Testing for brodifacoum resistance in invasive rodents on Lord Howe Island:

Summary of Work Undertaken by the Office of Environment and Heritage in 2013

Prepared for

The Lord Howe Island Board

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Introduction

The arrival of Ship Rats (*Rattus rattus*) and House Mice (*Mus musculus*) to Lord Howe Island (LHI) has resulted in significant changes to the Island's ecosystem, including the loss of several bird species (Hindwood 1940, Recher & Clark 1974), and impacts on reptiles, invertebrates and plants (Cogger 1971, Recher & Clark 1974, Hutton 2001, Priddel *et al*. 2003).

The Lord Howe Island Board (LHIB) has undertaken a concerted rat-control programme since 1986 to primarily protect the island's Kentia Palm industry (Harden and Leary 1992). In 2001 the LHIB contracted the Endangered Species Recovery Council to investigate the feasibility of eradicating rodents from LHI. The report on the investigation suggested that despite the difficulty, eradication was feasible (Saunders & Brown 2001).

Successful eradication is contingent on 1) 100% of target animals being exposed to a poison and 2) all of them being susceptible to that poison. Baits containing the anti-coagulant brodifacoum have been successful in eradicating introduced rodents from many of the world's islands (Howald *et al*. 2007). The bait used for rodent eradication in New Zealand, Western Australia and on Macquarie Island has been the Pestoff 20R cereal bait containing brodifacoum at a nominal concentration of 20 parts per million. Trials in 2007 and 2008 determined that the rodent populations on Lord Howe Island will readily consume non-toxic Pestoff 20R cereal baits (Wilkinson *et al*. 2008). However, as rodenticides containing brodifacoum have been used for more than a decade by residents and the Lord Howe Island Board, there is potential for rodents on Lord Howe Island to have developed a tolerance to this poison. Any such tolerance could undermine an eradication. Consequently it is important to establish if rodents are susceptible to the proposed poison (brodifacoum) to be used in the operation. To this end a captive-feeding trial using Pestoff 20R baits was conducted on LHI in 2013 to assess the likelihood of resistance in the mouse and rat populations located in the settlement or at the waste-treatment works. Rodents around human habitation were seen as having the most potential to be tolerant to brodifacoum. Full details of this trial are given in Appendix 1 which is an unpublished

manuscript (and therefore not for general circulation) written by David Priddel, Robert Wheeler, Nicholas Carlile and Ian Wilkinson.

Testing the Susceptibility of LHI Rodents to Brodifacoum

The feeding trial involved offering rodents various concentrations of brodifacoum expressed as multiples of the known lethal dose required to kill 50% (i.e., the LD_{50}) of a typical population of a specific rodent. The trial was divided into two parts for the test animals, with each part having five treatments. For mice in the first part of the trial, four groups were, respectively, offered pellets containing the equivalent of 1 LD_{50}, 2 LD_{50}, 3 LD_{50}, and 5 LD_{50}, of brodifacoum. Black Rats were also offered one of four poison diets in the first part of the trial, but in this case the LD_{50} equivalent was that for the Brown Rat, which is less than that for the Black Rat, the goal here being to determine if a relatively low dose of brodifacoum would still be effective in killing this species. For both the mice and rats, a fifth group served as a control to monitor the potential for subject rodents to die from other causes (e.g., such as being held in prolonged captivity). There were 10 rats and 10 mice in each initial treatment. Survivors from this first part of the trial were then fed an additional amount of brodifacoum equivalent to 10 LD_{50}.

The results indicated that the susceptibility of rats to brodifacoum was in line with that for the species as a whole. That is, judging by the results of this trial, all the rats on LHI are susceptible to low levels of brodifacoum. Based on an observed LD_{50} of 0.54 mg kg^{-1}, an average body weight of 196 g and a brodifacoum concentration in bait of 18.2 ppm (as determined by chemical assay of the Pestoff bait used in this feeding trial), the average rat on Lord Howe Island (in terms of both size and susceptibility) would need to consume 5.8 g of bait to ingest a lethal dose. The dosage needed to kill all rats on Lord Howe Island (LD_{100}), as determined in the feeding trial, is 0.81 mg kg^{-1}. Based on an observed LD_{100} of 0.81 mg kg^{-1} and a maximum body weight of 275 g (this feeding trial), the largest and least susceptible rat on Lord Howe Island would need to consume 12.2 g of bait to ingest a lethal dose. An adult rat will typically eat 25–30 g of food per day, taken in about ten small meals, with the maximum consumption per meal of around 3 g. Thus all rats on Lord Howe Island could consume a lethal dose in one day, but may require four or five meals to do so.

However, mice exhibited a tolerance to brodifacoum significantly in excess to the LD_{50} of 0.4 mg kg^{-1} prescribed for mice. Ingestion of brodifacoum at dose rates 1 and 2 LD_{50} by mice on the trial resulted in no mortality. A dose rate of 3 LD_{50} resulted in 10% mortality, and 5 LD_{50} resulted in 60% mortality. After 14 days, survivors from all dosage groups were weighed and fed additional bait containing a further 10 LD_{50}. Mortality for these treatments ranged from 67% to 100%, but mice consuming dosages equivalent to 12 LD_{50} (two individuals) and 13 LD_{50} (three individuals) survived despite consuming at least 4.8 mg kg^{-1} of brodifacoum. These survivors were still alive after 23 days (five days longer than any animal that died) and all appeared healthy, with no signs of bleeding or lethargy. These survivors did not originate from any particular location, but were captured in locations throughout the settlement including the nursery and waste management facility. These individuals were euthanized at the conclusion of the study, a condition of the Animal Ethics approval. The survival of these individuals demonstrated that some mice have developed a high level of tolerance to brodifacoum, but it is not firm evidence of complete resistance as it is possible that these individuals would have succumbed to higher doses of brodifacoum. In a similar study involving mice on Gough Island, two individuals (approximately 1% of those tested) survived after apparently ingesting doses of brodifacoum estimated to be 5 and 10 times the oral LD_{50} for the population, but subsequent exposure at higher doses resulted in mortality (Cuthbert *et al.* 2011). On Lord Howe Island, 28 mice that survived low doses of brodifacoum, died after subsequent feeding with the same toxic bait. Importantly, no mouse exhibited any inhibition to consume additional bait following its initial exposure to brodifacoum.

From the observations above, the observed LD_{50} for mice on Lord Howe Island was approximately five times the standard LD_{50} for mice, with some individuals showing a high level of tolerance, up to at least 13 LD_{50} (5.2 mg kg^{-1}). Although the LD_{50} for mice (0.4 mg kg^{-1}) was that reported for laboratory mice, similar values have been obtained for wild populations (0.52 mg kg^{-1}, O'Connor and Booth (2001); 0.44 mg kg^{-1}, Cuthbert *et al.* (2011)). The unusually high LD_{50} for mice on Lord Howe Island indicates that this population exhibits increased tolerance to brodifacoum. Based on an observed LD_{50} of 2.0 mg kg^{-1}, an average body weight of 16.5 g and a brodifacoum concentration of 18.2 ppm (this study), the average mouse on Lord Howe Island (in terms of both size and susceptibility) would need to consume 1.8 g of

bait to ingest a lethal dose. Mice typically consume approximately 3 g of food per day, in many small meals of up to 0.2 g (Morriss *et al.* 2008; Wade 2011). Thus, the typical mouse on Lord Howe Island could consume a lethal dose in one day, requiring up to nine meals to do so. However, the dosage needed to kill all mice on Lord Howe Island (LD_{100}) is at least 15 LD_{50}. Based on an observed LD_{100} of 6.0 mg kg^{-1} and a maximum body weight of 22 g (this study), the largest and least susceptible mouse on Lord Howe Island would need to consume at least 7.3 g of bait to ingest a lethal dose. This would take at least 37 meals or 3 days to complete, longer if alternative food was also eaten.

In August 2008, non-toxic Pestoff® 20R baits distributed at a density of 10 kg ha^{-1} within the palm forest on Lord Howe Island remained available above ground for at least seven days (Wilkinson *et al.* 2008). In these circumstances, bait would be available long enough for mice to find and consume a lethal quantity of bait following a single application. However, in sites with a high density of non-target consumers of bait (e.g. ducks and rails) bait may disappear much faster. In these situations, higher dose rates or multiple bait applications may be needed to increase the likelihood of mice receiving a lethal dose.

Efficacy of Brodifacoum in Eradicating Mice from LHI

Mice on LHI, at least those associated with the human environment, are less susceptible to brodifacoum than mice in other parts of the world. Although tolerance to the poison in a proportion of those mice used in the feeding trial was high, this, in itself, does not mean that some mice will survive baiting LHI with brodifacoum. However, it is crucial that further feeding trials are conducted before the eradication programme is undertaken. Not only should mice distant from human habitation be tested to determine how widespread this tolerance may be, but further tests should be conducted on mice from the settlement to gauge what is the minimum exposure to brodifacoum required to kill all mice. The feeding trial conducted in 2013 produced 100% mortality in those mice fed the equivalent of 15 LD_{50} but the sample size was small, too small to assume that the most tolerant mouse on LHI will succumb to such a dose.

Rats on LHI are susceptible to relatively small doses of brodifacoum, so it is likely that this species will be eradicated if all rats encounter baits. However, this is not necessarily so for mice. If rats are eliminated but not mice then there is likely to be:

- Increased seabird, and possibly land bird, numbers; e.g. Grey Ternlet and Little Shearwater. Note landbirds would no longer have the same predation pressure but will still have competition for food from mice. As mouse numbers are likely to significantly increase without rat predation, possibly decreasing the amount of food available for birds, the actual benefit is unknown.
- Likely recolonisation of the island by the Kermadec Petrel.
- Allow consideration of introducing closely related surrogate species to replace those driven to extinction by rats and or humans.
- Possibly some increase in recruitment by some tree species. Trials are currently being carried out to try to quantify this although removing rats will alter the dynamic with mice allowing them to potentially have a greater impact.
- Probable increase in the number of arboreal invertebrate species as mice seldom venture higher than one metre up into vegetation, therefore the successful re-introduction of the LHI Phasmid is feasible.
- Little if any change in most terrestrial invertebrate numbers as ground-dwelling invertebrates will still be vulnerable to rodent predation.
- Little change in recruitment by most plant species.
- Need for ongoing mouse control around the settlement and possibly key ecological sites.
- Likely increase in mouse numbers due to the absence of rat predation on mice. The relative impact of this is likely to increase as poison tolerance in mice increases.
- Some members in the community will see the whole project as a failure as the promoted social gains will be significantly reduced.
- Reduced community support for the required ongoing biosecurity systems.
- Unlikely to get political or social support for a mouse eradication in the foreseeable future (assuming any such eradication using a non anti-

coagulant poison would be possible, or any such eradication proposal would not elicit the same level of opposition as the current one).

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Recommendations

- A similar feeding trial to the one undertaken in 2013 is conducted on mice obtained from locations that are unlikely to have been subjected to brodifacoum baiting;
- A feeding trial is conducted on mice obtained from the same areas as those mice used in 2013 so as to determine the unequivocal LD_{100} dose;
- If brodifacoum resistance is only found in the settlement mice than consideration is given to increasing the concentration of brodifacoum in baits used in the settlement to the level of 50 parts per million (as per the baits currently used); and
- If brodifacoum resistance is only found in the settlement mice than a feeding trial involving brodifacoum and another poison (e.g., flocoumafen) is conducted on mice to determine the efficacy of using a combination of poisons.

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Appendix 1

The following is the manuscript detailing the feeding trials undertaken on Lord Howe Island in 2013. The manuscript was submitted to, but rejected by, Australian Wildlife Research.

The two referees that assessed the manuscript stated that there was insufficient evidence submitted by the authors to validate their assertion that the reduced susceptibility of the mice to brodifacoum on the island was due to long-term exposure to this poison. However, one referee did say “Most of the resistance problems in rodents has developed following the prolonged use of ineffective anticoagulants, in particular the first generation anticoagulants, and more recently, the less toxic second generation anticoagulants, bromadiolone and difenacoum.”

“In both species (*Brown Rats and House Mice*) a single dominant autosomal gene has been identified (the VKORC1 gene), mutations of which can confer a degree of resistance to anticoagulants, with a considerable degree of cross resistance between active ingredients. ……..”

“A low incidence of these genes appear to be present in many populations of rodent, and ineffective use of anticoagulant rodenticide raises the incidence of the gene in the population, selectively killing susceptible animals, and thus creating a resistance problem. Furthermore, the selection of a particular VKORC1 gene that confers a high degree of resistance to a second generation anticoagulant can be achieved using a first generation anticoagulant. It is not necessary for there to be a link between the toxicity of the anticoagulant used and the magnitude of the resistance selected.”

"The occurrence of high levels of resistance across Europe is primarily the result of the widespread use of ineffective active ingredients (initially from the use of first generation anticoagulants, and more recently bromadiolone and difenacoum). Currently, the most effective anticoagulants, brodifacoum, flocoumafen and difethialone, cannot be used in and around farm buildings and along hedgerows in the UK, **and there is a strong belief that the use of both brodifacoum and flocoumafen** could eradicate these highly resistant populations of Brown Rats."

One referee criticised the lack of a control treatment in the second part of the feeding trial. Although this is technically correct, the lack of a control does not invalidate the findings. A control group would be important if all the poisoned mice died but there were several survivors. Death occurring in any such control group would merely suggest that some deaths in the poisoned group may be due to other causes besides brodifacoum.

The following manuscript may be amended by the authors to cover the concerns expressed by the referees. As such it is not for general distribution but only for the information of the LHIB. It can be cited as *Priddel, Wheeler, Carlile and Wilkinson unpublished data*.

Resistance to second-generation anticoagulants adds to the challenge of eradicating exotic rodents on inhabited islands

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Abstract

Eradication of exotic rodents has become a powerful tool to prevent species extinctions and to restore degraded insular ecosystems. Current eradication techniques utilise rodenticide baits containing second-generation anticoagulant poisons. Success is dependent on all targeted individuals consuming toxic bait and dying as a result thereof. The long-term use of anticoagulant rodenticides to control commensal rodents on inhabited islands is likely to lead to local populations of these pests developing inherent resistance to anticoagulants. On Lord Howe Island, reduced susceptibility of mice to brodifacoum (a five-fold increase in the nominal LD_{50}) makes the planned task of eradication more challenging and increases the potential risk of failure. To ingest a lethal dose, some mice on Lord Howe Island will require numerous feeds, over many days. Current rodent-control practices on the island are likely to lead to further reduction in susceptibility to anticoagulants, eventually rendering these poisons ineffective and leaving no means of eradicating or controlling rodents on the island, with potentially catastrophic ecological and social impacts. Widespread resistance to anticoagulants could render current eradication techniques ineffective on islands with a history of rodenticide use. Possible modifications to current techniques include lengthening the period that bait is available to the target animal or using bait with a higher concentration of anticoagulant. Both changes increase the potential risk to non-target species and, on inhabited islands, have possible social ramifications.

Introduction

The presence of invasive rodents on islands is one of the prime causes of species extinction and ecosystem degradation (Groombridge 1992; Towns *et al.* 2006). Rats (*Rattus* spp) and house mice (*Mus musculus*) prey heavily on birds, bats, reptiles, snails, insects and other invertebrates (Atkinson 1985; Cuthbert and Hilton 2004; Towns *et al.* 2006). They consume vast quantities of seeds and

seedlings, severely reducing seedling recruitment and modifying vegetation communities (Rance 2001; Shaw *et al.* 2005; Brown *et al.* 2006). The loss of invertebrate fauna involved in plant decomposition or nutrient recycling can have devastating effects on soil fertility (Fukami *et al.* 2006). Similarly, suppression of seabird numbers by invasive rodents can result in a significant loss of marine-derived nutrients in the form of droppings, regurgitations, failed eggs and corpses, which in turn can profoundly affect the health and condition of island ecosystems (Holdaway *et al.* 2007).

Recognising the devastating impacts of invasive rodents on island ecosystems, conservation practitioners have developed techniques to eradicate these pests from islands. Rodents have been removed from at least 284 islands worldwide (Howald *et al.* 2007), and eradication has become a powerful tool to prevent species extinctions and to restore degraded insular ecosystems (Towns and Broome 2003). First developed in New Zealand in the 1980s (Moors 1985; Taylor and Thomas 1989), current rodent eradication techniques rely on the use of rodenticide baits containing anticoagulant poisons; substances that act by effectively blocking the production of vitamin-K in the liver, thereby reducing the ability of the blood to clot (Samama *et al.* 2003). Bait dispersal methods utilising novel computerised tracking and mapping technology (Lavoie *et al.* 2007) have improved to such an extent that eradications are now being attempted on increasingly larger and more complex islands, including those with human populations.

The success of any rodent eradication operation is dependent on all targeted individuals consuming toxic bait and dying as a result thereof. Anticoagulant rodenticides are freely available and commonly used throughout the world to control commensal rodents, and the sustained use of these products has seen the development of resistance in rodent populations worldwide (Bailey and Eason 2000; Pelz *et al.* 2005). Greaves (1994) described anticoagulant resistance as a major loss of efficacy in practical conditions where the anticoagulant has been applied correctly, the loss of efficacy being due to the presence of a strain of rodent with a heritable and commensurately reduced sensitivity to the anticoagulant. Rodents that are tolerant of a particular anticoagulant can still be killed by it, but population control or eradication generally requires ever-increasing doses to be efficacious. Over time, it becomes increasingly impractical to deliver a lethal dose and consequently the anticoagulant loses its utility for rodent control.

The use of anticoagulant rodenticides to control commensal rodents on inhabited islands could potentially lead to local populations of these pests developing resistance to anticoagulants. The current suite of second-generation anticoagulants is the only proven tool available for effectively eradicating rodents from islands. Reduced susceptibility to these compounds will make eradication challenging or impossible. Furthermore, if resistance to anticoagulants develops in island populations of invasive rodents there may be no effective way to control them, with potentially catastrophic environmental and social impacts.

The eradication of rodents from Lord Howe Island using brodifacoum baits is planned (LHIB 2009). The aim is to kill every rat and mouse on the island in a single operation that involves the distribution of baits containing brodifacoum (a potent second-generation anticoagulant) to all parts of the island in two applications several weeks apart. Specific measures will be undertaken to mitigate the risk to humans, pets, livestock and non-target species. Although challenging, such an operation is logistically feasible (Saunders and Brown 2001), provided that the populations of rats and mice remain susceptible to brodifacoum.

This study examined the susceptibility of both rats and mice on Lord Howe Island to brodifacoum by assessing the amount rodents needed to ingest to cause death. It also determined the time interval between ingestion and death, information that would help to identify the optimal time interval between sequential applications of bait.

Methods

Study Site

Lord Howe Island (31°31'S, 159°03’E), New South Wales, Australia, is located 760 kilometres north east of Sydney. The island is 1455 ha in area, 12 km long, 1–2 km wide and formed in the shape of a crescent with a coral reef enclosing a lagoon on the western side. Mount Gower (875 m), Mount Lidgbird (777 m) and Intermediate Hill (250 m) form the southern two-thirds of the island, which is

extremely rugged. The northern end of the island is fringed by sheer sea cliffs approximately 200 m in height.

The environmental significance of Lord Howe Island was formally recognised in 1982 when the entire island group was inscribed on the World Heritage Register for containing (i) superlative natural phenomena; (ii) areas of exceptional natural beauty and aesthetic importance; and (iii) important and significant natural habitats for the conservation of biological diversity, including threatened species of outstanding universal value (Deparment of the Environment 2013). Lord Howe Island is a hotspot for endemism; 44% of native plants and more than 50% of native invertebrates are endemic (Recher and Clark 1974; Green 1994).

Lord Howe Island falls under the jurisdiction of the New South Wales Government. The Lord Howe Island Board is responsible for the care, control and management of the island in accordance with the Lord Howe Island Act 1953. Approximately 75% of the main island plus all outlying islets and rocks within the Lord Howe Group are protected under the Permanent Park Preserve, which has similar status to that of a national park. First permanently settled in 1833, the resident population is now approximately 350 in 150 or so households. Lord Howe Island is the only island within the Lord Howe Group on which settlement has occurred. The settlement is restricted to the central lowlands and covers about 15% of the island. Islanders were given perpetual leases on blocks of up to 2 ha for residential purposes, and short-term leases on larger tracts for agricultural and pastoral activities (Hutton 1998). Today, there are approximately 1000 buildings or structures on the island.

Tourism is the island's major source of income. The island contains an airstrip with frequent commercial air services to Sydney and Brisbane. About 16 000 tourists visit the island each year, but numbers are regulated, with a maximum of 400 visitors allowed on the island at any one time. Until recently, the Lord Howe Island Board operated a nursery that produced and exported 2–3 million palm seedlings annually. The local palm industry was a prime source of revenue for the island, but the nursery closed in 2012, and its future is uncertain.

Two species of rodent—black rat (*Rattus rattus*) and house mouse (*Mus musculus*)—have been accidentally introduced to Lord Howe Island; mice probably around 1860, and rats in 1918. These pests have reduced, and continue to erode, the island's intrinsic biodiversity values (DECCW 2010), potentially threatening its World Heritage status. Predation by black rats on Lord Howe Island is listed as a *Key Threatening Process* under the environmental legislation of both national (Australia) and state (New South Wales) governments. Rodents also infest buildings and residences where they are a social nuisance and a threat to human health, destroying foodstuffs and contaminating homes with excrement. Rats also damage the kentia palm (*Howea forsteriana*), which resulted in economic losses to the local palm industry before it recently shut down.

Capture of rodents

Commensal rodents were captured from within the settlement; rats (n = 50) by the use of cage traps and mice (n = 50) using metal box traps (Elliott Scientific Equipment, Upwey, Victoria). Traps were placed throughout the settlement but concentrated in public areas with a long history of brodifacoum use, such as the nursery and the waste management facility. Traps were opened shortly before sunset and baited with a mixture of peanut butter and rolled oats. Traps were emptied and closed soon after sunrise. Trapping was conducted during 23–29 July 2013, eight weeks after routine broad-scale baiting. Captured rodents were transported back to the Lord Howe Research Centre in the trap, shielded from daylight, noise and wind inside a lidded plastic tub. Each individual was then weighed and housed separately in a polypropylene cage with a stainless steel lid (rat box RB-001and high top lid RL-001, mouse box MB-001-PP and lid ML-002; R.E. Walters Pty Ltd, West Sunshine, Victoria). Internal dimensions of cages were approximately 42 x 28 x 25 cm for rats and 29 x 16 x 18 cm for mice. All individuals had access to water from a polypropylene bottle fitted with a stainless steel sipper tube (600 ml for rats and 250 ml for mice; R.E. Walters Pty Ltd, West Sunshine Victoria) and feed pellets formulated for rodents (Rat and Mouse Nut, Vella Stock Feeds, Plumpton). A cardboard tube cut to form a half-cylinder was provided for shelter, along with shredded paper for bedding, and small blocks of wood to chew. The room holding the cages was maintained at ambient temperature and with natural light cycles, but windows were covered to block direct sunlight.

Resistance testing

The toxicity of a substance is usually expressed as the median lethal dose required to kill half the members of a population (LD_{50}) and is measured as the mass of substance per unit body mass of the animal. For brodifacoum the generally accepted acute oral LD_{50} for laboratory or brown rats (*Rattus norvegicus*) is 0.27 mg kg^{-1}, and for mice is 0.4 mg kg^{-1} (Redfern *et al.* 1976; Godfrey 1985). Hereafter, we refer to these values as the nominal LD_{50} (nLD_{50}). Although the published LD_{50} for black rats (*R. rattus*) is higher than that for brown rats, the lower LD_{50} value was used with the objective of determining the very minimal effective lethal dose required to kill rats on Lord Howe Island. Acute oral LD_{50} values for a particular species can vary depending on the laboratory procedures used and the population tested, thus toxicity values are indicative rather than absolute.

Food consumption by each captured individual was monitored until the animal was confirmed to be eating (0–2 days). Ten individuals of each species were then randomly assigned to one of four treatments that were fed cereal bait (Pestoff® 20R, Animal Control Products, Wanganui, New Zealand), the amount of bait varying among treatments such that different amounts of brodifacoum (1, 2, 3 and 5 times the relative nLD_{50}) were on offer. After the toxic bait was consumed (typically within 24 hours of it being offered) feeding with non-toxic food recommenced. The efficacy of each dosage was assessed by the percentage mortality. Another 10 individuals of each species were used as controls and were fed non-toxic pellets *ad libitum*.

All individuals were observed at 6-hourly intervals for signs of brodifacoum toxicosis including: pale extremities, bleeding from orifices, hunched posture, paresis, paralysis, prostration and death. Symptoms and time to death were recorded. As a requirement of Animal Ethics approval, any individual rendered prostrate by the effects of the poison was observed hourly, and if it remained prostrate for 3 hours it was euthanized. After death, all individuals were examined for internal bleeding.

The control group and some individuals receiving low dosages of brodifacoum were expected to survive. After 14 days, these individuals were weighed and fed additional bait containing the equivalent of 10 nLD_{50} for the respective test species. Observations of these individuals continued for a further 23 days.

Brodifacoum content of bait

Pestoff® 20R contains brodifacoum at a nominal concentration of 20 mg kg^{-1} (20 parts per million (ppm)). Twelve individual pellets (5.5 mm diameter, 0.5–0.8 g) were assayed for brodifacoum content by the Landcare Research toxicology laboratory, Lincoln, New Zealand using method TLM017 (the assay of brodifacoum baits and concentrates by high-performance liquid chromatography) based on the methods of Hunter (1983) and ICI (1983).

Results

Mortality

For rats, mean mass at the time of capture was 196.1 ± 44.8 g (range: 110–275 g). Ingestion of brodifacoum at a dose rate of 1 nLD_{50} resulted in no mortality (Table 1). Twice this dose rate (2 nLD_{50}) resulted in 60% mortality. Three or more nLD_{50} produced 100% mortality. After 14 days, survivors from the control and low-dosage groups were weighed and fed additional bait containing a further 10 nLD_{50}. Resultant mortality was 100% (Table 1). From these observations we conclude that the observed LD_{50} for Black Rats on Lord Howe Island was roughly twice the nLD_{50}, the latter being equivalent to the LD_{50} of the Brown Rat.

For mice, mean mass was 16.5 ± 2.5 g (range 11.0–22.0 g). Ingestion of brodifacoum at dose rates 1 and 2 nLD_{50} resulted in no mortality (Table 2). A dose rate of 3 nLD_{50} resulted in 10% mortality, and 5 nLD_{50} resulted in 60% mortality. After 14 days, survivors from all dosage groups were weighed and fed additional bait containing a further 10 nLD_{50}. Mortality for these treatments ranged from 67% to 100%, but mice consuming dosages equivalent to 12 LD_{50} (2 individuals) and 13 LD_{50} (3 individuals) survived (Table 2). These survivors were still alive after 23 days (5 days longer than any animal that died) and all appeared healthy, with no signs of bleeding or lethargy. These survivors did not originate from any particular location, but were captured in locations throughout the settlement including the nursery and waste management facility.

From the observations above we conclude that the observed LD_{50} for mice on Lord Howe Island was approximately five times the nLD_{50}, with some individuals showing a high level of tolerance, up to at least 13 nLD_{50} (5.2 mg kg^{-1}).

Time to death

For both rats and mice, the interval between ingestion and death was independent of the amount of brodifacoum consumed (rats: $F_{5, 44} = 0.2580$, $P = 0.933$; mice: $F_{5, 37} = 0.7714$, $P = 0.576$), so data from all dosages were combined. Rats died 3–13 days after ingestion of the bait (mean 6.9 ± 1.9 days, n = 50, Figure 1); mice died 1–18 days after ingestion (mean 7.3 ± 3.9, n = 44, Figure 2). Time to death was similar for both species ($t = 0.5729$, $P = 0.569$).
Mean time to death may be a slight underestimate because five rats and four mice were euthanized once rendered prostrate by the effects of the anticoagulant.

Brodifacoum content of bait

The assayed concentration of brodifacoum in baits (Figure 3) was 16–22 ppm (μg g^{-1}). The 95% confidence interval was ± 7%, equivalent to ± 1 ppm. Mean brodifacoum concentration was 18.2 ± 1.6 ppm, close to the nominal concentration of 20 ppm.

Discussion

Rats

This study has demonstrated that the dose of brodifacoum needed to kill 50% of the rats on Lord Howe Island (LD_{50}) is roughly twice the nominal LD_{50} (nLD_{50}) for rats. The nLD_{50} for rats was measured using laboratory brown rats. The LD_{50} for a laboratory population of black rats is 0.65 mg kg^{-1} for females and 0.73 mg kg^{-1} for males (Dubock and Kaukeinen 1978) and 0.46–0.77 mg kg^{-1} for wild populations (Mathur and Prakash 1981; O'Connor and Booth 2001), all similar to that obtained in this study (0.54 mg kg^{-1}). Thus, rats on Lord Howe Island show no signs of having developed increased tolerance to brodifacoum. Based on an observed LD_{50} of 0.54 mg kg^{-1}, an average body weight of 196 g and a brodifacoum concentration in bait of 18.2 ppm (this study), the average rat on Lord Howe Island (in terms of both size and susceptibility) would need to consume 5.8 g of bait to ingest a lethal dose. The dosage needed to kill all rats on Lord Howe Island (LD_{100}) is roughly three times the nLD_{50} for rats. Based on an observed LD_{100} of 0.81 mg kg^{-1} and a maximum body weight of 275 g (this study), the largest and least susceptible rat on Lord Howe Island would need to consume 12.2 g of bait to ingest a lethal dose. An adult rat will typically eat 25–30 g of food per day, taken in about ten small meals, with the maximum consumption per meal of around 3 g (Wade 2011). Thus all rats on Lord Howe Island could consume a lethal dose in one day, but may require four or five meals to do so.

Mice

The dose of brodifacoum needed to kill 50% of the mice on Lord Howe Island (LD_{50}) is roughly five times the nLD_{50}. Although the nLD_{50} for mice (0.4 mg kg^{-1}) was measured using laboratory mice, similar values have been obtained for wild populations (0.52 mg kg^{-1}, O'Connor and Booth (2001); 0.44 mg kg^{-1}, Cuthbert *et al.* (2011)). The unusually high LD_{50} for mice on Lord Howe Island indicates that this population has developed increased tolerance to brodifacoum. Based on an observed LD_{50} of 2.0 mg kg^{-1}, an average body weight of 16.5 g and a brodifacoum concentration of 18.2 ppm (this study), the average mouse on Lord Howe Island (in terms of both size and susceptibility) would need to consume 1.8 g of bait to ingest a lethal dose. Mice typically consume approximately 3 g of food per day, in many small meals of up to 0.2 g (Morriss *et al.* 2008; Wade 2011). Thus, the typical mouse on Lord Howe Island could consume a lethal dose in one day, requiring up to nine meals to do so.
The dosage needed to kill all mice on Lord Howe Island (LD_{100}) is at least 15 nLD_{50}. Based on an observed LD_{100} of 6.0 mg kg^{-1} and a maximum body weight of 22 g (this study), the largest and least susceptible mouse on Lord Howe Island would need to consume at least 7.3 g of bait to ingest a lethal dose. This would take at least 37 meals or 3 days to complete, longer if alternative food was also eaten. In August 2008, non-toxic Pestoff® 20R baits distributed at a density of 10 kg ha^{-1} within the palm forest on Lord Howe Island remained available above ground for at least 7 days (Wilkinson *et al.* 2008). In these circumstances, bait would be available long enough for mice to access and consume a lethal quantity of bait following a single application. However, in sites with a high density of non-target consumers of bait (e.g. ducks and rails) bait may disappear much faster. In these situations, higher dose rates or multiple bait applications may be needed to increase the likelihood of mice receiving a lethal dose.

Five mice survived the study despite consuming at least 4.8 mg kg^{-1} of brodifacoum (Table 2). These individuals were euthanized at the conclusion of the study, a condition of the Animal Ethics approval. The survival of these individuals demonstrated that some mice have developed a high level of tolerance to brodifacoum, but it is not firm evidence of complete resistance as it is possible that these individuals would have succumbed to higher doses of brodifacoum. In a similar study involving mice on Gough Island, two individuals (approximately 1% of those tested) survived after apparently ingesting doses of brodifacoum estimated to be 5 and 10 times the oral LD_{50} for the population, but subsequent exposure at higher doses resulted in mortality (Cuthbert *et al.* 2011). On Lord Howe Island, 28 mice that survived low doses of brodifacoum, died after subsequent feeding with the same toxic bait. Importantly, no mouse exhibited any inhibition to consume additional bait following its initial exposure to brodifacoum.

Time to death

The ingestion of a sufficient amount of brodifacoum can lead to death through internal haemorrhaging, which typically takes 3–10 days in rats (Hadler and Shadbolt 1975) and a few days longer in mice (Fisher 2005). For rats on Lord Howe Island, time to death following exposure averaged 6.9 ± 1.9 days, marginally less than that reported for this species in another study: 8.5–11.0 days (Lund 1981). For mice, time to death averaged 7.3 days, within the range reported for this species in other studies: 5.2 days (Cleghorn and Griffiths 2002), 5.5 days (Cuthbert *et al.* 2011) and 7.1–11.0 days (Lund 1981). Necropsy findings of free or clotted blood in the thoracic and/or abdominal cavity, kidney and subcutaneous tissues are consistent with the anticoagulant mode of action of brodifacoum. The rigours of living in the wild would probably reduce the time to death, as poisoned individuals would be exposed to movements and minor injuries that would probably exacerbate the likelihood of fatal haemorrhage caused by poisoning (Morriss *et al.* 2008).

Worldwide development of resistance

Anticoagulant rodenticide resistance is a worldwide phenomenon (Pelz *et al.* 2005) that occurs after sustained use of anticoagulant poisons for rodent control (Bailey and Eason 2000). Resistance to warfarin was first discovered in brown rats in Britain in 1958 (Boyle 1960), and in house mice shortly thereafter (Dodsworth 1961). Resistance to this and other first generation anticoagulants is now widespread across the globe and involves all three common commensal species: brown rat, black rat and house mouse (see review in Lund (1984)).

Second-generation anticoagulants initially proved effective at controlling rodents that were resistance to earlier anticoagulants. But within two decades, resistance to these more-potent second-generation anticoagulants was reported (Redfern and Gill 1978). Resistance to both bromadiolone and difenacoum has since been widely reported for brown rats, (e.g. Greaves 1994), black rats (e.g. Desideri *et al.* 1979) and house mice (e.g. Rowe *et al.* 1981; Siddiqi and Blaine 1982). Resistance to brodifacoum is less prevalent, possibly because significant constraints restrict the use of this substance in many countries. Notwithstanding, some degree of cross-resistance occurs (Lund 1984)) and increased tolerance to brodifacoum has been observed in brown rats (Greaves *et al.* 1982; Gill *et al.* 1992) and house mice (Siddiqi and Blaine 1982).

Development of resistance on Lord Howe Island

Mice on Lord Howe Island developed resistance to warfarin sometime before 2000, less than two decades after systematic baiting began. Little more than a decade later, the same population has developed a tolerance to brodifacoum, the most potent anticoagulant rodenticide available. This tolerance has developed through long-term exposure to bait containing brodifacoum (at the concentration of 50 parts per million) distributed throughout the settlement.

The potential for resistance to second-generation anticoagulant poisons to develop on Lord Howe Island has long been recognised. In 2001, an evaluation of the feasibility of eradicating rodents from Lord Howe Island (Saunders and Brown 2001) recommended that the ongoing use of brodifacoum baits be stopped to avoid the potential for resistance in the rodent population to develop. In 2009, the draft eradication plan (LHIB 2009) reiterated the same concerns.

Use of anticoagulants on Lord Howe Island

Widespread rodent control has occurred on Lord Howe Island for the past 90 years, aimed largely at reducing damage to the kentia palm seed, although more recently it has also been used for conservation purposes in specific areas. The use of warfarin, a first-generation anticoagulant, to control rats in palm

seeding areas began in the early 1960s (Harden and Leary 1992). Diphacinone was also trialled, but was withdrawn because of concerns of the risk to non-target birds (Harden and Leary 1992). In 1980, a more systematic control programme using warfarin began, but because the baits were simply placed out on the ground in sheltered sites, concerns about the risk to birds led to this programme being abandoned (Harden and Leary 1992). In 1986, baiting with warfarin was re-instigated, but this time in association with the use of bait stations. While changes have been made to the type of bait and baiting frequency, the locations targeted for control have remained essentially the same, albeit with a few minor additions.
Nowadays, approximately 1000 permanent bait stations are dispersed among 33 separate patches of palm forest around the island, covering a total area of approximately 140 ha (approximately 10% of the island). Between 1986 and 2009, approximately 119 tonnes of bait containing 83 kg of warfarin was distributed on the island (LHIB 2009). Initially, bait was available continuously. However, the mice developed resistance to warfarin and were feeding on the bait, which was being distributed in ever-increasing quantities of up to 7 tonnes per annum (Billing 2000; Billing and Harden 2000). To counter the mice, baiting frequency was reduced such that bait was available only intermittently. Bait is now replenished six times per annum (approximately every 8–9 weeks), and the amount of bait now dispersed is approximately 1.2 tonnes per annum (LHIB 2009). In 2012, the Lord Howe Island Board changed to using coumatetralyl, another first-generation anticoagulant but which has lower toxicity to birds.

In addition to protecting the palm seed crop, the Lord Howe Island Board also undertakes rodent control at strategic locations within the settlement, primarily at the waste management facility and, until recently, the now-defunct commercial palm nursery. First-generation anticoagulant baits (currently coumatetralyl, previously warfarin) are used to control rats, and second-generation anticoagulant baits (brodifacoum 50 ppm) used to control mice. Until the nursery closed in 2012, approximately 100 kg of brodifacoum-based bait was used annually (LHIB 2009).

Baiting with anticoagulants has long been undertaken by the Lord Howe Island community to reduce the social impacts of rats and mice within the area of human habitation. Residents use coumatetralyl (previously warfarin) bait supplied by the Lord Howe Island Board as well as brodifacoum and other second-generation anticoagulant baits purchased from shops on the island and on the mainland. The amount of bait supplied to residents by the Lord Howe Island Board was estimated at approximately 380 kg per annum (Saunders and Brown 2001). In the absence of any records, the quantity of brodifacoum-based rodenticide used by residents on the island is difficult to determine, but probably exceeds 100 kg per annum (LHIB 2009).

Based on the usage estimates above, the Lord Howe Island Board and local community together distributed a total of approximately 2.6 tonnes of brodifacoum baits within the settlement between 2000 and 2012. Although usage by the Board has declined significantly since the closure of the nursery, use of brodifacoum baits by the Lord Howe Island community continues largely unabated.

Conservation implications

Eradication of exotic rodents on Lord Howe Island will deliver significant biodiversity benefits to the local ecosystem (LHIB 2009), and end the ongoing use of rodenticides on the island. The presence of mice that are tolerant to brodifacoum increases both the difficulty of eradicating this species from the island and the potential risk of failure. The objective, however, remains unchanged—to provide each individual rodent on the island with access to a lethal dose of bait. This study has provided the first experimental estimate of the size of that lethal dose.

Mice on Lord Howe Island are known to be resistant to warfarin (Billing 2000), but this study provides the first evidence that they have also developed a tolerance to brodifacoum. This situation is already parlous but will get worse if the current use of anticoagulants continues. Extensive and prolonged use of resisted compounds increases the severity of the resistance as the baiting programme selects for the most resistant individuals. Experience from Britain (Buckle 2013) suggests that, within a decade or so, anticoagulants will soon prove ineffective on Lord Howe Island, leaving no other means to effectively control mice on the island. This will have both biodiversity and social costs. For example, resistant mice containing high concentrations of anticoagulants spread to control rats would increase the risk of secondary poisoning of native predators and scavengers, and companion dogs. Also, businesses such as shops and restaurants may be unable to fulfil their statutory obligations with respect to human health.

Reduced susceptibility of mice to brodifacoum may also reduce the effectiveness of the use of anticoagulants to control rats. Baiting would provide resistant mice with a supplementary food resource that may enable them to sustain higher population numbers than they otherwise would. By consuming large quantities of bait, resistant mice would reduce the amount of rodenticide available to rats, leading to a situation where more and more rodenticide has to distributed to maintain the same level of control on rat numbers; a scenario that mirrors the history of warfarin use on Lord Howe Island. Also, if current practices persist, rats are also likely to further increase their tolerance to anticoagulants, as has occurred elsewhere (Pelz *et al.* 2005), with catastrophic results for biodiversity and tourism as well as the general well-being of the islanders.

Conclusions

This study has (1) confirmed that on Lord Howe Island rats are more susceptible to brodifacoum than mice; (2) demonstrated that mice on Lord Howe Island have a much greater variability in susceptibility to brodifacoum than do rats, and (3) identified low susceptibility to brodifacoum by a small proportion of the mouse population. In essence, mice on Lord Howe Island will need to consume relatively large amounts of brodifacoum over several days for it to be fatal, and thus mice will be much more difficult to eradicate than rats. Consequently, a priority objective for the proposed eradication on Lord Howe Island must be to maintain a continuous supply of bait for long enough to ensure that the entire mouse population has ample opportunity to ingest a lethal dose.

Globally, the failure rate for mouse eradications is greater than that for rats (MacKay *et al.* 2007). Mice have smaller home ranges than rats (MacKay 2011) so are less likely to have access to bait dispersed thinly or unevenly. Mice also have a higher natural tolerance and greater individual variability in susceptibility to anticoagulants. Mice also appear to have a high propensity to develop inherent resistance. These traits make them difficult to eradicate, particularly on islands with a long history of anticoagulant use.

Techniques to eradicate rodents from islands have essentially been designed for rats. Anticoagulant baits for aerial dispersal, for example, have been formulated primarily for highly susceptible rats on islands with little or no history of rodenticide use. Eradications targeting mice (or resistant rats) should consider the use of higher concentrations of brodifacoum to increase the likelihood of all individuals obtaining a lethal dose when small quantities of bait are consumed. This option would need to be considered in relation to the increased risks to non-target species, particularly those that are not taken into temporary captivity during the eradication operation. If bait stations are used in particular areas, rather than hand- or aerial distribution, high toxicity baits could probably be used within these stations without significantly increasing the risk to non-target species.

Widespread use of anticoagulants on inhabited islands may mean that eradication techniques developed on uninhabited islands need to be modified on an island-by-island basis if they are to be effective on inhabited islands, or on islands with a long history of anticoagulant use. Second-generation anticoagulants are often described as single-feed rodenticides, i.e., a lethal dose is consumed in a single meal. This is seldom the case, but if baits are palatable and available in sufficient quantity, non-resistant individuals can generally consume a lethal dose in a single day, albeit over numerous feeds. Resistant individuals, however, will require many more feeds, spread over several days. Therefore, if eradication operations on rodent populations with any level of tolerance are to be successful, bait must be available over a sufficiently long period to enable a lethal dose to be consumed.

The possibility of some resistant rodents receiving a sub-lethal dose of poison emphasises the need to undertake a second or third application of bait. Undertaking multiple applications will provide the opportunity for the targeted species to consume repeat doses. However, to maximise bait availability for any initial survivors the second application of bait should not occur until after the majority of rodents that have consumed a lethal dose have died (up to 18 days for mice on Lord Howe Island). This study found that captive mice would readily consume bait after an initial sub-lethal exposure. The apparent absence of bait avoidance upon second exposure suggests no short-term inhibition to consume a second and toxic dose of brodifacoum. Whether or not wild mice, with access to alternative natural foods, behave similarly is unknown.

Although invasive rodents have been eradicated from approximately 300 islands worldwide (Howald *et al.* 2007), the use of anticoagulants, largely on inhabited islands, makes eradication much more

challenging. Also, time is of the essence. Rodents, particularly mice, can quickly develop resistance to even the most potent anticoagulants (Rowe *et al.* 1981; Siddiqi and Blaine 1982). Once rodents have developed a high level of resistance to these substances, the opportunity for both eradication and effective control is lost.

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Table 1. Mortality rate and interval to death for black rats following ingestion of various concentrations of brodifacoum

	x LD_{50}							Combined
	1	**2**	**3**	**5**	**10**	**1 + 10**	**2 + 10**	
Dosage (mg kg^{-1})	0.27	0.54	0.81	1.35	2.70	2.97	3.24	
Mortality ***n***	0% (10)	60% (10)	100% (10)	100% (10)	100% (10)	100% (10)	100% (4)	
Days to death **Mean ± SD** **Range** ***n***		7.5 ± 2.3 4–11 (6)	6.6 ± 0.7 6–8 (10)	6.7 ± 1.8 4–10 (10)	7.2 ± 2.4 5–13 (10)	6.7 ± 2.3 4–12 (10)	7.0 ± 1.4 5–8 (4)	6.9 ± 1.9 4–13 (50)

Table 2. Mortality rate and interval to death for house mice following ingestion of various concentrations of brodifacoum

	x LD_{50}									Combined
	1	**2**	**3**	**5**	**10**	**1 + 10**	**2 + 10**	**3 + 10**	**5 + 10**	
Dosage (mg kg^{-1})	0.40	0.80	1.20	2.00	4.00	4.40	4.80	5.20	6.00	
Mortality ***n***	0% (10)	0% (10)	10% (10)	60% (10)	100% (9)	100% (10)	80% (10)	67% (9)	100% (4)	
Days to death **Mean ± SD** **Range** ***n***			6.0 (1)	6.3 ± 2.6 3–10 (6)	8.1 ± 3.6 4–13 (9)	8.8 ± 5.5 1–18 (10)	5.5 ± 3.3 3–13 (8)	6.7 ± 2.7 3–11 (6)	7.8 ± 5.3 1–14 (4)	7.3 ± 3.9 1–18 (44)