predators and therefore, given the data in the Results, this seems to have been the case for Nangeen and Mt Caroline. Pentland's (2014) studies conform to most of these findings.

A legacy caused by fear: habitat degradations

The four photographs that follow illustrate the sequence of events that progressively led to the life-threatening degradation of the habitat on Nangeen Hill. During the prebaiting period (1978–82), the Nangeen colony (~20–25 individuals) confined their foraging to the outcrop itself (Fig. 4). Eventually, the population increase (~100) exceeded the carrying capacity of the outcrop, and the RWs began to graze the meadow, but only to a limited extent (Fig. 5). By 2000, invasion of the outcrop by cape weed (*Arctotheca calendula*) became conspicuous on Nangeen; this was subsequently succeeded by ice plant

(*Mesembryanthemum* sp.) (Fig. 6). These circumstances ultimately forced hungry RWs to resort to digging for rhizomes on the meadow (Fig. 5) and stripping bark from small trees and shrubs on the outcrop (Fig. 7). Numbers then crashed precipitously after a year of very low rainfall (Figs 2, 3).

More conundrums: bait interference by non-target species

In response to the threat posed by foxes, the Western Australian government embarked on a wide-area fox-control program known as 'Western Shield'. It embraced National Parks and large nature reserves, which were baited aerially: smaller areas were ground-baited, typically on a monthly basis. Dundas *et al.* (2014) have reported on the efficacy of a ground-baiting protocol designed to protect a mainland Quokka population (*Setonix brachyurus*). Bait uptakes were monitored using cameras. Most of the baits were taken or disturbed by non-target species (including *S. brachyurus*), thus reducing the exposure of baits to foxes. Their finding suggests that a negative feedback loop may be operative caused by such interference: (1) initially fox densities were high, prey densities very low, and baitings reduce fox numbers; (2) prey numbers increase and prey start interfering with baits; (3) fox control becomes less effective, fox numbers increase, and prey decline due to a higher level of predation; and (4) a new equilibrium is reached with
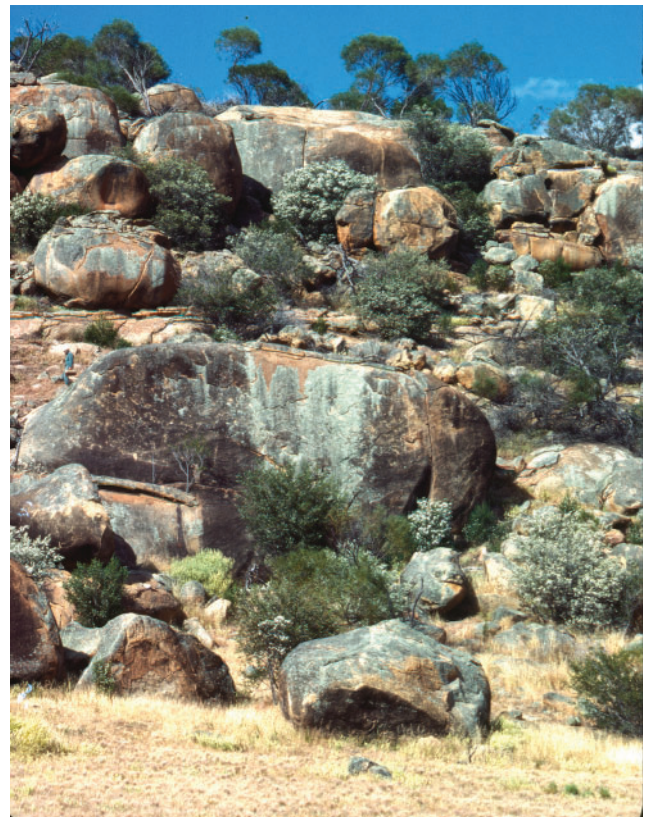

Fig. 4. Nangeen Hill, November 1978 (no fox control): a dry-season view taken from the meadow (population 20–25) showing the absence of grazing on the meadow. Foraging was strictly limited to the outcrop itself. (Photograph by J. E. Kinnear)

Fig. 5. An extended view of the meadow (2007) delineating the foraging range of the RW population, creating a 'virtual boundary' beyond which they feared to forage (Pentland 2014). The foreground is ungrazed, but the outcrop and adjacent meadow are heavily overgrazed and devoid of ground cover, causing the RWs to dig and eat rhizomes. (Photograph by C. Pentland)

Fig. 6. Weed invasion by ice plant (*Mesembryanthemum* sp.), which succeeded cape weed (*Arctotheca calendula*), the first weed invader. The former is a salt accumulator that salinises the soil when it dies. It is also known to be toxic. Wallabies were observed to selectively graze the tips of the plant. (Photograph by C. Pentland)

Fig. 7. Small trees, shrubs, forbs and grasses are found wherever soil accumulates on the Nangeen outcrop. In the final stages before the crash, hungry RWs were observed stripping the bark from *Acacia lasiocalyx* (shown), *Ricinocarpos tuberculatus* and *R. muricatus*. (Photograph by N. Moore)

prey densities below carrying capacity with prey still interfering with baits. The solution to this conundrum remains unresolved. Whatever the solution, it will involve more intensive management.

The Marlow mesopredator conundrum

Another more insidious side-effect of baiting is the rise of a mesopredator less amenable to control (Marlow *et al.* 2015a). Evidence supporting mesopredator release involving cats has been gathered on Dryandra Woodland, a site in the Western

Australian Wheatbelt with a long history of fox control. Prior to baiting this remnant in the 1980s, woylies (*Bettongia penicillata*) and other marsupials were so rare that they were practically undetectable (see Kinnear *et al.* 2002). Baiting produced a dramatic reversal, with woylie capture rates approaching 70%. Many years later a decline occurred, as reflected by more recent trap success rates that had plummeted to 10%. Their research has shown that cats are now the apex predator affecting woylie densities, but there is also evidence of bait interference by possums (*Trichosurus vulpecula*). The conundrum arising here is this: a bait-susceptible apex

predator, the fox, has been replaced by a bait-recalcitrant mesopredator, the feral cat.

A remote area baiting conundrum: a likely mesopredator effect

During the 1990s, reports of RW sightings on the remote Calvert Ranges in Western Australia came to our notice. The site was visited and although the RWs were not conspicuous, they were nonetheless widely distributed along the rocky escarpment. An aerial baiting program was implemented and the site was monitored. We expected to detect a population increase but, in contrast to Wheatbelt colonies, this remote desert population responded to fox baiting by contracting and retreating to the more deeply fractured areas, presumably serving as refugia. (It represents the converse scenario illustrated by Fig. 1.) Furthermore, the population did not respond to further baitings (Kinnear, unpubl. data), but the population has subsequently responded to cat baiting (unpubl. data in DPaW files). The mesopredator effect offers an explanation: fox baiting would have targeted both the dingo and the fox, releasing the cat. The message here is that managing predators in remote regions by baiting is not only logistically difficult and costly, one has to also deal with the full predator mix of dingoes, foxes and cats and their interrelationships under a control regime.

The ecology of predator control: the dingo, another conundrum?

It is sometimes forgotten that predator-removal (control) programs by baiting in Australia have a long history spanning centuries; indeed, the viability of the wool industry was dependent on dingo control. This vast predator-removal program most likely facilitated the spread and the establishment of foxes and cats. Later, when the Australian conservation community adopted baiting as a means of controlling foxes (and feral cats), the conservation community itself became an *apex* predator (Fleming *et al.* 2012). As an apex predator, conservation agencies need to fully understand the ecological consequences in becoming so. We need to know whether we are equally efficient, i.e. are we the same predator doing the same thing across the continent? If we are not the same predator, then a lack of consensus among researchers will happen, replicated experiments will produce different outcomes, and debates will rage in the literature. This is likely to continue, for baiting is a messy mishmash of protocols, lacking standards, and subject to constraints and numerous shortfalls.

This situation is particularly evident concerning the dingo, which has been promoted as a fauna protector in areas where it enjoys apex status. Moseby *et al.* (2012) demonstrated experimentally that dingoes readily kill foxes and feral cats. These observations and other instances have led to the proposal that dingoes should be protected and restored as the apex predator, playing a role of fauna benefactor by killing foxes and cats (Nimmo *et al.* 2015, and references therein). Others dispute this proposal or advise caution (Fleming *et al.* 2012; Claridge 2013; Hayward and Marlow 2014). Allen *et al.* (2013) argue that the evidence is as 'clear as mud'. It will remain largely muddied until more basic research is carried out on these predator interrelationships.

1080 baitings: currently essential, but a deeply flawed management tool

During the lifetime of the rock-wallaby project (and elsewhere), lessons have been learned about controlling foxes using 1080 baits (Kinnear *et al.* 1988, 2002). Foxes are sometimes seen as the consummate scavenger with an insatiable appetite, but bait palatability can be an issue. The first bait tested in the Wheatbelt was the 'crackle' bait, widely used to control dingoes in Western Australia, but despite lengthy trials, *absolutely* none were taken. Subsequently, over three dry seasons, numerous trials revealed that fresh meat baits were preferred by foxes, but they also took fish-flavored baits and fowl eggs (foxes have been photographed robbing malleefowl mounds). Eggs have useful properties, especially if they are injected with 1080 under sterile conditions, for they retain their toxicity for longer periods regardless of the weather. The toxic life span of baits is affected by wet conditions, which enable microorganisms to readily degrade 1080 (defluoroinating microorganisms are ubiquitous in the environment: see Wong *et al.* 1991*a*, 1991*b*, 1992, 1995; Saunders *et al.* 2000). We also noted that uptake of palatable baits was erratic: on encountering bait stations, foxes would sometimes inspect baits only to move on (more recent monitoring using cameras has captured foxes urinating on baits: Moore, unpubl. data). Often they would ignore baits entirely, while on other occasions, multiple baits would be taken.

Additional constraining factors

Baiting is often constrained because it is not target specific, placing native fauna at risk (especially outside of Western Australia, where faunal tolerance of 1080 is lower). Precautions need to be factored in to allow for such risks, and this can affect the efficacy of control. Some people in the community regard 1080 baiting as inhumane and in the past have mounted determined, recurrent campaigns arguing that 1080 should be banned – one day they may succeed. The rural community is always at risk of being alienated if their dogs are poisoned. For example, in response to land owner complaints concerning farm dog deaths, we became aware that ravens (*Corvus coronoides*) were carrying off baits and causing fatalities on nearby properties. Management responded to these complaints by burying baits on Nangeen Reserve for a period, but this practice was stopped due to a bout of serial killing of RWs that followed. A Boyagin farmer described another hazard (Kinnear *et al.* 2002; pers. comm.): baiting events on a nearby reserve were typically associated with corvids congregating around dams as they would remove dried meat baits and soak them to make them palatable; unfortunately, this behaviour exposed hot, thirsty farm dogs to baits.

Devising an effective baiting protocol, can therefore be a daunting task, fraught with obstacles. Operationally one has to deal with numerous factors and impediments, e.g. regulations, public opposition, costs, non-target species, palatability, frequency, timing, fox densities, bait spacing and distribution, bait caching, toxin dosage, bait potency-longevity, bait presentation, prey vulnerability and bizarre Boyagin-like mishaps, not to mention indiscriminate razor-gang cuts to program funding. Sublethal baits caused by microbial activity (e. g. following bait caching or rainfall) can potentially create bait-

shy predators who become intractable killers. Apart from failing to protect endangered fauna, this lengthy list has the potential to confound experiments.

The future of 1080 baiting: time to embrace a CRISPR solution

From the outset, the use of the toxin 1080 to control foxes has always been recognised as a holding action to quote: 'In the long term the ultimate solution would be some method of biological control' (Kinnear *et al.* 1988). Given a recent breakthrough in genomic engineering (CRISPRCas9), there are now compelling grounds to believe that this goal is within reach in the future. (CRISPR – as in 'whisper' – is an acronym that defies meaningful translation; for the record, it translates as: 'clustered regularly interspersed short palindromic repeats'. See references for non-specialist explanations.)

Genomes can be read (sequenced) inexpensively, and genes written (synthesised), but the one aspect that has eluded cell biologists is the ability to readily edit genes and genomes. This was rectified following a collaboration involving two cell biologists (Jennifer Doudna and Immanuelle Charpentier) which produced a revolutionary breakthrough. They and their colleagues worked out a way to adapt elements of a bacterial immune system (CRISPRCas9) so that they could precisely locate and edit genes in a eukaryote organism (Jinek *et al.* 2012). To use an analogy and to greatly oversimplify, they invented a 'genomic word processor' complete with some familiar manuscript editing functions: e.g. the genome (or a chromosome) can be scanned (the find function) for a specific DNA sequence and selected (i.e. a gene or fragment of DNA); following this, a gene can be cut (disrupted), or a gene can be cut and pasted (inserted). The molecular hardware came from a bacteria immune system (an enzyme/RNA complex, i.e. Cas9), and the cell biologists provided the 'software' making the system programmable so that any part of a eukaryote genome can be targeted with precision and manipulated. Moreover, in contrast to existing editing systems, it is simple and inexpensive, and it produces results in days rather than weeks or months.

CRISPR and invasive species control

The applications of the CRISPR technology extend across many biological disciplines including the control of invasive species. As to how it will be applied in the case of a specific invasive species, it is too early to speculate, but nonetheless a possible mechanism would be to alter the sex chromosomes so that only males are born.

For a gene-editing solution to work, it must be able to spread throughout the target species' population. For example, if one inserts a deleterious gene into a species' genome, it will make it less fit, and natural selection will eliminate it. Fortunately, this can be overcome by harnessing gene drive mechanisms (referenced as follows).

For the interested reader, we include a sample list of references that provide a range of technical details and information of interest to ecologists. For non-specialist accounts see: Esvelt *et al.* (2014*a*); Le Page (2015); Miller (2014); Saey (2015) and Hall (2016). For accounts involving more technical details concerning CRISPRcas9, gene silencing,

gene drives and invasive species, see: Esvelt *et al.* (2014*b*), Regalado (2015) and Johnson *et al.* (2016). For a lucid account of cell biology, see Alberts *et al.* (2015).

Predation research: moving forward towards a CRISPR era

At best, 1080 baiting is a crude research tool that has played a pivotal role in revealing that conservation in Australia is faced with an exotic predator problem. Kinnear *et al.* (2010) emphasised the need to measure the functional and numerical responses of predators for without such data, understanding predation remains a black box. By gaining such information, we would acquire knowledge about three important processes and outcomes affecting wildlife conservation in Australia: (1) who is doing the killing and exactly when, (2) who is being killed, and (3) how often do kills take place? Currently, researchers have just managed to prise open the black box ever so slightly.

As an apex predator baiting RW sites, all we know is that baiting kills foxes and RWs respond by increasing until another factor intervenes, although strictly it remains a correlation, not cause and effect; nevertheless, this association has gained credence through replication, but baiting provides few insights regarding the predation process. Data about the three processes listed above would resolve many the issues affecting our ability to conserve Australia's threatened species, including the roles of the dingo, the feral cat and the mesopredator effect. It is a demanding task requiring sophisticated remote-sensing technologies (Kinnear *et al.* 2010), but it needs to be done, because the exploitation of CRISPR technology will require a fuller understanding of the biology/ecology of invasive species, as well as the consequences that follow pending their control or removal.

Concluding remarks

In summary, we believe that it is now clear that the era of 1080 baiting needs to come to an end. Apart from its many constraints and limitations, conundrums are now emerging that are proving difficult to resolve. As a management tool, it begs more questions: must baiting be forever? Is that all researchers have to offer? The recent advances in gene engineering proffer a potential means of getting off the treadmill of forever laying poison baits. Conservation biologists now need to forge relationships with molecular biologists to exploit these new promising methods for controlling invasive species in Australia.

Paradoxically, we should point out that, despite the many shortfalls associated with 1080 baiting, and because of the prospect that a future solution may be within reach, predator control by baiting (fear aside), or by any other means, should be intensified and improved in order to rescue and maintain as many endangered populations as possible in the short term. Looking further ahead, it is not inconceivable that gene engineering may also provide a means of restoring genetically impoverished genomes or enhancing genomes challenged by global warming. In the former case, steps are being taken towards this goal involving black footed ferrets (*Mustela nigripes*) in the USA (Biello 2016).

Finally, the Wheatbelt RW project validates one of the tenets of wildlife management – the benefits of long-term studies

and adaptive monitoring (Lindenmayer *et al.* 2011). Monitoring sounded the alarm and management has responded accordingly by reversing the RW decline and by seeking new solutions (unpublished work in progress, Western Australia Department of Parks and Wildlife).

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