# ANNEX 2: RODENT TRAPPING AND DNA SAMPLING

## Contents



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## 1. Why and when do you need to trap rodents?

1.1. There are a number of reasons why trapping rodents is required, most usually when you need the rodent itself, rather than just evidence that a rodent is present. There are occasional other uses for trapping, also. **However, animal welfare must be a primary consideration when using traps. It is important to ensure that the right trapping methods are used, and in the right way. If there are animal welfare concerns this may render traps - both live capture and kill - unfit for use.**

1.2. Trapping can/should be used:

a) To obtain genetic material (DNA) to **help assess a project's feasibility - is eradication sustainable** or are rats likely to reinvade quickly?

- A genetic comparison should be made between the animals on the target island with those of likely/possible source populations on the mainland or neighbouring islands, particularly those within twice the known swimming distance of the target species. This involves taking representative DNA samples from each population (See Section 5 below).
- Results are used to estimate the frequency of animals invading the island, or the 'connectivity' of the island's rodent populations with potential source populations, which will support a decision on whether or not eradication is the best course of action.
- However, recent research shows that newly arriving rats are less likely to become established on islands where rats are already present (Fraser et al. 2015). This means that rat populations on two islands may be genetically distinct despite rats being able to move between them. The results of DNA genetic comparison studies should therefore be interpreted very cautiously, especially where the islands are within or close to the known swimming distances for rats.

b) To obtain DNA to **assess rodenticide resistance** in the island rodent population, and so be able to identify a rodenticide that is likely to achieve a 100% kill rate, i.e. **is eradication technically feasible**?

- Resistance to a number of rodenticides is known in the UK, including from some islands. Tests for some types of resistance are available and require DNA samples in order to be conducted.
- **N.B.** There is a trade off between applying early for rodenticide permissions and conducting early resistance testing & conducting resistance testing closer to the time of eradication and applying later for the rodenticide permits. **Seek advice**.

c) To obtain DNA for **biosecurity planning** purposes prior to, and following, eradication.

- DNA samples can be used as the basis for a genetic comparison if rodents are discovered and collected on the island after the eradication in order to gauge whether there was a reinvasion or the eradication attempt failed. This can only be done if DNA samples are taken prior to the eradication. It may be possible to get a university or resistance testing facility to store the DNA strand information for the project in one of their long-term DNA/resistance mapping projects.
- This knowledge is crucial: if there has been a reinvasion you will need to revisit biosecurity arrangements. If the initial eradication failed, you need to re-evaluate the Operational Plan and Operation Log and determine the cause of failure and whether or not it can be addressed if a fresh eradication attempt is made.

d) To determine the exact rodent/target species present on the island, gain insight into abundance and spread across the island to assist with the **Feasibility study** and also **Operational planning,** and gain insight into their diet (e.g. to help demonstrate the **project need**).

- Ensuring correct rodent identification is important, for example, to determine the required grid size across the different project areas (See Annexes 1 & 3).
- An idea of abundance and spread can be gained by index trapping (Section 3 below). If there are hotspot areas, a smaller grid may be required in these areas. In areas of low rodent density (e.g. in poor habitats such as bogs) it may be possible to reduce the number of grid points.

e) If the presence of house mouse/other invasive rodents is unknown – i.e. as **part of the assessment of the project's environmental-acceptability (Feasibility Study).** It is important to determine whether or not there is a rat-suppressed house mouse population with potential for mesopredator release[1], for example.

- If mice are present, your project objectives may need to change (you may need to target mice as well). If you do not target mice, you will need to assess the likely environmental impact of replacing a rat population with a potentially invasive mouse population.
- To determine if mice are present, 'trap out' rats from an area of good house mouse habitat (e.g. around buildings), protect the area from rat reinvasion (e.g. by rat-proof fencing), allow time for mice to respond/population to recover and implement a mouse surveillance programme. An area approximately 1-5 ha should be trapped to ensure that there are enough mice present to produce a measurable response to the lack of rats within a reasonably short time. Historical information and/ or consultation with residents may also provide information on the presence of mice. Setting traps specific for small mammals (e.g. Longworth live-capture traps), tracking tunnels and camera traps can also be useful ways of determining the presence of mice

f) In the lead up to an eradication attempt using rodenticides, routine rodent control using such products must stop to improve the likelihood of eradication success (**implementation of Operational Plan**). This is to prevent rats getting used to the presence of bait and possibly developing an aversion to the bait and/or bait station, especially as they could obtain a sub-lethal dose depending on what bait is being used. It also covers the possibility that any existing bait stations have not been placed in optimal positions, or are not using the correct methods to control rats. When used for control, often bait is put in a station and left for a long period of time, which could prompt the rat to ignore the station due to bait attractiveness issues. There are also resistance issues to consider – the continued use of bait could add to the possibility that resistance may occur and cause issues for the eradication operation.

- This is most likely to be necessary on inhabited islands, but control using rodenticide may also be in place on uninhabited islands.
- Rodenticide use for routine control purposes should cease at least six months (ideally a year) before the start of the eradication operation. In between times, residents may wish to have an alternative control method to the use of poisoned bait. Traps should be provided to the residents by the project (free of charge), and a suitably skilled person should provide training in proper trap use. You must ensure guidelines (Section 2 below) are followed.

[1] a process whereby mid-sized carnivorous mammals became far more abundant after being "released" from the control of a larger carnivore.

g) As a **complementary eradication technique** used, for example, around homes and food stores.

**N.B.** Traps are not effective tools for eradication unless used in combination with anticoagulant rodenticides (although future developments in trap design or efficiency may increase their role in eradication projects).

- You must ensure guidelines (Section 2 below) are followed.

h) To respond to a confirmed rodent sighting towards the end of the eradication operation or at any point after it (**eradication** and **biosecurity implementation**).

- If a rat/mouse is discovered on a recently cleared island, an emergency response should be initiated to locate and kill it. As many different detection and capture devices as possible should be deployed in the area, including traps. **N.B.** Traps are not usually used as part of on-going surveillance for rodents, but are deployed in response to a rodent being detected by other devices.

- Any captured rodent should be necropsied to determine whether or not they are already breeding on the island – and so to help assess at what stage the invasion may be at/whether there are more rodents that require capture (Section 4 below).

i) Research value e.g. rat colonisation phylogeny.

1.3. In addition to the DNA samples, other information important to the project can also be obtained from trapped rodents (e.g. breeding status of population, population structure). Follow the guidance in Section 4 (below) to obtain this information from the animals you capture.

## 2. Guidance on the use of live and kill traps for rodents

2.1. **You must ensure that all trap use as part of the project, or resulting from the project, is in accordance with these guidelines.**

2.2. **ANIMAL WELFARE SHOULD BE A PRIMARY CONSIDERATION WHEN USING TRAPS: IT IS IMPORTANT TO ENSURE THAT THE RIGHT TRAPPING METHODS ARE USED, AND IN THE RIGHT WAY.** Any deviation from this will render traps – both live capture and kill – unfit for use.

2.3. **DO NOT USE LIVE TRAPS IF THERE MAY BE ACCESS ISSUES WHICH MEANS DAILY CHECKING CANNOT BE GUARANTEED.**

2.4. Traps should be set at dusk and checked and disarmed at dawn. The target species are most active at night, so this increases the chances of trapping successfully whilst also reducing the chances of catching diurnal non-target species.

2.5. Live capture traps may be useful if there is a high risk of non-target casualties resulting from the use of kill traps. However, live traps will need to be checked ***at least twice a day*** as any animal caught in them is protected by the Animal Welfare Act (2006)/ Animal Health and Welfare (Scotland) Act (2006) (making it an offence to cause unnecessary suffering). Live traps must be placed so that any captured animal is protected from weather and temperature extremes or flooding.

2.6. Spring traps (e.g. Fenn and DOC traps) should be checked ***at least once per day*** as a kill cannot be guaranteed. Only spring traps designed to catch and kill rats or mice humanely, listed by the relevant Spring Traps Approval Order and used in accordance with the stipulations of the Order may be used, therefore should be consulted before use. Approved kill traps have to cause irreversible unconsciousness within 5 minutes (300 seconds) in 80% of captures. Fenn traps are widely used, but failed recent testing in New Zealand for humaneness and can be difficult to set. New Zealand DOC traps are more expensive, bulky and must be used inside bespoke wooden tunnels, but they achieve very high kill rates, with a catch efficiency much higher than that of Fenn Traps. Neither Fenn nor DOC traps are suitable for catching mice.

2.7. Spring traps approval is now a devolved issue. Relevant documents can be found on http://www.legislation.gov.uk Check you are referencing the correct Order (and for updates):

**England (2012)**

**Northern Ireland (2012)**

**Scotland (2011)**

**Wales (2012)**

2.8. Break-back (snap) traps may be used against rats and mice. They are not subject to the Spring Traps Approval Order so any models can be used. However, Baker et al. (2012) assessed the performance of break-back traps available in the UK, with a view to their humaneness and found that strength and performance varied widely between models. Seek advice from professionals in the field before selecting trap types. Trapper T-Rex™ traps and Victor Professional™ break-back traps are easy to carry in the field and set, but may not kill particularly large brown rats. Ideally, break-back traps should also be checked once a day.

2.9. It is illegal to use leg-hold traps (gin traps) in the EU. There are serious welfare concerns surrounding the use of glue boards and these should not be used (unless set upside down to capture hairs as part of surveillance (see Annex 3), though using Velcro is preferable.

2.10. Goodnature A24 traps (self re-setting multi-kill devices) have been approved for use in England, and approval is pending for their use in Scotland against rats (as well as stoats in areas where they are not native). Approvals for use in the other UK nations are also likely. Additional work on target specificity of the traps in a UK context may be required before use to assess risks to non-target species.

2.11. Even approved kill traps may not kill all animals that enter them: all personnel involved in checking traps should be trained in killing injured or maimed animals in a humane, legal and efficient manner. It is illegal to release some non-target species in the UK (such as grey squirrels – see Section 14 of the Wildlife and Countryside Act for full lists), and in Scotland it is an offence to release any animal to a location outside of it's natural range. You must have a plan for dealing with the accidental capture of such species, e.g. personnel must be trained and prepared to kill these species also, even if they are not harmed in the trapping process, or to transport them to a rescue centre where they will live in captivity.

2.12. **All set kill traps must be covered** so as to reduce the likelihood of non-target species being maimed or killed. Covers should be designed so as to guide the rodent into the front of the trap to increase the likelihood of a clean kill: e.g. by building a natural or artificial tunnel, or placing in a bespoke rodent surveillance box (see Figure A2.1).

2.13. A full assessment of risks to non-target species should be conducted prior to setting traps and appropriate mitigation measures installed. For example wire, or similar, should be placed across the entrances of covers to **reduce the entrance size.** This should be covered in the Environmental Impact Assessment (as part of the Feasibility study). Be aware that such measures may make it less likely that your target species will enter them.

2.14. Place traps where there is plenty of natural cover and where rodents are likely to be active e.g. alongside walls, buildings or large rocks, around the base of trees, or near any rodent sign.

2.15. **Break-back (snap) traps must be tied firmly** with strong string or wire to vegetation or held by a firmly set peg, so that injured animals cannot drag them away or be dragged away by scavenging predators.

2.16. Break-back traps should be set in pairs, back to back. Leave a slight gap between them so one trap can be set off without triggering the second. If both rats and mice are present, or if you are unsure which rodents are present, set a mouse and a rat trap at each site. If only rats or only mice are present, set two traps of the appropriate type. Always use the right trap: large rats may escape from mouse traps (or not be killed cleanly), whilst mice may not trigger rat traps.

2.17. A lure (commonly known as 'bait' but not be confused with poison bait containing rodenticide) should be placed between traps if one cannot be placed in each trap. A mixture of rolled oats and peanut butter is recommended, but chicken eggs, chocolate, fish oil, and bacon can also be used. Use a strong-smelling protein lure for brown rats.

2.18. Ensure traps are on a level surface and are stable so that they don't move/rock if pressure is put on any corner or side of the trap.

2.19. Traps need to be maintained. Un-galvanised traps may rust quickly if used outside, reducing their efficacy. Metal parts can be treated with fish oil or wax but anti-rust sprays may deter rodents. Disarming traps each morning helps prevent jamming. Take traps in when not in use.

2.20. Some traps are capable of breaking a person's fingers. Ensure you only ever handle a set trap from the back. Refer to guidelines for individual trap types and seek assistance if unsure. Cover safe trap handling within the Health and Safety Plan.

2.21. Rodents are carriers of diseases which can be fatal to humans, and can carry ticks which may harbour Lyme disease. Appropriate measures should be taken to ensure people handling rodents and rodent carcasses are protected, e.g. cover scratches and cuts, wear gloves and wash hands thoroughly before eating, drinking or smoking. Cover safe rodent handling within the Health and Safety Plan.

**Figure A2.1 -** Above: Two (set) Trapper T-Rex™ traps back to back in a homemade wooden tunnel. The entrance to the tunnel restricts entry by larger species and prevents the trap being dragged away. The gap between traps prevents them setting each other off. Below: The same in a natural tunnel made of branches (shown both open and covered). Images © WMIL.

**Figure A2.2 -** (left): trap set and placed within a lockable plastic box (Protecta™) and (right) station closed and secured to post with wire. Single trap use like this is most likely in response to an incursion or reinvasion (biosecurity breach), rather than initial scoping and planning phases. Images © WMIL.

## 3. Index trapping

3.1. Index trapping helps assess the density and distribution of rodents over the island, which in turn can inform operational planning (e.g. grid density in different parts of the island). It can also be used to compare populations between years, although it is important to recognise that lower abundance does not equate to 'easier to eradicate'. For best results, use a mixture of methods to determine abundance (e.g. index trapping and tracking tunnels or wax chew blocks – see Annex 3 for information on using surveillance devices).

3.2. Abundance (or rat density) is recognised as low (less than 10%), moderate (between 11-25%), high (between 26-50%) and very high (over 50%) (King 1990, Moors 1985). Islands usually vary between 5-25%. The % figure is also known as 'rats per 100 trap nights' (see Section 3.7).

3.3. Always use the same brand of trap and the same lures for index trapping to enable comparison (differences in their effectiveness will otherwise bias your results). Break-back (snap) traps are the most commonly used type for index trapping.

3.4. An index line should consist of at least 25 trapping sites evenly spaced within the same habitat type (e.g. grassland, woodland), with two traps per site. Try to place traps in sites likely to appeal to rats – see Section 2.14 for guidance while keeping to the correct distance spacing. The distance between pairs of traps should be as large as possible, within the range of 25-50m. Keeping the distance between pairs of traps equal within and between trap lines in different habitats means their results can be more meaningfully compared. Index lines are usually run for three consecutive nights. A minimum of 100 corrected trap-nights should be achieved in each habitat: 50 traps (one index line) for three nights gives a maximum of 150 trap-nights; this number will be reduced if any traps are found to have been set off without capture ('empty & sprung').

3.5. Place trap lines in different habitats across the island to determine relative abundance.

3.6. Number each trap and record its position using GPS. Keep accurate records for each trap (e.g. 'no take & unsprung', 'bait taken but unsprung', 'empty & sprung', 'rodent captured') and make note of the weather conditions overnight.

3.7. The Abundance Index is a measure of the number of individuals captured adjusted by the number of traps deployed (Cunningham & Moors 1996), see Table A2.1.

**Table A2.1 –** Example of a calculation of an Abundance Index.

| Factor | Calculation |
|---|---|
| 50 traps run<br>3 nights<br>16 rats caught<br>9 sprung, empty traps | Total trap nights (TTN) = number of traps x number of nights<br>• TTN = 50 x 3 = 150<br>Lost trap nights (LTN) = ½ (captures + sprung, empty traps)<br>• LTN = ½ (16+9) = ½ (25) = 12.5<br>Corrected trap nights (CTN) = TTN – LTN<br>• CTN = 150 – 12.5 = 137.5<br>Index of Abundance (IoA) = captures x 100 / CTN<br>• IoA = 16 x 100 / 137.5 = 1600 / 137.5 = 11.6 **rats per 100 trap nights**<br>= **11.6%** |

3.8. A well designed Capture-Mark-Recapture study using live trapping could potentially provide more accurate evidence of population size, as well as information on sex and age distribution and ranging behaviour.

## 4. Necropsy, measuring and sexing rodents

Try to collect the following information from the animals you capture (most vital information indicated with (*) below):

1. **Date of capture** and **trap location** (*)**;**
2. **Head-body length (mm)** – taken from tip of nose to end of anus;
3. **Tail length (mm)** – taken from middle of anus to tip of tail;
4. **Hind foot length (mm) –** taken from heel to tip of longest toe, record measurements both including and excluding claw (to allow comparison with results from other researchers);
5. **Ear length (mm) –** taken from bottom of the notch of the ear to furthest point along the rim;
6. **Weight (g);**
7. **Age** (*) – juvenile or adult (see Figure A2.3);
8. **General body condition** – condition of coat (shiny/ dull), presence of scabs, damage to the tail and ears, injuries, parasites and other general comments;
9. **Sex** (*) **–** see Figure A2.3;
10. **Breeding condition** (*) – see Figure A2.3. If nipples are large with little hair around them, check to see if the female is lactating. Check ovaries for size and colour to see if coming to breeding condition and check uterus for embryos (usually recorded as the number per branch – 3/2 means 3 embryos on the left and 2 on the right, size of embryos is also handy to know for stage of breeding cycle). Note unperforated females as well; and
11. **Stomach contents** – identification of content groups and estimate percentage (e.g. 40% vegetation, 10% invertebrate etc.).

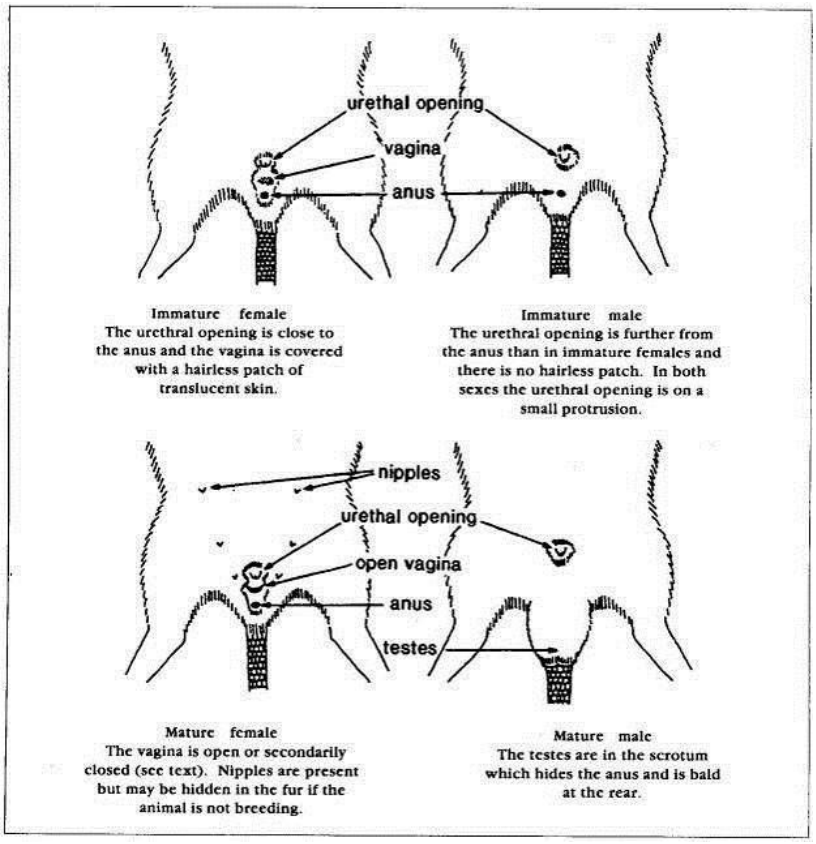


**Figure A2.3 -** Guide to rat breeding condition. Secondarily closed vaginas can be easily opened with a probe, those of immature rats cannot. From Cunningham & Moors (1996).

## 5. Guidance on the collection of DNA samples

Adapted from Russell (2006) (contained within New Zealand DOC, 2008).

5.1. **Sample collection:** DNA can be found on almost any item that has been in prolonged contact with a living body. For island biosecurity purposes the best sources of DNA are from bodies (tissue), hair or faeces. DNA can only survive outside of living organisms if it is preserved correctly. For island biosecurity purposes DNA will become very hard to isolate after the sample has been exposed for more than 3-4 weeks.

- Collect samples from at least 20 individuals per target species for each DNA study: more is better.
- For each rodent, cut off a 3-4 cm length of tail using sterile equipment (scalpel/knife/scissors), place it in a collecting vial and immerse it in 70-95% ethanol (note, DNA can be denatured in absolute alcohol). ANY piece of flesh can be used if the tail is not intact.
- NEVER mix samples from different individuals in the same vial. Ensure blood or tissue from one individual never contaminates the sample of another (from a dirty knife/scissors/gloves etc).
- The closer the sample is taken to the rodent's death, the better.
- If possible, keep the sample refrigerated or frozen.
- If you cannot use ethanol, samples should be frozen - triple bag each sample.
- Label your samples properly with date, type of rodent, location (preferably GPS-ed), collector and part of the rodent sampled. Consider double labelling on both the outside and inside of vials.
- Procedure for faeces – faeces should be preserved in ethanol or frozen. Collect as much as is available (i.e. 20 droppings are more valuable than just one).
- Procedure for hair – microscopically visible features on the hair itself may allow for species identification, however, by preference you want to pluck hair so as to obtain hair follicles. Aim for at least ten hairs and obtain guard hairs if possible. Hair needs only to be wrapped in paper and placed in a paper envelope with some silica desiccant beads.

5.2. **Analyses:** It is important to choose the right type of genetic analysis. Methods for identifying the species may be different to those for identifying the likely origin of an individual for example.

DNA can be used to determine where an individual has come from. Generally microsatellite markers are the most informative at the population (island) level. You will need genetic samples from all likely locations that you think an individual might have come from; otherwise you can only tell that it didn't come from the place(s) from which you collected samples.

5.1. **Contamination**: Contamination from different species is not a major issue in DNA analysis. If a person has touched rodent faeces then the DNA extracted from them will still clearly appear as rodent DNA. This will be obvious to the scientist doing the analysis. Contamination from the same species, however, is a major issue: ensure equipment is thoroughly cleaned/sterilised after each sample is taken.

5.2. **Costs:** Costs will depend on who you get to perform the analysis for you, what type, how well preserved the DNA is, and whether the laboratory is set up for analysing this species. As an indication, to analyse one rat sample is likely to cost more than £100. To analyse many samples costs may go down to as low as around £25 a sample. If the lab is already doing some work on this species, then costs may be lower still. Most costs are in buying start-up chemicals specific to the species you are working on so analysis may be cheaper if a laboratory is already set-up for rodent DNA analysis. You may be able to get some analyses done for free, e.g. if the samples can be used by a researcher or student in their work.

5.3. While the financial cost of such DNA analysis can be significant for larger islands or those with multiple possible source locations (where more sampling is required), it is far lower than the financial and social costs of having rodents quickly reinvade an island.

5.4. **Contacts**

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New Zealand experts with specific island restoration experience:

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## 6. Rodent trapping equipment list

- Kill traps (break-back traps are the usual choice) (e.g. 150)
- Trap covers (half the trap number, if setting in pairs – every trap must be covered)
- Lures/ non-toxic bait, such as peanut butter (e.g. 450 'doses' for three nights of trapping: assume you need to replace each night)
- Wire/sturdy tent pegs to secure every trap / cover
- String to tie the traps to the tent pegs (to prevent them being dragged away by injured rats)
- Marking poles & flagging tape (to help locate traps)
- Plastic tags (to number traps) and warning labels
- GPS device
- Notebook & pencils/pens
- Nitrile gloves
- Vernier callipers
- Scales/ balance for weighing rats – electronic or spring balance or even basic kitchen scales
- Ruler/tape measure
- Sharp scissors, knife, scalpel
- Forceps
- Collecting vials (e.g. at least 30) and sealable bags (e.g. zip lock bags)
- Ethanol
- Sterilising equipment

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