Lord Howe Island Rodent Eradication Project

EPBC Public Environment Report December 2016

Appendix D - LHI Trials Package

D.1 LHI Non Toxic Bait Trial Report
D.2 LHI Bait Uptake Trial Report
D.3 LHI Placostylus Trial Report
D.4 LHI Bait Efficacy Trail Report
D.5 Efficacy of Pestoff 20R on LHI Mice
Executive Summary

In August 2007 a non-toxic bait trial was conducted at Lord Howe Island to support preparations for a planned eradication of ship rats (*Rattus rattus*) and mice (*Mus musculus*) that are widespread on the island and have significant adverse impacts. The study examined palatability of bait to rodents, risks posed to non-target species, bait longevity in the environment, and trialed the use of aerial baiting methodology which will be critical for an eradication attempt.

Palatability of baits to rodents was tested by baiting large (23 and 34 ha) areas with baits of two sizes (5.5 mm and 10 mm diameter pellets) at a rate of 13 and 9 kg/ha and then trapping animals over a 7 days period commencing 2 days after the bait drops. Baits were non-toxic and contained a biomarker which fluoresces under ultra violet light. Bait ingestion was confirmed by the presence of fluorescence in trapped rats and mice. Prior to baiting, each area was trapped for between 3 and 7 days and live captured rodents were ear marked and released. Residency of rodents on the trapping grids and thus access to bait prior to capture was assumed if trapped animals were ear marked. 83.9% of mice, and 87.5% of marked rats in the 5.5 mm bait area had eaten bait, and 100% of animals in the 10 mm bait area consumed bait. Robust comparison of the two rates of uptake was prevented by low capture rates with only 1 mouse and 9 rats were captured on the 10 mm grid areas. While results on bait uptake are equivocal, circumstances relating to those animals not consuming bait in the 5.5 mm suggest that bait palatability may not necessarily have been the reason for no observed uptake.

Non-target species were assessed for uptake by baiting a 30 ha area adjacent to the islands golf course with 5.5 mm bait at a rate of 10.1 kg/ha and capturing animals over the following 9 days.

Four bird species were shown to be at risk from the baiting, and would therefore be at risk during a poison drop. Of these, woodhens were the only threatened island endemic to test positive for bait uptake, and confirmed the view that they would be vulnerable during a bait drop. The threat posed to woodhens from a poison bait drop will necessitate the capture and holding of a significant proportion of the population in captivity for the duration of any eradication operation. The period of captivity will be determined by the time it takes for baits remaining in the environment after rodent deaths to breakdown to a stage where they are no longer a risk to non-target species.

Other threatened island endemics; currawongs, golden whistlers and silvereyes did not appear to ingest bait, notwithstanding the findings, currawongs are at high risk of secondary poisoning during any operation as they would prey on dead and moribund rats and mice. Consequently they would also be captive managed along with woodhens.
Several invertebrate species were observed either fluorescing under UV light indicating bait ingestion, or feeding on baits.

Condition of baits placed in cages in three habitat types was monitored over 55 days and indicated that the smaller 5.5 mm baits disintegrated at a faster rate than the 10 mm which would reduce the period any at risk non-target species were held in captivity during an eradication, and livestock in confined holding facilities.

Aerial baiting was shown to be an effective technique that could be utilised in an operation on Lord Howe Island. The trial provided an opportunity to establish the correct flight configuration: air speed and aperture ring size to produce the required flow rate of bait during operations. Methodologies for loading procedures, and determination of bait usage on flight runs were developed for use in future baiting operations.
Introduction

In common with many oceanic islands Lord Howe Island has unique faunal and floral assemblages, with high degrees of endemism. The introduction of house mice (*Mus musculus*) in 1860, and ship rats (*Rattus rattus*) in 1918 has had extensive adverse impacts on the natural flora, fauna and ecological processes on the island. Rats have been implicated in the decline and extinction of a number of bird, reptile and invertebrate species. They also have significant impacts on the vegetative parts of a number of plant species on the island. While the impacts of mice have not been intensively studied at Lord Howe Island evidence from other locations would suggest that they are likely to be significant predators of invertebrates, the eggs of smaller birds, and of plant seeds.

Attempts at control of rats have been attempted since shortly after their arrival in 1918. Since 1986 the Lord Howe Island Board has undertaken control at 33 sites on the island primarily to protect the palm industry which is heavily impacted by rats. While control may temporarily reduce number, it can not prevent the ongoing biodiversity impacts by both rats and mice (which are not controlled due to their resistance to the Warfarin used in the programme).

With developments in eradication techniques during the past 20 years, and in particular the use of aerial baiting methods, the eradication of both rodent species on Lord Howe Island in a single operation is considered feasible (Saunders and Brown 2001). To achieve this, while minimising impacts on native species, will require detailed technical and logistical planning. A single eradication operation would have a the major advantages of minimising disturbance to native wildlife, cost efficacy, and limiting the possibility of a dramatic mouse population increase which may occur in the absence of rats on the island.

A prerequisite of all eradications is that all target individuals must be put at risk by the methods used, and impacts on non-target species should be minimised. To this end, this study aims to: determine the palatability of proposed bait types to both rats and mice and assess the risk posed to non-target species. It will also determine the longevity of baits in the environment, and trial and refine aerial bait delivery for use on Lord Howe Island.

Methods

*Study Site*

Lord Howe Island (31°33'S, 159°05'E) is a crescent shaped, volcanic remnant on the Lord Howe Rise, approximately 600 km east of Port Macquarie, New South Wales. It is 1455 ha in area with very rugged relief, rising to 875 m in the south on the summit of Mount Gower. The central lowland areas have been cleared for agriculture or settlement and are dissected by a network of 11 km of
narrow roads. Patches of uncleared evergreen closed forest (Pickard 1983) adjoin grazing leases and urban settlement. Lord Howe Island was included in the World Heritage List in 1982.

Three baiting areas were chosen on the island, two of approximately 30 ha on Transit hill for the rodent trapping study and a third area (~30 ha) to the east of Intermediate hill used for non-target species capture (Fig. 1). Four trapping grids (numbered 1 to 4) of 49 Elliot traps and 49 cage traps spaced at approximately 10 m intervals (60 x 60 m) were established in the area to the east of Transit Hill (the 5.5 mm bait area) and three grids (numbered 5 to 7) on the western slopes of Transit Hill, the 10 mm bait area. Each of the trapping grids was at least 100m from the nearest adjacent grid and from the edge of the baiting area.

Fig. 2 shows the planned extent of the proposed 5.5 mm baiting area to the east of Transit hill which contained trapping grids 1-4. Prior to aerial baiting, but after commencement of live trapping, it became clear that the paddocks on the western side of the area were being used for grazing cattle, and a decision was made to avoid a bait drop over the paddocks as it was unclear as to how the green dye on the bait would impact milk production, quality, or colour. The baiting area was redrawn to exclude the paddocks (Fig. 3), and in the process resulted in a reduction in area, and the exclusion of part of trapping grid 1 from the baited area.

Fig. 1. An aerial photograph of Lord Howe Island showing the location of aerial baiting areas.
Fig. 2. Proposed 5.5 mm (30 ha) baiting area to the east of Transit hill containing rodent trapping grids 1 – 4. Grid 1 is shown darker than the remaining three grids.

Fig. 3. Revised 5.5 mm baiting zone (23 ha) excluding paddocks on the western edge of the area. Note how trapping grid 1 has been partially excluded from the baiting zone.

The location of the 10 mm bait area to the west of Transit hill is shown in Fig. 4, and the golf course bait area and its proximity to the other two areas is seen in Fig. 5.
Fig. 4. 5.5 mm baiting zone containing four trapping grids to the east of Transit hill, and the 10 mm area (34 ha) containing the three trapping grids to the west of Transit hill.

Fig. 5. Location of Golf course baiting area (30 ha) and its proximity to the other two bait areas.

**Live capture of rodents**

Rodents were live trapped over a period of 8 nights (3-11 August) prior to aerial baiting. Elliot and cage traps (containing leaf litter to prevent trap mortalities) were set in grids, baited with peanut butter and rolled oats. All rats and mice captured were transferred from traps to catch bags to facilitate handling (Fig. 6), and then ear punched (Fig. 7) to allow identification to the grid on which they were captured, and subsequently released. Traps were opened at 16h00 and
then checked at 06h00 before closing traps during the day. Any previously marked animals were recorded.

**Fig. 6.** Transfer of rat from cage trap to facilitate handling

**Fig. 7.** Ear punching a rat to enable catch bag to identification to grid on which captured

**Aerial Baiting operation**

All three areas (Fig. 1) were aerially baited on August 14th using a squirrel helicopter and a custom made bait spreader bucket (Fig. 8) slung under the helicopter (Fig. 9) Flight lines over each area were determined using a differential GPS system fitted in the aircraft, to ensure accurate bait coverage, at a targeted rate of ~10 kg per hectare. Baits dropped were non-toxic PESTOFF 2OR produced by Animal Control Products, Wanganui, New Zealand. The baits are cereal based, dyed green, and contain the non-toxic biotracer, Pyranine 120 which when exposed to ultra violet light fluoresces green. Both 5.5 mm (~0.5 g) and 10 mm (~2 g) baits were dropped to allow a comparison to be made as to which would be the most appropriate for a two species eradication. Baits were in all ways, other than presence of a toxin, identical to those that would be used in an eradication operation. The 10 mm baits were spread on the western side of Transit hill and the 5.5 mm baits on the eastern side. 5.5 mm baits were spread over an area to the west of Intermediate hill overlapping the island’s golf course which had been identified as an appropriate area to trap non-target species (Fig. 10). A baiting rate of 10 kg/ha results in approximately 1 10 mm bait every two square metres on the ground, while 5.5 mm baits will fall at a density 4 times that giving a ground coverage of 2 per square metre.

While exact baiting areas were calculated prior to flight operations, problems with uploading these areas to the onboard GPS system necessitated the manual establishment of areas during flight. Flight lines were set at the effective
swath width provided by the bucket manufacturer, using a flow rate aperture (Figs 11 and 12) to give a rate of approximately 5kg per hectare. A second flight was then conducted along lines midway between those of the first flight. This flight plan allowed a 100% overlap in baiting producing the desired baiting rate of 10kg/ha. All flight lines were run in parallel to minimise bait gaps which might occur on right angle flight paths as a result of errors in calculating the effective swath width of the bait spreader.

Fig. 8. Custom built bait spreader bucket being prepared for use on LHI.

Fig. 9. Squirrel helicopter with bait bucket during baiting operations.

While the size of the bucket would have enabled a single loading to conduct both bait runs on each area, the aircraft landed after the first baiting run to allow confirmation of baiting rates. This was facilitated by determining the amount of bait used during the flight. The inside of the bucket was calibrated prior to use
by filling with the contents of 25 kg bait bags, raking each 25 kg flat and marking the inside of the bucket to show the amount of bait. At the start of the baiting operation, approximately two thirds of the estimated bait required for the whole area was loaded into the bucket, and the remaining bait quantity determined when the aircraft returned by raking the bait in the bucket flat and recording the amount. Changes to the aperture size at the base of the bucket were made, if required, to achieve required flow rates.

Fig. 10. 5.5 mm bait on the golf course after the aerial baiting operation.

Fig. 11. Adjustable bait flow rate aperture.
Post baiting trapping of rodents

The previously established grids on Transit hill were trapped for 7 days, commencing on the second day after the bait drop (evening of 16 August). Both rat and mouse snap traps were used at each site, placed under cover to prevent non-target bycatch. Subsequent to the first night’s trapping, during which there were few captures, Elliot and cage traps were redeployed to provide additional potential for captures. All animals captured in live traps were euthanased using blunt trauma techniques in accordance with DECC animal ethics guidelines. Captured animals were weighed to the nearest 2 g and checked for ear marking to determine if they had been captured during the live trapping phase prior to aerial baiting.

All rodents captured were assessed for bait uptake by visual inspection under UV light for pyranine dye (green fluorescence) in their mouth, rectum and in faeces. Any animals which showed no external signs of dye were dissected and examined internally. The proportion of previously marked (an indication of residency), and unmarked (assumed to have originated outside the baited area) rodents was determined. Separate analyses were conducted for the 5.5 mm and 10 mm bait areas.

Assessment of non-rodent impacts

Birds were captured on the golf course area adjacent to Intermediate hill commencing 2 days (16 August) after the bait drop using mist nets and butterfly cage traps, and trapping continued for 9 days. Additional captures using butterfly cage traps were made in the 5.5 mm baiting zone to the east of Transit
hill. Once captured, birds were placed in a drawstring bag to minimise handling stress. Mouth linings, and cloaca of all birds were checked under UV light for fluorescence indicating consumption of bait. They were colour banded for identification if recaptured, and then transferred into lined aerated boxes in a quiet, dark place to minimise disturbance until a faecal sample had been produced. Each bird was held for the minimum period necessary for them to produce faeces, which did not exceed 1 hour. All faecal samples were checked for fluorescence under UV light, and then frozen for further analysis if required.

In addition to trapping, opportunistic observations were made of foraging animals, faecal material collected when species producing it were observed, and on several occasions baits were directly presented to birds to determine palatability.

A harp trap was set for five nights on the golf course, and for three in the bait zone to the east of Transit hill, to catch Large Forest Bats (*Vespadelus darlingtoni*).

**Bait longevity**

Rodent cage traps were covered with 6 mm aperture wire mesh to prevent access by rodents or non-target species to trial baits. Cages containing 5.5 mm and 10 mm baits were placed at three locations: an open site (Fig. 13) with zero canopy cover, a medium cover site with a broken canopy and a full canopy cover site to monitor bait longevity. 100 baits were placed in each cage and samples removed at approximately weekly intervals and photographed to assess the status of the baits, 10mm and 5.5 mm baits are shown in cages in Fig 14. Bait condition was assessed according to a 6 point scale developed by the New Zealand Department of Conservation (Fig. 15).

![Fig. 13. Bait cages in ‘open’ area.](image-url)
Results

Live capture of rodents

A total of 95 mice and 147 rats were captured and marked during the 8 night period of trapping prior to the aerial baiting operation. Numbers of rats and mice in each trapping grid are shown in Table 1. An estimate of minimum numbers of rodents per hectare was calculated by dividing the total number of marked animals by the area of the grid on which they were captured (Table 1).
Table 1. Numbers of trapping days, trap nights, trapping grid areas, rats and mice caught and marked on LHI, and estimates of minimum numbers of mice and rats per hectare.

<table>
<thead>
<tr>
<th>Grid</th>
<th>Days grid trapped</th>
<th>Trap nights (nights * # of traps)</th>
<th>Area of grid (ha)</th>
<th>Mice marked</th>
<th>Minimum Mice/ha</th>
<th>Rats marked</th>
<th>Minimum Rats/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>492</td>
<td>0.37</td>
<td>37</td>
<td>100.0</td>
<td>13</td>
<td>35.1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>336</td>
<td>0.38</td>
<td>28</td>
<td>73.7</td>
<td>15</td>
<td>39.5</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>420</td>
<td>0.31</td>
<td>29</td>
<td>93.5</td>
<td>23</td>
<td>74.2</td>
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<tr>
<td>4</td>
<td>3</td>
<td>252</td>
<td>0.30</td>
<td>0</td>
<td>0.0</td>
<td>22</td>
<td>73.3</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>686</td>
<td>0.40</td>
<td>0</td>
<td>0.0</td>
<td>25</td>
<td>62.5</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>686</td>
<td>0.37</td>
<td>1</td>
<td>2.7</td>
<td>23</td>
<td>62.2</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>588</td>
<td>0.40</td>
<td>0</td>
<td>0.0</td>
<td>26</td>
<td>65.0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td></td>
<td>95</td>
<td></td>
<td>147</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unmarked rats and mice were still being captured on most grids at the cessation of the live trapping period (Figs 16 & 17), indicating numbers marked represented minimum numbers of animals on each grid. Only one mouse was captured during the live trapping period on the western group of grids, which is not shown on Fig. 16.

Fig. 16. Cumulative numbers of mice marked on trapping grids prior to aerial baiting.
Aerial Baiting

Aerial baiting was conducted during 7 flights on 14 August. A total of 920 kg of non-toxic bait was spread during the flights. Two measured bait drops were undertaken over the first two areas baited. During the 10 mm bait drop to the west of Transit hill 170 kg bait was used on the first run with a 70 mm aperture on the bucket, and a swath width of 70 metres. This resulted in a delivery rate of 4.9 kg/ha over the 34.8 hectares baited. The second run used the remainder of the bait with flight lines offset by 50% of the swath width from the first run.

During the baiting over the golf course the first flight used a 60 mm aperture to spread the 5.5 mm baits resulting in only 75 kg of bait being used over the 29.6 ha. The second run used a 70 mm aperture and 150 kg were used providing a baiting rate of 5.1 kg/ha which was consistent with the figure for the 10 mm runs. A third run dropped a further 75 kg of bait over the area. All baiting with 5.5 mm bait used a swath width of 60 metres.

The details of the baiting, with baiting rates and numbers of baits spread per hectare are shown in Table 2.

Table 2. Details of aerial baiting conducted on LHI on 14 August.

<table>
<thead>
<tr>
<th>Zone</th>
<th>Bait size (mm)</th>
<th>Area (ha)</th>
<th>Bait (kg)</th>
<th>Baiting rate (kg/ha)</th>
<th>Baits/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>West</td>
<td>10</td>
<td>34.8</td>
<td>320</td>
<td>9.2</td>
<td>4600</td>
</tr>
<tr>
<td>East</td>
<td>5.5</td>
<td>23.1</td>
<td>300</td>
<td>13.0</td>
<td>26000</td>
</tr>
<tr>
<td>Intermediate hill</td>
<td>5.5</td>
<td>29.6</td>
<td>300</td>
<td>10.1</td>
<td>20200</td>
</tr>
</tbody>
</table>
The modification to the planned baiting area to the east of Transit hill (Fig. 2) resulted in baits only being distributed over part of trapping grid 1. In flight changes to the baiting area resulted in an area of 23.1 ha (area shown in Fig. 2) being sprayed, rather than the planned 25 ha. All baiting for the east area was conducted in a single flight, with bucket apertures set for a 5 kg/ha baiting rate. At the start of the flight sufficient bait to achieve the 10 kg/ha coverage was loaded (250 kg), along with an extra 50 kg to cover variation on flow rate, and to allow extra baiting along the boundaries of the area which may be missed during the flight lines. The reduction in the actual size of the East bait area, combined with a slight increase in bait loaded resulted in higher baiting rate ~13 kg c.f. ~10 kg/ha for the other two areas.

Within 7 days of the aerial operation (21 August), baits which had been easily visible on the ground in both baiting areas had all but disappeared, presumably as a result of removal by rodents, and invertebrate activity.

Bait uptake by rodents

A total of 132 mice, and 39 rats were caught over 7 nights on the trapping grids. 10 of 24 (41.7%) adult rats, 1 of 15 (6.7%) of juvenile rats, and 56 of 132 (42.4%) mice were ear marked indicating capture prior to aerial baiting. All marked animals were captured in the grid in which they were marked indicating a high degree of fidelity to the area. Fifty six (58.3%) of the 96 mice marked on the grids were captured, compared to only 11 (7.5%) of the 147 rats.

Mass of 122 mice and 37 rats were recorded. Adults rats weighed 207.4 ± 10.2 g (range 92 – 266 g, n = 24), juveniles 43.8 ± 3.0 g (range 28 – 62 g, n = 13), and mice 19.2 ± 0.4 g (range 8 – 28 g, n = 122). mean 43.8 ± 3.0 g), and mice (n=122) ranged from 8–28g with a mean of 19.2g.

Uptake of 5.5 mm bait for 131 marked and unmarked mice inferred from the presence of pyranine fluorescence (Fig. 18) is estimated at 78.6%, with corresponding figures of 88.9% for 18 adult rats and 91.7% for 12 juvenile rats (Table 3). Both rats and a single mouse showed 100% uptake of 10 mm bait

Table 3. Estimates of rates of uptake of 5.5 mm and 10 mm non-toxic baits indicated by pyranine fluorescence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Consume 5.5 mm bait</th>
<th>% Positive</th>
<th>Consume 10 mm bait</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Mouse</td>
<td>28</td>
<td>103</td>
<td>78.6</td>
<td>0</td>
</tr>
<tr>
<td>Rat - Adult</td>
<td>2</td>
<td>16</td>
<td>88.9</td>
<td>0</td>
</tr>
<tr>
<td>Rat - Juvenile</td>
<td>1</td>
<td>11</td>
<td>91.7</td>
<td>0</td>
</tr>
</tbody>
</table>

The corresponding values for marked animals, those assumed to be resident in the area, are shown in Table 4.
Table 4. Estimates of rates of uptake by previously marked rodents of 5.5 mm and 10 mm non-toxic baits indicated by pyranine fluorescence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Consume 5.5 mm bait</th>
<th>% Positive</th>
<th>Consume 10 mm bait</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Mouse</td>
<td>9</td>
<td>47</td>
<td>83.9</td>
<td>0</td>
</tr>
<tr>
<td>Rat - Adult</td>
<td>1</td>
<td>7</td>
<td>87.5</td>
<td>0</td>
</tr>
<tr>
<td>Rat - Juvenile</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The marked adult rat which showed no signs of bait consumption was captured in grid 3 on 16 August, the second night after the aerial baiting. The juvenile rat was captured on 21 August on grid 1 in an area that was missed during the baiting (see Fig. 3).

Nine marked mice showed no sign of bait uptake during the trial. Seven of these animals were captured on the partly baited grid 1, the two remaining animals were trapped in grid 3, 7 and 9 days after the aerial baiting. Data for mice in grids other than the partially baited grid 1, show 100% positive results until day 6 after baiting (20 August), and a significant drop by 9 days post baiting. (Fig. 19).

Fig. 19. Numbers of mice in grids other than grid 1 recording positive (solid bars) and negative (hollow bars) pyranine fluorescence by day through the trapping period, and the percentage of inferred bait uptake (line)
Numbers of adult rats captured in the 5.5 mm bait area showed an increase towards the end of the trapping period with more captured in the final 2 days of trapping than in the previous five (Fig. 20). Juvenile rats showed a similar, non-significant pattern (Fig. 20), while mice, after the first day, showed no difference in capture rates through the period. (Fig. 21). In the 10 mm area, the total numbers of captures were very low (13 rats and mice), but numbers of adult rats showed an increase on the final day of captures (Fig. 22).

Fig. 20. Daily captures of juvenile and adult rats in the 5.5 mm bait area

Fig. 21. Daily captures of mice in the 5.5 mm bait area

Fig. 22. Daily captures of juvenile and adult rats, and mice in the 10 mm bait area
Non-target bait uptake

11 species of birds were examined during the study for indication of bait uptake (Table 5). Woodhens, Buff banded rails, blackbirds and Mallards all provided fluorescing faecal samples (Fig. 23) indicating consumption of the dyed bait. In addition to the confirmation provided by the positive faecal samples, woodhens and mallards were both seen feeding directly on baits, while a single case of an emerald dove picking up bait and then discarding it was recorded. The remains of an owl kill were found on the golf course and the gizzard fluoresced brightly indicating that the owl's prey had ingested bait. The identity of the prey species was thought to be a woodhen.

Table 5. Results of pyranine fluorescence to assess uptake of bait for bird species caught in mist nets and traps, for faecal samples of known source, and autopsied* animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pyranine Fluorescence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Currawong</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Emerald dove</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Silveryeye</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Buff Banded Rail</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Whistler</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Woodhen</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Kingfisher</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Blackbird*</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mallard</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Owl Kill - Gizzard (Woodhen?)</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Magpie Lark</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Purple swamp hen*</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>32</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 23. Duck faeces under natural light (left), and fluorescing under ultra violet light confirming ingestion of bait (right).
Seven currawongs captured in clap traps showed no signs of pyranine fluorescence, either in faecal samples, or during physical inspection of their mouth or cloaca. Physical inspection of the 21 large forest bats captured in the harp trap provided no positive results (Fig. 24).

Fig. 24. All 21 large forest bats captured showed no signs of pyranine fluorescence in the mouth or anus during physical inspections.

Baits, both 10 mm and 5.5 mm, presented directly to buff banded rails, emerald doves, currawongs and whistlers elicited no response. Similar non-toxic bait dyed red, or un-dyed (beige in colour) was immediately taken when presented to buff banded rails.

Observations of baits in the field showed invertebrate damage occurred within a day of the bait drop. Several species of invertebrates were scanned externally with UV light to determine if they had ingested bait. Slugs, and snails (not *Placostylus*) fluoresced brightly indicating bait uptake (Fig. 25), and ants, cockroach and slugs were observed feeding directly on bait (Table 6). A single delicate skink, *Lampropholis delicata*, was scanned with UV light but did not show any evidence of bait consumption.

Fig. 25. Slug sp. feeding on bait viewed in natural light (left) and viewed under UV light (right), fluorescence indicates bait consumption.
Table 6. Results of pyranine fluorescence to assess uptake of bait for non-avian species collected, and * those observed feeding directly on baits.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pyranine Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Slug spp.</td>
<td>1</td>
</tr>
<tr>
<td>Snails (not Placostylus)</td>
<td>4</td>
</tr>
<tr>
<td>Delicate Skink</td>
<td>1</td>
</tr>
<tr>
<td>Millipede sp</td>
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</tr>
<tr>
<td>Termite sp.</td>
<td>1</td>
</tr>
<tr>
<td>Ant sp.</td>
<td></td>
</tr>
<tr>
<td>Large wing Cockroach (Sp. A)</td>
<td>1</td>
</tr>
<tr>
<td>Cockroach sp B</td>
<td></td>
</tr>
</tbody>
</table>

Bait longevity

Observations of bait integrity showed that 5.5 mm baits in the medium cover site had completely broken down after 55 days, and 164.2 mm of rainfall (Table 7). The other 5.5 mm sites showed advanced decomposition by this time, but still retained recognisable pieces of bait (code 5). All samples of 10 mm baits showed less decomposition than the corresponding 5.5 mm baits after 55 days in the field.

Table 7. Rates of decomposition of bait following NZ Department of Conservation scale measured at intervals up to 55 days after being placed in decomposition cages on 10 August. Rainfall figures provided by the Bureau of Meteorology.

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>Rainfall (mm)</th>
<th>5.5 mm bait</th>
<th>10 mm bait</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Open</td>
<td>Medium cover</td>
</tr>
<tr>
<td>10/08/07</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>31/08/07</td>
<td>21</td>
<td>14.2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
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<td>31</td>
<td>70.8</td>
<td>3</td>
<td>2</td>
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<tr>
<td>14/09/07</td>
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<td>50</td>
<td>164.2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5/10/07</td>
<td>55</td>
<td>164.2</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Discussion

The primary goals of the non-toxic bait trial were four fold, to determine uptake rates of 5.5 mm and 10 mm bait by rodents, uptake of bait by non-targets, to determine longevity of bait in the environment, and to trial the use of aerial baiting techniques on Lord Howe Island. While some of the results in the study are equivocal they provide important data on which further planning towards an eradication can be based.

The motivation for comparing two size baits in the trial was a direct result observations from global eradications which indicate that mouse operations are less successful than those for rats and the failures for mice have been linked with inadequate baiting densities which reduce encounter rates (Howald et al.
2007). Changes to bait densities can be addressed by increasing the amount of bait distributed (kg/ha), or by reducing the size so that each individual bait is smaller, and there are more for a similar baiting rate (kg/ha). By using 5.5 mm baits weighing ~0.5 g it is possible to achieve 400% of the coverage, in terms of numbers of baits, that you achieve with 10 mm (~2 g) baits, for the same baiting rate i.e. 10 kg/ha.

**Live capture of rodents and bait uptake**

The justification for conducting trapping prior to aerial baiting in the current study was to provide a pool of marked individuals that we knew were present in the grid areas, and thus would be exposed to the baits when dropped. Given that all marked animals were recaptured in the grid in which they were marked, there is likely to be very limited movement by both species on LHI, and based on that observation allows conclusions to be drawn from the entire capture sample, as they are likely to have been ‘resident’ in the grid areas at the time of the baiting and thus exposed to bait. Previous work on LHI rats found that 70% of animals were recaptured within 40 m of the initial capture site, and mean distance moved was approximately 45 m, with a maximum distance moved of 450 m (Billing 1999). The high rate of residency found in the current study is consistent with previous data.

The lack of mouse captures on the 10 mm bait grids, 1 was caught, prevented a robust comparison of palatability of 5.5 mm and 10 mm baits. During the live trapping, prior to aerial baiting, there was evidence that mice were present but not being caught, this included numerous observations of cage traps being triggered and associated bait removal, and removal of bait from untriggered cage traps by burrowing under the trap to access the bait sitting on the floor of the cage. In the case of the closed traps, mice are able to squeeze between through bars of the cage to escape, and burrows under cages were too small to have enabled a rat to access the bait. Assuming that mice were present on the grids it is puzzling that there was only a single capture in an Elliot trap on 686 trap nights on grid 6, and on a combined total of 1960 trap nights in the 10 mm bait area. Despite the lack of mice captured in the area it had been hoped that the use of snap traps to catch animals after aerial baiting would result in the capture of mice that were believed to be in the area, and have escaped from cage traps and avoided Elliot traps. This did not occur and only one mouse was captured during this period.

The ability to assess the uptake of bait by these species is also dependent on trapping animals to examine them for pyranine fluorescence with a UV light. Post baiting trapping was characterised by very low captures of rats with only 7.5% of those marked being recaptured, compared to 58.3% of marked mice. However, similar proportions of marked to unmarked adult rats and mice (41.7% c.f. 42.4%) were captured indicating that the low overall rate of marked rats in the sample was not a result of their previous capture experience, but rather a consequence of the low trapping rates.

Captures of rats were almost zero for first 5 days of trapping, i.e. 7 days from aerial baiting. One explanation is that rats were foraging as normal during this
period but were feeding entirely on the abundant cereal baits that were dropped, and were not attracted to the peanut butter and rolled oat baited traps. As the availability of the preferred food, in this case the bait, declined animals would have been more likely to seek alternative food and increase their probability of approaching a trap baited with peanut butter which would have increased probability of capture and translated to more captures.

An indication of bait available to each animal can be determined by estimating the numbers of animals inhabiting each grid. If we consider a mouse (mean wt 19.2 g) to be equivalent to ~0.1 rats (mean mass 207.4 g), and assume the population inhabiting the grid equates to the numbers of marked individuals (rats + mice – see Table 1), and then divide this into the product of the number of baits dropped per hectare (Table 2) and the size of the trapping grid (Table 1) then rats in the 5.5 mm zone had between 310 and 580 pellets (155 – 290 g) available to each of them, while mice had 31 and 58 pellets (15.5 – 29.0 g) and in the 10 mm zone rats had between 70 and 75 of the larger pellets (140 - 150 g), and mice 7 to 8 (14 – 15g).

An alternative suggestion is that rats cached pellets in the first few days after the bait drop, and then were not active on the grid until several days later when again searching for food, with the associated higher risk of capture. It would seem from the low proportion of marked rats caught compared to mice, that rats may show a stronger preference for the cereal baits to the exclusion of other food sources, which is beneficial in an eradication to ensure bait is consumed. If the rats did cache baits it increases the probability that during a toxic bait drop they would be more likely to succumb to toxicosis underground, and thus not pose a secondary poisoning threat to species that prey upon them.

The situation with mice differed in that captures did not show any changes during the trapping period, suggesting that while mice fed on the bait, they were also willing to take other available food as evidenced by their attraction to peanut butter in the traps.

Despite the apparent willingness of mice to take alternative food when bait is in abundance, uptake of 5.5mm bait was still 100% up to 6 days after the bait drop (Fig. 18), with the rate declining to 44% by day 9. In the context of an eradication operation, even if bait is in abundance and mice eat both bait and alternative food, based on a lethal dose of brodifacoum, (the toxin of choice for current eradications) of 0.4 mg/kg (Haydock and Eason 1997), a 20 g mouse would have to consume only 80% of a single 5.5 mm bait or 20% of a 10 mm bait to get a lethal dose of the toxin. Based on the uptake rates in the first days after the bait drop, it would appear that as long as bait is available at sufficient density to mice they will ingest it, and succumb to the effects of the toxin. At a baiting rate of 10 kg/ha, 20000 5.5 mm baits would fall per hectare, and 5000 10 mm baits. Given a combined rat and mouse density of 85 (75 rats and 100 mice rat equivalents based on a mouse being ~0.1 rat) each rat would have access to approximately 200 small baits and 50 larger baits, while the figures for mice would be 20 and 5. The available baits represent 25 times the lethal dose for mice, suggesting that there would be sufficient bait available.
The supposition in the study that rats are feeding intensively on the baits provides confidence that they would consume the required quantity of a toxic bait to facilitate eradication. A lethal dose of brodifacoum in ship rats is 0.46 mg/kg (O’Connor & Booth 2001), and therefore a 200 g rat would need to consume 2.5, 10 mm baits or 9, 5.5 mm baits to ingest this amount of toxin. Calculations above of bait availability to rats at a baiting rate of 10 kg/ha indicate that there would be around 20 times the required level of toxin available to kill animals. It is unclear why the marked adult rat captured in grid 3 on the second night after baiting had not consumed bait, but it may be reasonable to expect that if it had not been trapped it would have had the opportunity to consume the amount of bait required to receive a lethal dose. The marked juvenile rat that had not ingested bait was trapped in grid 1 which was only part baited during the aerial operation, and so during its movements it may not have encountered bait. This would not occur during an eradication given the comprehensive coverage across the entire island.

In addition to the single mouse capture in the 10 mm bait area compromising the bait size comparison, the low numbers of rats captured at the two sites also prevented a statically robust assessment. Despite this shortcoming in the data, it is important to note that all rats and the single mouse captured in the 10 mm bait area had consumed the bait, while uptake in the 5.5 mm bait area is discussed above.

**Bait longevity**

The period during which bait remains intact in the field is a critical factor in operational planning for any proposed eradication to be undertaken on LHI. The primary requirement is that the bait remains intact for long enough for the target species to encounter and consume it, once that criterion is met, any undue delay in decomposition of the remaining bait increases the risks to non-target species. In the case of LHI persistence of toxic bait will determine the period of high risk to human residents and pets, it will also determine when non-targets being held in captivity can be returned to the wild, and livestock returned to paddocks.

The observations suggest that both sizes of bait will persist for at least 55 days which is long enough for uptake by the target rodent species, but the more rapid breakdown of the 5.5 mm bait would facilitate a shorter holding period for island endemics such as Woodhen and Currawong, and livestock. At the time of writing this report, baits had been observed in the field for 55 days, after 164.2 mm rainfall. The only baits that had completely degraded (decomposition code 6) within this period were the 5.5 mm baits in medium cover, but all 5.5 mm baits were at a more advance rate of decomposition than the larger 10 mm baits (Table 7).

Decomposition rates may be slower than would be expected during an eradication operation as the cages in which they were held kept the baits off the ground which may reduce invertebrate and microbial breakdown. The elevation of baits off the ground also facilitates the drying of bait through air movement.
after rainfall events, which assists in maintaining bait integrity. This may explain why baits in the open test area seemed to exhibit slower rates of decomposition than those in the higher humidity medium and full canopy cover areas.

All planning of captive management of island endemics and holding periods for livestock will utilise the slowest decomposition rates for a given bait size in the current study. Given the observation of the delayed decomposition of caged baits utilising the slowest decomposition rates will provide a conservative and safe estimate of the point at which risk to livestock and endemics is eliminated.

While final figures for decomposition times (in excess of 55 days) will only be known after this report has been submitted, it would appear that from an environmental risk standpoint, the more rapid breakdown of the smaller 5.5 mm baits would enable shorter captive periods for island endemics, livestock and risks posed to island residents through the presence of the toxin in the environment.

Non-target impacts

The potential for impact on non-target species is a very important planning issue for rodent eradications. While brodifacoum has been widely shown to be effective in eradicating mice and rats (Howald et al. 2007), it can pose risks to non-target species, both through primary and secondary poisoning (Eason and Spurr 1995, Towns and Broome 2003). These non-target issues are particularly important when the at-risk species are threatened endemic species such as the case with the Lord Howe Island Woodhen Gallirallus sylvestris, and LHI Currawong Strepera graculina crissalis. While the impacts of invasive rodents on offshore islands are widely accepted (Towns et al. 2006), and have been the catalyst for many eradications globally (Howald et al. 2007), non-target issues must be taken into consideration and methods of mitigating risk be incorporated into eradication planning processes.

The iconic status of woodhens on LHI, and their probable vulnerability to both primary and secondary brodifacoum poisoning, given the susceptibility of the congeneric New Zealand weka, Gallirallus australis, (Eason and Spurr 1995), focuses attention during any planned rodent eradication on non-target issues. On Tawhitinui island in New Zealand the entire weka population was exterminated during a brodifacoum baiting for ship rats (Taylor 1984).

The observation of woodhens consuming non-toxic bait during the study, and producing faeces that fluoresced confirmed expectations for this species. While the techniques used in the non-toxic trial do not enable us to determine the quantity of bait consumed, given the threatened status of this species it is prudent to prepare mitigation measures. In New Zealand weka were captured prior to a rodent eradication on Kapiti Island and successfully housed in captivity until release after bait disintegration (Empson and Miskelly 1999). A similar solution is suggested for woodhens on LHI. In addition to woodhens, currawongs are also thought to be at high risk of exposure to brodifacoum. The current study examined seven currawongs and none showed signs of bait ingestion. Despite the lack of evidence of either primary or secondary exposure
to bait, the potential risks posed to this threatened species during an eradication can not be ignored given the high probability of birds feeding on either dead or moribund brodifacoum poisoned rats and mice. Captive management of currawongs during any eradication operation is recommended.

Other bird species which showed signs of bait ingestion species during the study were blackbirds, mallards and buff banded rails. Both blackbird and mallard mortality resulting from brodifacoum poisoning have been recorded in New Zealand eradications (Dowding et al. 1999). None of these three species is threatened, nor are they endemic to LHI. It is not recommended that any measures be taken to mitigate impacts of toxins. Island endemics the LHI Golden Whistler, *Pachycephala pectoralis contempta*, and the LHI Silvereye, *Zosterops lateralis tephropleur* were both negative for bait uptake.

Several emerald ground doves were examined during the trial and despite the expectation that they would be vulnerable to ingestion of the bait, there was no evidence collected to support that view. An individual was also observed picking up bait, but soon dropped it and showed no further interest. Kingfishers, magpie lark and purple swamp hen also showed no evidence of bait uptake, although kingfishers may be vulnerable through secondary poisoning, and purple swamp hens are known to suffer significant (~50%) mortality during New Zealand rodent eradications (Dowding et al. 1999).

While no Masked Owls (*Tyto novaehollandiae*) were captured during the trial an opportunistic discovery of the remains of an owl kill indicated it had fed on a bird which had ingested bait. In cases where such prey species had fed on toxic baits predators are vulnerable to secondary poisoning. Work in New Zealand has shown that Moreporks (native owls), *Ninox novaeseelandiae*, have been killed during brodifacoum operations (Stephenson et al. 1999). The removal of rodents as a source of prey for Masked owls will result in them switching prey, possibly to endemic species, and it would be appropriate to undertake a cull or attempted eradication of the owl during any rodent eradication. In addition to avian non-target species, 21 large forest bats were examined and found negative for bait uptake. This species is potentially at risk from secondary poisoning from invertebrates it may consume.

Several invertebrates either fluoresced under UV light, or were observed feeding on the bait. While invertebrates are known to consume anticoagulant baits (Ogilvie *et al.* 1997, Spurr and Drew, 1999) they do not have the same blood clotting systems as vertebrates and are therefore thought to be at low risk of toxicosis from ingesting brodifacoum. Indeed a review of brodifacoum impacts on non-target species in New Zealand reported no mortality to invertebrate species as a result of brodifacoum baiting (Hoare and Hare 2006). More importantly brodifacoum residues of up to 7.47 µg/g have been recorded in NZ terrestrial invertebrates (Craddock, 2003). Residue levels take in excess of four weeks to return to background levels, and trace levels are detectable up to ten weeks following brodifacoum baiting operations, which potentially poses a risk to insectivorous bird species (Booth *et al.*, 2003; Craddock, 2003).
Notwithstanding the potential risk of secondary poisoning, the only reported case of insectivorous birds succumbing to brodifacoum poisoning was in a zoo, where several species died in an aviary after feeding on pavement ants and cockroaches that had eaten brodifacoum baits (Godfrey 1985).

While brodifacoum clearly impacts non-target species (Hoare and Hare 2006), short term losses of individuals are more than offset by population level benefits resulting from rodent eradication (Towns and Broome 2003).

**Aerial baiting**

Aerial broadcast by helicopter is becoming the most common method of rodenticide delivery (Towns & Broome 2003), and the current study provided valuable experience in planning and conducting an aerial baiting operation. The spreader bucket worked flawlessly, and we were able to establish the correct flight configuration: air speed and aperture ring size to produce the required flow rate of bait during operations. Methodologies for loading procedures, and determination of bait usage on flight runs were developed for use in future baiting operations.

Problems with the interface between office computers and the aircraft’s onboard digital GPS system to allow the uploading of baiting areas and flight lines have been resolved since the trial and will be incorporated into all future operations.

The aerial baiting operation attracted considerable attention from island residents, and provided an opportunity to further discuss eradication plans with them.

**Conclusions**

While the primary function of the bait used in an eradication attempt is to remove rodents, its impacts on non-target species must be taken into consideration when planning an operation. Results on uptake of bait while equivocal, suggest that both are palatable to both species of rodents. Further testing of the two sized baits should be undertaken, with some modifications to experimental design to try to achieve 100% bait uptake. Assuming both bait sizes produce the required result relating to uptake, then what other factors should be considered when choosing the bait for an eradication?

Risk to non-target species can largely be mitigated in an operation on LHI by putting populations of high risk species (woodhens, currawongs and possibly *Placostylus* snails) into captivity to prevent them accessing baits, or consuming dead and dying poisoned rodents. However, captive management poses its own risks and periods of captivity should be kept to a minimum. The period of captivity will be determined by the length of time that uneaten baits remaining in the environment take to break down to a point at which they are no longer in a form that they may be ingested. Preliminary data on bait decomposition suggests that the smaller 5.5 mm baits decompose at a more rapid rate than the larger 10 mm baits, thus posing a risk for a shorter time period.
The success of the aerial baiting operation during this project confirms that this technique can be used to bait a significant proportion of the island outside of the settlement area during an eradication. Problems associated with uploading of bait areas during the project have subsequently been solved, and future aerial baiting will utilise accurate bait maps prepared prior to flying uploaded onto the aircraft’s GPS system.

Work conducted during the project has provided valuable input to the planning of a future rodent eradication on LHI.

Acknowledgements

This study forms part of the planning phase of the Lord Howe Island rodent eradication programme. The Northern Rivers Catchment Management Authority provided funding for the work through and NHT grant to the Lord Howe Island Board (LHIB); along with support from the LHIB and the NSW Department of Environment and Climate Change (DECC). Field work was made possible by assistance from Josh Keating, Phil Tennant, Dianne Brown, Geoff Smith, and numerous members of the LHIB environment staff. Christo Haselden is thanked for monitoring caged baits after 26 August, and along with Nicholas Carlile for permission to use photographs to illustrate this report.

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References


Howald et al. 2007.


Measuring uptake of non-toxic baits by ship rats (*Rattus rattus*) and house mice (*Mus musculus*): essential information for planning a rodent eradication programme on Lord Howe Island
Department of Environment and Climate Change
(Primary Author Dr Ian Wilkinson)

Summary

A non-toxic bait trial was conducted on Lord Howe Island (LHI) to inform preparations for a proposed eradication of ship rats *Rattus rattus* and house mice *Mus musculus* that are widespread on the island and have significant, adverse environmental impacts. The study examined the palatability of two sizes of bait to rodents, a critical input to project feasibility and planning.

Non-toxic baits were distributed across two study areas on LHI, each approximately 3 ha in size. Each area was dosed at a rate of approximately 10 kg/ha, one using 10 mm diameter pellets, the other using 5.5 mm pellets. Baits of both sizes contained a biomarker that fluoresced under ultraviolet (UV) light. Bait ingestion was confirmed by the presence of fluorescence in the gut of trapped rats and mice. Prior to baiting, each area was trapped for seven days, and captured rodents were ear marked and released. After baiting, rodents in the study areas were sampled using live traps and snap-traps. Rodents trapped after the baiting and which had previously been marked were assumed to be resident and thus would have had access to bait. All resident rats and mice captured after baiting had consumed bait.

Two of the 47 mice captured after baiting had not consumed bait. Both these animals were unmarked and both were caught at the end of the trapping period when bait had largely gone from the forest floor. It is likely that these individuals were transients and had not encountered baits. Three of the 43 rats captured after baiting had not consumed bait. All three were juveniles, had only recently emerged from the nest, and almost certainly had yet to encounter baits. Bait distribution during the proposed eradication would have placed all five of these individuals at risk from the poison, as bait would be distributed over the entire island on two separate occasions, each about 10 days apart.
Baits of both sizes (10 mm and 5.5 mm) were highly palatable to both rats and mice, and so their suitability for use in the proposed rodent eradication programme on LHI is now confirmed. However, given the advantages of large baits in aerial operations and the need for a higher encounter rate for mice in the settlement area on LHI, it is recommended that 10 mm baits be used for aerial operations and 5.5 mm baits for hand broadcast operations.

Introduction

In common with many oceanic islands, Lord Howe Island (LHI) has unique faunal and floral assemblages, with a high degree of endemism. The introductions of house mice *Mus musculus* in c.1860 and ship rats *Rattus rattus* in 1918 have had extensive adverse impacts on the natural flora and fauna of the island, and have disrupted numerous ecological processes (DECC 2007). Rats have been implicated in the decline and extinction of a number of bird, reptile and invertebrate species (DECC 2007). They also have significant impacts on the survival and reproductive processes of a number of plant species on the island. While the impacts of mice have not been intensively studied at LHI, evidence from other locations suggests that they are likely to be significant predators of invertebrates, the eggs of smaller birds and plant seeds (Towns *et al.* 2006).

The economy of LHI has long been dependent on the export of the endemic kentia palm *Howea forsteriana*. In recognition of the destructive impact that rats have on the seeds of this palm, attempts to control the rats commenced shortly after their arrival. These attempts, albeit using different methods, continue to the present day. Since 1986, the LHI Board (LHIB) has undertaken rat control at 33 sites on the island, primarily to protect the palm industry but more recently to also minimise their impact on a few select species of endemic flora and fauna. The total area of these 33 treated sites is approximately 140 ha, about 10% of the island. Mice are not controlled due to their resistance to the particular toxin (warfarin) used (LHIB 2009). The community also undertakes rat and mice
control within the settlement area. While control may temporarily reduce rat numbers in selected areas, it does not eliminate the broader biodiversity impacts caused by either rats or mice.

Developments in eradication techniques during the past 20 years (Howald et al. 2007), in particular the use of aerial baiting methods, now make it feasible to eradicate both species of exotic rodent on LHI in a single operation (Saunders and Brown 2001). A single eradication operation is not only cost-effective it has the advantage of minimising disturbance to native wildlife and preventing any increase in the mouse population that may occur in the absence of rats. Achieving eradication of both species of exotic rodents, while minimising potential impacts on native species, requires detailed technical and logistical planning.

An essential prerequisite for any eradication is that all target individuals be put at risk by the methods employed. It is critical, therefore, to test the palatability of proposed baits to ensure that they are taken up by each target species. Observations from other eradications indicate that operations aimed at eradicating mice are less successful than those targeting rats. In some instances the failure to eradicate mice has been linked to inadequate bait encounter rates (Howald et al. 2007, MacKay et al. 2007). Bait encounter rates can be increased by either increasing the amount of bait distributed (kg/ha) or by reducing the size of the bait pellet. The smaller the pellet the more individual baits are broadcast for any given dose rate (kg/ha). In addition to assessing the palatability of the proposed bait formulation, it is important to assess whether the size of the bait is appropriate for the species targeted.

Previous studies, conducted on LHI investigated the longevity of bait in the environment and assessed the risks to non-target species from aerial baiting with baits laced with brodifacoum. Baits were found to persist for about 100 days and a number of bird species were found to be at risk, including woodhens, blackbirds, buff-banded rails and mallard ducks. This earlier work also examined the palatability of Pestoff 20R bait to rats and mice on LHI. Bait palatability was tested by aerially baiting large areas (23 and 34 ha) and then
trapping animals to assess whether they had consumed bait. Baits were non-
toxic and contained a biomarker that fluoresced under ultraviolet (UV) light. Bait
ingestion was confirmed by the presence of fluorescence in the gut of trapped
rats and mice. Although these earlier studies demonstrated that Pestoff 20R
baits are palatable to both rats and mice on LHI, the effect of pellet size was not
adequately resolved. The current study aims to confirm the palatability of the
proposed bait type to both rats and mice on LHI, and examine any differences
related to size of baits. This information will provide critical input into the
planning of a rodent eradication on LHI.

Methods

Study site

Lord Howe Island (31°33’S, 159°05’E) is a crescent shaped, volcanic remnant
on the Lord Howe Rise, approximately 600 km east of Port Macquarie, New
South Wales. It is 1,455 ha in area with very rugged relief, rising to 875 m in the
south on the summit of Mount Gower. The central lowland areas have been
cleared for agriculture or settlement and are dissected by a network of 11 km of
narrow roads. Patches of uncleared evergreen closed forest (Pickard 1983)
adjoin grazing leases and urban settlement. The LHI Group was inscribed on

The study site was on the eastern side of Transit Hill in the vicinity of the Clear
Place (Figure 1). Two baiting areas were established to test uptake of 5.5 mm
baits (Area 1; 3.4 hectares) and 10 mm baits (Area 2; 3.2 hectares). A single
trapping grid was established within each area. Each trapping grid (~60 x 60 m)
consisted of 49 grid points spaced at approximately 10 m intervals. Trapping
grids were at least 50 m from the edge of the baited area.

Live capture of rodents

Rodents were live trapped for seven nights prior to baiting. Two Elliott and two
cage traps (containing leaf litter to provide bedding and concealment from
predators) were placed at each grid point. Each trap was baited with a mixture
of peanut butter and rolled oats. Traps were opened in the afternoon (commencing about 1600 h), checked soon after dawn (commencing about 0600 h) and then closed. Captured animals were transferred from traps to cloth bags to facilitate handling. All rats and mice were weighed to the nearest 2 g and then ear punched in either the left or right ear to identify the grid on which they were initially captured. They were then released. Any retrapped animals were recorded and released.

Baiting operation

Both areas were baited by hand on a single day. Approximately 10 kg/hectare of bait was distributed over each area. Baits were non-toxic Pestoff® 20R produced by Animal Control Products, Wanganui, New Zealand. The baits were cereal based, dyed green and contained the non-toxic biotracer pyranine 120, which, when exposed to ultraviolet light, fluoresces bright green. Both small (5.5 mm, ~0.5 g per pellet) and large (10 mm, ~2 g) baits were used to allow a comparison to be made as to which would be the most appropriate for the proposed two-species eradication. Baits were in all ways, other than presence of pyranine and the absence of a toxin, identical to those that would be used in an eradication operation. Small baits were spread in Area 1 and large baits in Area 2. A baiting rate of 10 kg/ha results in approximately one large bait every two square metres, while small baits give a density of approximately two per square metre (i.e. 4 times that of the large bait).

Post-baiting sampling of rodents

Both areas were trapped for seven days, with traps set on the evening of the day following bait application. Two snap traps and two Elliot traps at each grid point were baited with peanut butter and rolled oats, set and placed under cover to minimise the likelihood of capturing non-target species such as birds. All animals captured in live traps were euthanased using blunt trauma techniques in accordance with the Department of Environment, Climate Change and Water (DECCW) animal ethics guidelines. Captured animals were weighed to the
nearest 2 g and checked for ear marking to determine if they had been captured during the live trapping undertaken prior to baiting.

All rodents captured were assessed for bait uptake by visual inspection under UV light for pyranine dye (green fluorescence) in their mouth, rectum and faeces. Any animals which showed no external signs of dye were dissected and examined internally. The proportion of previously marked (an indication of residency), and unmarked (assumed to be non-resident) rodents was determined. Separate analyses were conducted for each of the two grids.

Results

Live capture of rodents

A total of 53 mice and 34 rats were captured and marked during the seven nights of trapping prior to the baiting operation. Numbers of rats and mice in each trapping grid are shown in Table 1. Estimates of the density of rodents on each grid were calculated by dividing the total number of marked animals by the area of the grid on which they were captured (Table 1).

Unmarked mice were still being captured on both grids, and rats on grid 2 at the cessation of the live trapping period (Figs 2 & 3), indicating numbers marked represented less than the total number of animals on each grid.

Bait removal

While no formalised monitoring of bait removal was undertaken, baits had all but disappeared from both areas within 7 days (6 trap nights) of the baiting operation.

Bait uptake by rodents
After the bait drop, a total of 47 mice and 43 rats were caught over seven nights on the trapping grids. Five of 21 (24%) adult rats, none of 22 juvenile rats, 25 of 45 (56%) adult mice and neither of the two juvenile mice were ear marked, indicating they had not been captured prior to baiting. All marked animals were captured in the grid in which they were originally captured. Of the 53 mice marked on the grids before baiting, 25 (47%) were recaptured, compared to only 5 (15%) of the 34 rats.

Both adult rats ($\chi^2 = 16.0$, df = 6, P<0.05) and mice ($\chi^2 = 36.1$, df = 6, P<0.01) showed a significant departure from a constant capture rate through the trapping period (Fig 4). Mouse captures increased dramatically on day 6 and rat captures increased from day 4 onwards. In sharp contrast, there was a relatively constant capture rats of juvenile rats.

Adult rats weighed 197 ± 9 g (range 110–265 g, n = 21), juveniles 51 ± 5 g (range 21–79 g, n = 22), adult mice 20 ± 1 g (range 15–26 g, n = 45), and juvenile mice 14 ± 2 g (range 12–15 g, n = 2).

Uptake of small bait by both marked and unmarked individuals was 100% for rats and the single juvenile mouse. One of 28 adult mice did not consume baits, but this animal was not marked (Table 2). Uptake of large bait was 100% for both adult mice and rats, but lower in juveniles.

When results for adult and juvenile rats are combined there was no difference in the proportions of the population consuming either small or large baits (Fishers Exact test P=1). A similar finding is evident from the mouse data (Fishers Exact test P=1).

All marked animals that were captured after baiting had consumed baits (Table 3). Three unmarked rats and two unmarked mice captured in snap traps showed no sign of ingestion of baits. All three rats were juveniles ranging in mass from 21–23 g, and all three were caught in the same trap, two at the same
time (Fig. 5). One mouse was juvenile caught on the 7th night of trapping, the other was an adult caught on the 6th trapping night.

Three blackbirds (*Turdus merula*) were live captured on Grid 1 on trap nights 3, 4 and 5. Inspection of the birds under UV light indicated that all had passed faecal material containing pyranine. Characteristic markings on each of these birds indicated that they were three different individuals.

**Discussion**

The goal of the non-toxic bait trial was to determine if 100% of rats and mice would consume the non-toxic baits, and to determine if there were any differences between uptake of differing sized baits to inform decisions of bait choice in an eradication on LHI.

The reason for conducting trapping prior to baiting was to provide a pool of marked individuals that were known to be present before bait was distributed. If these individuals were recaptured on the same grid after the baiting it could be reasonably assumed that these individuals had been exposed to the bait. The high rate of residency found in the current study is consistent with previous findings from LHI. Billing (1999) found that 70% of rats were recaptured within 40 m of the initial capture site, and mean distance moved was approximately 45 m, with a maximum of 450 m. Elsewhere, mice have been shown to have average movements as low as 6 m (Goldwater 2008), although they have been recorded moving up to 90 m (Wanless *et al*. 2008). Based on these collective observations, it is likely that most animals captured in the grid were ‘resident’ at the time of the baiting and thus exposed to the bait, however the potential exists for movements of individuals into the area.

Both mice that had not consumed bait were non-residents (unmarked) and captured at the end of the trapping period (nights 6 and 7) when there was little bait remaining on the forest floor. Thus, it is likely that these individuals came from outside the baited area, and had not encountered baits. This scenario would not occur during an eradication operation when bait would be present across the entire island. A previous study (Wilkinson unpublished data) showed
similar findings: that the proportion of mice consuming bait declined after the 6th day post baiting, in association with a decline in availability of bait on the forest floor.

All three rats that had not consumed bait were juveniles and were caught at the same trap at the same location. Given their size (21–23 g) and the fact that two individuals were captured in the same snap trap (see Figure 5) it is probable that all these animals had recently emerged from a nest (a hole was situated within centimetres of the trap) and had not yet had the opportunity to encounter baits. Again, this scenario would not occur during an eradication operation because any juvenile rats that emerged from the nest would be exposed to bait delivered in second bait drop.

The immediate kill of all individuals may not be necessary to achieve eradication. Courchamp et al. (1999) noted that populations occurring at extremely low densities can sometimes become extinct through the ‘Allee Effect’. This occurs when not all target animals are killed, but survivors are few and separated by distances sufficient to prevent them meeting and breeding. Notwithstanding, a central tenant in planning the eradication of exotic rodents on LHI (LHIB 2009) has been to ensure that each and every rat and mouse is exposed to sufficient toxic bait to ensure it succumbs to the poison.

The ability to capture rats and mice in traps after baiting occurred indicates that both species will consume food other than baits, if alternative food is available. However, increases in captures for rats from day 4 and mice from day 6 suggests that prior to this time they were preferentially taking baits, and ignoring the peanut butter in the baited traps. It seems that as baits disappeared on the forest floor, they were more likely to seek alternatives, resulting in the observed captures. Importantly, all rats and mice captured early in the trapping period (prior to the increase in capture rates) tested 100% positive for bait uptake.

In the context of an eradication operation, each mouse would need to consume only 80% of a single small bait or 20% of a large bait to get a lethal dose of toxin (based on a lethal dose of brodifacoum of 0.4 mg/kg; Eason and
Wickstrom 2001). Each rat would need to consume 2.5 large baits or 9 small baits to ingest a lethal dose (0.46 mg/kg, O’Connor and Booth 2001). These quantities represent approximately 2% of the body weight of the two species, which is a fraction of the daily consumption estimates of 10% of body weight for rats (mass ~200 g) and 10–20% for mice (mass ~20 g, Billings 2000).

This study confirms that, provided bait is available at sufficient density, both mice and rats will ingest it. At a dose rate of 12 kg/ha (the proposed baiting rate on the first drop during an eradication on LHI, LHIB 2009) there will be 24,000 small baits or 6,000 large baits available per hectare. In the current study densities of rats ranged from 31–64 per hectare, and mice from 67–81 per hectare. Densities in a previous trial ranged from 35–74 for rats and 74–100 for mice (Wilkinson unpublished data). At the highest densities recorded (74 rats 100 and mice per hectare), each rodent would have access to numerous baits containing many times the lethal dose.

The rapid disappearance of baits, together with the low capture rates of rats and mice immediately after baiting, suggests that rodents may have cached pellets in the first few days after the bait drop. These animals were not active on the grid until several days later when less bait was available and these animals were again searching for alternative food. Caching of baits increases the probability that, during a toxic bait drop, rodents would succumb to toxicosis underground, and thus not pose a secondary poisoning threat to species that may potentially prey upon them.

The lower proportion of marked rats (compared to mice) caught immediately after baiting is possibly because rats exhibit a greater tendency for trap shyness after initial capture than do mice. Alternatively, rats may have a stronger preference for cereal baits to the exclusion of other food sources. This behaviour may potentially explain why eradications targeting rats have been more successful than those targeting mice (Howald et al. 2007).

There were no differences in bait uptake among rats and mice based on bait size. This finding has important implications for planning the eradication of
rodents on LHI. Typically, 10 mm (or larger) diameter bait pellets are used for eradications targeting rats (Broome 2009), but the most appropriate size bait to target mice is less certain. Mice typically have smaller home ranges than rats and are less likely to be exposed to bait when it is broadcast relatively sparsely (Goldwater 2008). This is thought to have been the reason for some mice eradications failing (Howald et al. 2007). For operations involving bait stations, a solution is to put the stations as close as 10 m apart. For aerial operations, a possible solution is to use smaller bait that provides a greater number of pellets per unit area. On average, each 5.5 mm bait pellet weighs approximately half a gram, and each 10 mm pellet weighs approximately two grams. Therefore, when smaller bait pellets are applied at the same number of kilograms per hectare, there is four times the number of pellets on the ground compared to when 10 mm baits are used. This provides a greater number of pellets per unit area and increases the chances of mice encountering bait, thus improving the chances of all individuals having access to bait. The recent successful eradication of mice on Montague Island, NSW, also demonstrated that both bait sizes are capable of eradicating mice (LHIB 2009).

The reasoned explanations for the lack of bait uptake by 3 juvenile rats and 2 mice in this study offered above, allow an assumption of full bait uptake by both rats and mice for both bait sizes. These data are critical to the successful planning of an eradication on LHI, and every contingency will be considered in planning to ensure that each and every rat and mouse is exposed to sufficient toxic bait to ensure the success of the operation. Notwithstanding the prerequisite for 100% uptake by target animals of any toxin used in an eradication, a 100% kill is not necessarily required to achieve a positive outcome. Courchamp et al. (1999) noted that populations occurring at extremely low densities can become extinct through the Allee Effect: ie. the probability of encountering potential mates is too low. In any eradication attempt it is possible that if all rodents are not killed, then eradication may still be achieved as long as survivors are few and separated by distances sufficient to prevent them meeting and breeding.
It is anticipated that the most difficult component of the proposed eradication of exotic rodents on LHI will be removing mice from the settlement area, where alternative foods may be more readily available. Accordingly, a high encounter rate (i.e. smaller bait) may be preferable. On the other hand, there are practical advantages of using 10 mm baits over 5.5 mm baits for aerial operations. These include (i) 10 mm baits have been used successfully in aerial sowing buckets in large quantities, (ii) the pilot can see baits as they are being spread which can be an advantage when distributing baits next to exclusion zones or sensitive boundaries, and (iii) it is feasible to retrieve baits accidentally over-sown into exclusion zones during aerial baiting operations. Considering the advantages and disadvantages of each bait size, it is proposed that 10 mm baits be used for all aerial operations on LHI, and 5.5 mm baits for all hand-baiting operations. While the use of two bait sizes adds complexity to the operation, it is justified by the benefits associated with each.

Ingestion of bait by blackbirds in the current study is consistent with other eradication operations (Dowding et al. 1999), and indicates that numbers of this introduced species are likely to drop during an operation to eradicate rodents on LHI. The impact on exotic blackbirds is of no concern from a conservation perspective, but their loss highlights the potential risks to non-target species that can occur through both primary and secondary poisoning (Eason and Spurr 1995, Towns and Broome 2003). Previous research has identified that the endemic species most at-risk on LHI are the Lord Howe woodhen Gallirallus sylvestris and Lord Howe currawong Strepera graculina crissalis. The proposed eradication operation incorporates significant mitigation measures to ensure that these and other non-target species are not adversely affected (LHIB 2009).

Conclusions

Both small (5.5 mm) and large (10 mm) baits were shown to be palatable to rats and mice. Consequently, either baits would be appropriate for use in an eradication operation on LHI. Each bait size has its advantages and disadvantages, and each is best suited to different aspects of the operation.
Large baits are recommended for aerial operations, and small baits for hand broadcasting where it is critical to increase bait encounter rates for mice.

Acknowledgements

This study forms part of the planning phase of the LHI rodent eradication programme. The Australian Government provided funding for the work through a Caring for our Country programme grant to the LHI Board (LHIB). Additional funding and support was provided by the LHIB and the NSW Department of Environment, Climate Change and Water (DECCW). The study was conducted under National Parks and Wildlife Service (NSW) Scientific Licence S12340, and Department of Environment and Climate Change (NSW) Animal Ethics Committee research licence 070618/03

References


Eason, C. T. and Wickstrom, M. Vertebrate pesticide toxicology manual (poisons): Information on poisons used in New Zealand as vertebrate


LHIB 2009, Draft Lord Howe Island Rodent Eradication Plan, Lord Howe Island Board, Lord Howe Island.


Pickard, J. 1983. Vegetation of Lord Howe Island. Cunninghamia 1, 133–266.


Table 1. Numbers of trapping days, trap nights, area of trapping grid, numbers of rats and mice caught and marked, and estimates of the density of each species.

<table>
<thead>
<tr>
<th>Grid</th>
<th>Days grid trapped</th>
<th>Trap nights</th>
<th>Area of grid (ha)</th>
<th>Mice marked</th>
<th>Mice/ha</th>
<th>Rats marked</th>
<th>Rats/ha</th>
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<tbody>
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<td>29</td>
<td>80.6</td>
<td>11</td>
<td>30.6</td>
</tr>
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<td>7</td>
<td>1372</td>
<td>0.36</td>
<td>24</td>
<td>66.7</td>
<td>23</td>
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<td>53</td>
<td>34</td>
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</tr>
</tbody>
</table>
Table 2. Estimates of rates of uptake of small and large non-toxic baits, as indicated by pyranine fluorescence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Consume small bait (Grid 1)</th>
<th>Consume large bait (Grid 2)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
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<td>Mouse - adult</td>
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<tr>
<td>Mouse - Juvenile</td>
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<td>4</td>
</tr>
<tr>
<td>Rat - Juvenile</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 3. Estimates of rates of uptake by marked rodents of small and large non-toxic baits, as indicated by pyranine fluorescence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Consume small bait</th>
<th>Consume large bait</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Mouse</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Rat</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Captions for figures

Figure 1: Map of Lord Howe Island showing the locations of baiting areas and trapping grids for the non-toxic bait trial at the Clear Place.

Figure 2. Cumulative numbers of mice marked on trapping grids prior to baiting.

Figure 3. Cumulative numbers of rats marked on trapping grids prior to baiting.

Figure 4. Daily cumulative captures of adult and juvenile rats and mice.

Figure 5. Juvenile rats captured in the same snap trap.
Figure 1: Map of Lord Howe Island showing the locations of baiting areas and trapping grids for the non-toxic bait trial at the Clear Place.
Figure 2. Cumulative numbers of mice marked on trapping grids prior to baiting.
Figure 3. Cumulative numbers of rats marked on trapping grids prior to baiting.
Figure 4. Cumulative captures of adult and juvenile rats and mice.
Figure 5. Juvenile rats captured in the same snap trap.
Assessing the risk of Pestoff® 20R brodifacoum baits to the Lord Howe Island flax snail (*Placostylus bivaricosus*)

Dr Ian Wilkinson and Ian Hutton 2013

Introduction

The Lord Howe Island flax snail (*Placostylus bivaricosus*) is endemic to Lord Howe Island (LHI), and listed as endangered on both the NSW *Threatened Species Conservation* (TSC) Act 1995 and the Australian Government’s *Environment Protection and Biodiversity Conservation* (EPBC) Act 1999. Ship rats (*Rattus rattus*) are recognized under both state and federal legislation as a key threat to *Placostylus* on LHI (DECC 2007, LHIB 2009).

The planned eradication of exotic rodents—ship rats and house mice (*Mus musculus*)—on LHI is considered a critical action to mitigate negative impacts on both *Placostylus* and the island’s biodiversity as a whole (DECC 2007). While eradication will eliminate the threat of rodent predation to the *Placostylus* population, there are potential risks associated with the use of toxins to achieve the eradication (Booth *et al.* 2003).

Brodifacoum, the toxin proposed for use in the eradication, is a second generation anticoagulant. Brodifacoum, like other anticoagulant toxicants, acts by interfering with the normal synthesis of vitamin K-dependent clotting factors in the liver of vertebrates (Hadler & Shadbolt 1975). This results in an increase in blood clotting time until the point where no clotting occurs, resulting in haemorrhaging and death. While brodifacoum is thought to lack insecticidal properties because invertebrates do not possess the same blood-clotting systems as vertebrates (Shirer 1992), several studies have been conducted to examine its impacts on invertebrates (see Booth *et al.* 2001).

Captive studies with large-headed tree-weta (*Hemidenina crassidens*) and Ascension Island land-crab (*Gecarcinus lagostoma*) indicate that neither of these species are particularly susceptible to brodifacoum, with no brodifacoum residues being detected in weta four days after sub-lethal exposure and in land crabs one month after sub-lethal exposure. Arthropods
exposed to brodifacoum during captive trials were unaffected (Booth et al. 2001), and earthworms only showed toxic effects at extreme doses (Booth et al. 2003), several orders of magnitude higher than would occur during an eradication. Field evaluations following aerial application of brodifacoum at a number of sites in New Zealand indicate that few insect species are at risk of primary poisoning, and no deleterious effects on arthropod populations have been detected (Spurr and Drew 1999, Booth et al. 2001).

There is however, some indication that molluscs may be susceptible to brodifacoum poisoning. Gerlach & Florens (2000) reported 100% mortality of two Seychelles Islands snail species (*Achatina fulica* and *Pachnodus silhouettanus*, a common species used as a model for the threatened *P. fregatensis*) that had consumed brodifacoum bait in a laboratory trial. They also suggested that brodifacoum poisoning may have contributed to observations of significantly higher numbers of recently dead *Pachystyla bicolor*, lower numbers of live adult *P. bicolor*, and shells of the critically endangered *Erepta stylodon* at Mauritian field sites subject to rodent baiting.

Given the conservation status of *Placostylus* on LHI, it is important to assess the risk posed to this species by the widespread distribution of brodifacoum baits to eradicate rodents. This study investigated whether *Placostylus* snails fed on baits, and if toxic baits were consumed, whether ingestion resulted in mortality.

**Methods**

Snails were collected from the property Arajilla on LHI and held in captivity for seven days to acclimate to conditions before commencement of experimental protocols.

Animals were held in 9-litre plastic containers (300 x 200 x 150 mm) with small holes in the bottom to drain excess water. Each container had a 30 mm deep layer of gravel placed at the bottom, covered by a 50 mm layer of
calcareous sand. A top layer of leaf litter comprised of Banyan (*Ficus columnaris*), Cottonwood (*Celtis conferta*) and Sallywood (*Lagunaria Patersonia*), both fresh and dead, was added as a source of natural food. Small shallow dishes to hold water were placed in the sand layer as per farming protocols developed for a congeneric in New Caledonia (Brescia *et al.* 2008).

The whole tank was sprayed with water, and any free water drained from the bottom of the tank. The gravel layer enabled sand to remain moist, but not waterlogged, permitting animals to burrow into it. Leaf litter was kept moist by spraying every second day, and high humidity maintained by covering the tank with damp hessian. High humidity was maintained in the tanks by placing both ends of the Hessian in water-filled containers, thereby keeping the hessian damp.

Animals were exposed to two experimental protocols. The first, using non-toxic baits, involved a choice-based feeding trial to ascertain if snails fed on the baits. The second, using toxic baits, aimed to determine if *Placostylus* were killed by the toxin.

**Non-toxic bait uptake trial**

Two groups of 5 snails were exposed to 10 g of intact Pestoff® 20R non-toxic baits placed in the tank along with the natural food described above. The remaining two groups of 5 animals were exposed to 10 g of crushed baits to simulate a later stage of bait disintegration. The baits used contained the biomarker pyranine, which fluoresces under ultraviolet light. Baits were placed in tanks along with natural food providing a choice for the snails in the tank.

Snails were left for seven days and daily checks of each tank were made to locate their faeces. These samples were then scanned with UV light to confirm whether or not pyranine was present, thus indicating whether animals had or had not ingested baits. Results were recorded as presence or absence of fluorescing faecal samples over the period the trial.
**Toxic bait trials**

Four tanks were used in the experiment, and all natural food was removed prior to the commencement of the trial.

In the first tank, 10 g of intact Pestoff® 20R baits containing brodifacoum at a concentration of 20 mg/kg was added. In the second treatment, 10 g of crushed toxic bait was placed in the tank. Two additional tanks were set up using non-toxic baits containing pyranine, one with intact baits and one with crushed baits. Five snails were placed in each of the four tanks. All tanks were then monitored for 30 days to observe if any mortalities occurred. Faecal material in the tanks containing non-toxic baits, were examined to assess whether or not snails had ingested baits.

**Return of captured animals to the wild**

All *Placostylus* exposed to non-toxic baits were returned to the site from which they were captured at the completion of the experiment.

**Results**

Four groups of snails (n=5) that were fed non-toxic baits both intact and crushed produced no fluorescing faecal samples (Table 1), and there was no mortality associated with the treatment over 7 days.

**Table 1. Results of non-toxic bait uptake trial**

<table>
<thead>
<tr>
<th>Tank</th>
<th>Number of individuals</th>
<th>Toxic/Non-toxic baits</th>
<th>Intact/crushed baits</th>
<th>Fluorescent faecal sample</th>
<th>Ingested baits</th>
<th>Mortality recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>5</td>
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</tr>
<tr>
<td>3</td>
<td>5</td>
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<td>Non-toxic</td>
<td>Crushed</td>
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<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
*Placostylus* exposed to toxic baits over a period of 30 days suffered no mortality associated with the treatment. Those animals exposed to non-toxic baits (either intact or crushed) produced fluorescing faecal samples confirming ingestion of baits, but there was no mortality associated with the ingestion of non-toxic or toxic baits (see Table 2).

**Table 2. Toxic bait uptake trial**

<table>
<thead>
<tr>
<th>Tank</th>
<th>Number of individuals</th>
<th>Toxic/non toxic</th>
<th>Intact/crushed baits</th>
<th>Fluorescent faecal sample</th>
<th>Ingested baits</th>
<th>Mortality recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>Toxic</td>
<td>Intact</td>
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<td>?</td>
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<td>Toxic</td>
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<td>Non-toxic</td>
<td>Crushed</td>
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</tr>
</tbody>
</table>

**Discussion**

The eradication of rats and mice from LHI is an important management measure to prevent ongoing environmental damage, and further erosion of the Island’s World Heritage values. However, it is critical that such an operation does not endanger populations of any of the Island’s endemic species (DECC 2007, LHIB 2009).

This research was directed at the collection of data that could inform a risk assessment dealing with the impact of the proposed eradication on the *Placostylus* population. Any risk assessment considers both the likelihood of occurrence and the consequence of occurrence, with their product providing a measure of overall risk (LHIB 2009). Consequences of risk are measured on a 5-point scale from insignificant to catastrophic. Similarly, likelihood is measured on a 5-point scale ranging from very unlikely to almost certain.
The lack of pyranine fluorescing faeces in the non-toxic food choice test suggests that when presented with a choice, as would occur in an eradication where poison baits would be distributed on the forest floor among the leaf litter *Placostylus* prefer their natural diet. This finding is significant as it indicates that the potential for a significant proportion of the population to ingest toxic pellets would be extremely low. Thus, whatever the result of brodifacoum ingestion, the overall impact on the population is likely to be low, as it would only involve a small proportion of the threatened population. Non-toxic bait trials conducted in 2007 on LHI to examine uptake of baits by the two rodent species showed that some snails (not *Placostylus*) and slugs would feed on bait pellets (Wilkinson unpubl. data).

The second finding from this study—that no mortality occurred after bait consumption—indicates that the consequences of the eradication operation would be insignificant to minor. Consequently the overall risk posed by the operation to *Placostylus* is minimal. This is an important finding that is in sharp contrast to that found by Gerlach and Florens (2000). While Gerlach and Florens (2000) noted the mortality of both *Pachnodus silhouettanus* and *Achatina fulica* snails in laboratory trials, they failed to explore whether these species would feed on baits if given the choice between baits and their natural diet. In risk assessment terms, while consequences may be at the high end, there is no information regarding the likelihood of them consuming baits. Therefore, there is no ability to adequately assess the overall risk posed by such a baiting operation.

An eight-year research project on the congeneric *Placostylus ambagiosus* in northern New Zealand showed that pulse baiting four times a year to control rodents (Sherley *et al.* 1998) resulted in increased adult recruitment which was attributed to the reduction in predation pressure by rodents. The potential impacts of the toxin on snails was not considered in this study, but the clear increases in population indicate that any impacts the toxin may have had were more than offset by the benefits that accrued due to the removal of predation pressure. This finding, in conjunction with the lack of bait ingestion and
mortality seen in the current study, provide a basis for assessing risks posed to four recently listed snail species on LHI.

The critically endangered land snails: Masters’ charopid land snail (*Mystivagor mastersi*), Mount Lidgbird charopid land snail (*Pseudocharopa lidgbirdi*), Whitelegge’s land snail (*Pseudocharopa whiteleggei*) and an unnamed land snail (*Gudeoconcha sophiae magnifica*) are all highly threatened by rat predation (LHIB 2009) and it is likely that if rats are not removed these species may become extinct; indeed some may already be extinct. The extreme rarity of these species precludes any testing of their susceptibility to brodifacoum. However, given the findings of this study, the threats for these species associated with not removing rodents likely exceed the potential risk associated with a rodent eradication operation.

This study has found that there is negligible risk posed to *Placostylus bivaricosus* by the eradication operation proposed for rodents. Notwithstanding, given the endemicity of *Placostylus bivaricosus* and its threatened status under both NSW and Australian Government environmental legislation, it would be prudent to hold a captive population until bait breakdown in the environment is complete. This recommendation is consistent with that for other endemic species including the LHI silvereye (*Zosterops lateralis tephroleura*) and LHI golden whistler (*Pachycephala pectoralis contempta*). Although the eradication operation poses no obvious threat to these species, it would be prudent to hold a small captive population on the island during the operation (LHIB 2009).

**References**


DECC – see Department of Environment and Climate Change.

DEWHA – see Department of Environment, Water, Heritage and the Arts.


Department of Environment, Water, Heritage and the Arts (2009). Threat Abatement Plan to reduce the impacts of exotic rodents on biodiversity on Australian offshore islands of less than 100 000 hectares. DEWHA, Canberra.


LHIB - see Lord Howe Island Board

Lord Howe Island Board 2009, *Draft Lord Howe Island Rodent Eradication Plan*, Lord Howe Island Board, Lord Howe Island.


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

By

Robert Wheeler and Nicholas Carlile

New South Wales Office of Environment and Heritage
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 \( \text{LD}_{50} \) of 0.4 mg kg\(^{-1}\) prescribed for mice. Ingestion of brodifacoum at dose rates 1 and 2 \( \text{LD}_{50} \) by mice on the trial resulted in no mortality. A dose rate of 3 \( \text{LD}_{50} \) resulted in 10% mortality, and 5 \( \text{LD}_{50} \) resulted in 60% mortality. After 14 days, survivors from all dosage groups were weighed and fed additional bait containing a further 10 \( \text{LD}_{50} \). Mortality for these treatments ranged from 67% to 100%, but mice consuming dosages equivalent to 12 \( \text{LD}_{50} \) (two individuals) and 13 \( \text{LD}_{50} \) (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 \( \text{LD}_{50} \) 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 \( \text{LD}_{50} \) for mice on Lord Howe Island was approximately five times the standard \( \text{LD}_{50} \) for mice, with some individuals showing a high level of tolerance, up to at least 13 \( \text{LD}_{50} \) (5.2 mg kg\(^{-1}\)). Although the \( \text{LD}_{50} \) 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 \( \text{LD}_{50} \) for mice on Lord Howe Island indicates that this population exhibits increased tolerance to brodifacoum. Based on an observed \( \text{LD}_{50} \) 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 (Morris 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).

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$\textsubscript{100}$ 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.
REFERENCES


Harden, R.H., Leary, C. 1992. The Lord Howe Island Board rat control program: report to the Lord Howe Island Board. Unpublished report to the Lord Howe Island Board.


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 LD₅₀) 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 eradication is 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 (Department 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-001 and 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 (Morris 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 (Morriess 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 be 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 have 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 anticoaguants (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.

Acknowledgements

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References


ICI (1983). Method PPSM 500. The determination of brodifacoum in baits, concentrates and technical material by high performance liquid chromatography. ICI Plant Protection Division, Yalding, UK.


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

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

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<td>6.0 (1)</td>
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Testing the efficacy of Pestoff® 20R to kill House Mice *Mus musculus* on Lord Howe Island.

Prepared for

The Lord Howe Island Board

By

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MAY 2016
Executive Summary

An invasive rodent eradication programme targeting Ship Rats (*Rattus rattus*) and House Mice (*Mus musculus*) is proposed for Lord Howe Island in the winter of 2017. The proposed bait to be used in the trial is Pestoff® Rodent Bait 20R containing brodifacoum at 20 ppm. The bait will be distributed by hand broadcasting or in bait stations within the settlement area, and by helicopter outside the settlement area. The aims of this study were to demonstrate that Pestoff 20R will be effective against mice when the bait is provided in a manner that is consistent with its application in the field.

Between 4 and 8 April 2016, 90 mice were captured at various locations across the settled parts of the island. After an up to seven day acclimatisation period the mice were placed in three treatment groups: Control (C) where 29* mice were fed *ad libitum* with commercial pet feed; Aerial Simulation (AS) where 30 mice were given Pestoff 20R for three days followed by seven days of pet food, followed by another three days of Pestoff 20R; Bait Station Simulation (BS) where 30 mice were provided with Pestoff 20R *ad libitum*.

The first death in the AS group occurred after 2 days and the last mouse died (euthanized as per animal ethics requirements^) after 22 days. In the BS group, the first death occurred after 4 days and the last mouse died after 22 days (euthanized as per animal ethics requirements^). After 16 days more than 90% of mice had died or had been euthanized in both the AS group and the BS group. All 29 mice in the Control Group were alive at the end of the trial.

This study shows that, while there is a wide range in the time until death following ingestion of Pestoff 20R, the bait will kill Lord Howe Island mice when the bait is provided in a manner that is consistent with field conditions. Initially, the mice in the AS trial died faster than those in the BS trial. Only six mice of the original 30 in the AS group survived to receive the second dose of poison. This indicates that a single ingestion of the bait (from a limited exposure) will be sufficient to kill the majority of mice relatively quickly. During the actual eradication, the period between poison exposure and death is likely to be faster than in this simulation. The mice in this trial were not challenged physically due to the confinement of their holding cages. In a natural setting with normal physical activity and exertion, there should be more likelihood of bleeding leading to death.

*One mouse died during the acclimation period, presumably from poison consumed prior to being captured.*

^In a moribund state as measured by immobility and a lack of response to stimuli
Introduction

An invasive rodent eradication programme targeting Ship Rats (*Rattus rattus*) and House Mice (*Mus musculus*) is proposed for Lord Howe Island in the winter of 2017. The proposed bait to be used in the trial is Pestoff® Rodent Bait 20R (Pestoff 20R) in the form of pellets containing the anticoagulant brodifacoum at 20 ppm. In 2013, a trial was performed to test the efficacy of brodifacoum on Lord Howe Island rodents (Wheeler & Carlile 2013). In those trials, rats and mice were fed a measured and restricted amount of brodifacoum in line with their respective accepted LD50. The results showed that, while rats died as expected, the LD50 of mice caught from within the settlement area of the island was five times the accepted value of 0.4 mg/kg. Moreover, some mice could survive a dose of 15 times the accepted LD50. As brodifacoum has long been used by island inhabitants in an effort to control rodent numbers (particularly in and around the settlement area), the results of the 2013 trial suggested that the mice had developed some resistance to brodifacoum.

In the proposed rodent eradication, Pestoff 20R will be applied across the entire island. Within the settlement area, pellets will be either broadcast by hand or made available to rodents in bait stations. Outside of the settlement area, pellets will be broadcast by helicopter in two drops. The first drop will spread pellets at a density of 12 kg/ha (one bait every two square metres). The second drop will occur 14 to 21 days later (depending on conditions) and will spread bait at a density of 8kg/ha. A single 2 g pellet of the bait will provide mice with the LD50 of brodifacoum as determined in the 2013 study.

This toxicity trial was designed to simulate potential exposure to bait that mice will experience under field situations. The main aim of the trial was to examine the efficacy of Pestoff 20R to kill Lord Howe Island mice, when the bait is provided in a manner that is consistent with its application in the proposed eradication.

Methods

With the aim of catching 90 mice, 250 Elliot traps were set between 4 and 6 April 2016, at various locations across the island: Southern Settlement (200 trap nights); Waste Management Facility (WMF)/Airport (100 trap nights); and Nursery (200 trap nights). The locations were chosen to include mice from within and on the edge of the settlement area to reflect potential differences in previous exposure to brodifacoum in mice from different parts of the inhabited sections of the island.

The majority of the 90 mice were caught in the Nursery area (63%), followed by the WMF/Airport (37%). No mice were caught at the Southern Settlement. The mice were weighed, and then placed in individual purpose-built mouse cages. Every seven days the cages were cleaned and the bedding was replaced. The mice were allowed to acclimatise for up to seven days in a mouse housing facility which provided 12 hours of natural/artificial light/12 hours of darkness each day throughout the trial. On 12 April 2016 the mice were placed in three treatment groups:

Control (C; N =29*. Mice fed *ad libitum* with pet food pellets and mixed seeds)

Aerial Simulation (AS; N = 30. Mice given Pestoff 20R for three days followed by seven days of pet food, followed by another three days of exposure to Pestoff 20R)
Bait Station Simulation (BS; N = 30. Mice provided with Pestoff 20R *ad libitum*)

The distribution of mice by treatment group and capture location is shown in Fig 1. At the beginning of the trial, there was no significant difference in the mean body mass of mice in different treatment groups ($F_{2,86} = 3.10, P = 0.12$; Fig. 3).

![Graph showing the proportion of mice used in the trial by treatment group and capture location.](image)

**Figure 1. The proportion of mice used in the trial by treatment group and capture location.**

The condition of mice was checked every six hours. The characteristics examined included, activity level, gait, posture, respiration, condition of fur, and condition of eyes. If a mouse was found to be prostrate, it was checked every hour for the next three hours. As per Office of Environment and Heritage Animal Ethics Committee requirements (AEC 160202 02), the mouse was euthanized if its condition had not changed after those three hours. Mice were also euthanized if they became moribund to the extent that they were found to be immobile and unresponsive to stimuli in two consecutive 6-hourly checks.

**Results**

The first death in the AS group occurred after two days of exposure to toxic bait and the last death occurred after 22 days after commencement of exposure (the mouse was in a severe moribund state and was therefore euthanized). After 15 days, more than 90% of the mice in the AS group had died or been euthanized. The average time until death in the AS group was 8.7 ± 4.4 days. In the BS group, the first death occurred four days after exposure and the last mouse died after 22 days after commencement of exposure (the mouse was in a severe moribund state and was therefore euthanized). More than 90% of mice were dead 16 days after commencement of exposure. The average time until death in the BS group was 9.9 ± 4.8 days. There was no significant difference in the mean number of days until death between the AS and BS groups ($t_{11.58} = 2.00, P = 0.33$). All 29 mice in the Control group were alive at the end of the trial, at which time these mice were euthanized as per ethics licence requirements. The attrition of mice in each treatment group is shown in Figure 2.
Mice in all three groups had a lower body mass at the end point (i.e. death in the baited groups or at 29 days in the control group); however the average decrease in the baited mice was more than twice that of the control mice (Fig 3).

*One mouse died during the acclimatisation period. The mouse had blood around its nose and mouth and thus had presumably consumed poison prior to being captured.*
Discussion

The finding that no mice died in the Control group while all mice died in the groups given Pestoff 20R, indicates that the poison was effective against Lord Howe Island mice when provided in a manner that is consistent with field conditions. Initially, the mice in the AS group (mice that had access to bait for three days) died faster than those in the BS group (mice with ad libitum access to bait). Only six of the original 30 mice in the AS group survived to receive the second dose of poison on day 10. This indicates that a single ingestion of the bait will be sufficient to kill the majority of mice relatively quickly.

The results also confirmed that there is a broad range in tolerance to brodifacoum, with the time until death following ingestion of Pestoff 20R varying from just two days to 22 days. The fact that 90% of mice were dead after 16 days in both of the baited groups and that only three mice made it past 18 days, suggests that an extensive range of tolerance levels in the mouse population have been captured in the trial. The results of this trial, therefore, provide some level of confidence that all wild mice will receive a lethal dose of brodifacoum in the proposed eradication. Indeed, death from ingestion of brodifacoum is expected to be faster during the eradication. The mice in this trial were not challenged physically due to the confinement of their holding cages. In a natural setting percussive damage from normal activities, and therefore the likelihood of more excessive bleeding leading to death, would be expected. In addition, once mice in the trial became strongly affected by the poison they became unresponsive to gentle stimuli and did not readily seek refuge within their cardboard tubes or beneath shredded paper but rather sat out in the open. Wild mice displaying these behaviours would be vulnerable to predation and, because the eradication is planned for winter, they would be exposed to cold temperatures, both of which are likely to reduce the survivability of wild mice following consumption of bait.

One mouse died during the acclimatisation period. Bait is used by residents in and around the settlement area, and there was evidence that this mouse had been poisoned prior to capture. It is possible that the mice that were the quickest to die during the baiting trial had also eaten bait prior to being captured. However, no mice in the control group died once the baiting component of the trial had begun, suggesting that few if any of the mice used in this trial had previously ingested a lethal dose of bait and that the deaths in the baited groups were a result of ingestion of Pestoff 20R during the trial.

While there is little doubt that the death of mice was due to ingestion of Pestoff 20R (i.e. no mice in the control group died), necropsies of dead mice were performed to provide confirmation that the cause of death was brodifacoum poisoning. A number of mice examined showed external signs of haemorrhaging as evidenced by bleeding around the nose and mouth and darkening in the rear leg joints. Mice that showed no external signs of haemorrhaging were dissected. These mice exhibited various signs of being affected by brodifacoum including bleeding in the pericardium, subcutaneous bleeding along the flanks, discoloured kidneys, and blotchy lungs.

Mice in the baited groups lost more body mass throughout the trial than did mice in the Control group. It is likely that this loss of body mass is due to illness and a loss of appetite once the poison had begun taking an effect rather than an aversion to the bait and therefore a lack of overall food intake. Pestoff 20R does not contain the bitter compound (Bitrex®) found in commercially available rodenticides containing brodifacoum. Previous trials on LHI
have shown that Pestoff 20R is palatable to LHI rodents (Wheeler & Carlile 2013) and in this trial a number of mice were seen to almost immediately begin to chew the pellets following the provision of them. Conversely, mice that were fed commercial pet food during the acclimatisation period and those in the control group, were rarely seen consuming food.

In conclusion, this trial demonstrated that, despite there being a broad range of tolerance levels to brodifacoum in the Lord Howe Island mouse population, Pestoff 20R, when provided in a manner consistent with the methods proposed for the rodent eradication, will be effective against Lord Howe Island mice.

REFERENCES