Invertebrates: search and extraction methods

Version 1.1

This specification was prepared by Greg Sherley and Alison Evans in 2016.

Contents

Department of Conservation
Te Papa Atawhai

Synopsis

Manual methods are usually employed to collect invertebrates opportunistically for survey and inventory work when the objective is to determine what species are present. They are occasionally adapted for taking quantitative samples when monitoring or for comparative purposes. If you want to use these methods quantitatively then we encourage you to consult Southwood & Henderson (2000) as this is one of the most authoritative texts for this. Quantitative work for survey, monitoring or study requires an experimental design whereby decisions are made on the number of samples required, and what randomisation and replication is required. This generally requires specialist statistical input once you have clarified what the purpose is for taking the samples or what question you want answered by taking the samples.

This specification describes the following four basic search and extraction methods, which can be used in a variety of different ways and are often used together.

- Hand searching (including using artificial habitats and stratified sampling)
- Foliage beating
- Sweep netting
- Soil and litter extraction

All four methods can be used for simple inventory of species found and in some cases can be adapted to collect quantitative measurements (e.g. catch per unit effort or per unit of area).

Hand searching simply involves manually looking for invertebrates in their habitats and moving objects such as logs or rocks with your hands. It can involve targeting particular species by concentrating on places where they are known or suspected to be found. It also includes searching at night with lights. Manual searching does not normally require specialised equipment, but forceps (tweezers) and a pooter (aspirator) (Figure 1) may be useful for collecting small specimens. Hand searching may be supported by the use of artificial habitats such as logs, wooden discs or wētā motels. Artificial habitats that are introduced to an area may take several months to become occupied but can be an effective monitor tool. Although hand searching is generally used to collect qualitative data for inventory, searching can be stratified into units of area or time spent searching if monitoring is necessary. Hand searching may also include collection of lice or mites from birds. Collecting these groups of invertebrates requires specialist training to extract the specimens.

Foliage beating involves using a stick to repeatedly hit branches and dislodge invertebrates that fall into collection trays. This method is typically used to collect invertebrates resting on the foliage of shrubs or trees (e.g. spiders, caterpillars, aphids, flies, beetles). Only foliage which is accessible is sampled. The method is usually used for inventory purposes but has also been used to collect quantitative data (Memmott et al. 2000).

A sweep net can be used either to catch individual insects or to sweep through vegetation to capture a variety of invertebrates. It is usually used in combination with other manual search

methods when making an inventory for a given location. Nets are best used to confirm if a species is present, but it is difficult to standardise their use so they are not employed for quantitative work.

Most specimens caught by hand, by foliage beating or using a sweep net will need to be collected in suitably sized containers such as glass or plastic vials or a killing jar for later identification by specialists. They usually need to be preserved in alcohol if they are soft bodied or they are wanted for later DNA analysis, or they can be killed in a killing jar or frozen. Moths and butterflies, together with many other insects, may need to be pinned when dead, but whether this is necessary or not depends on the taxonomist employed to identify them.

Extraction methods include taking leaf litter samples or soil cores (usually of the upper soil horizons) and extracting the invertebrates from them either by searching through the samples by hand or using a Berlese or Tullgren funnel. Soil samples are obtained with a corer or spade whereas litter samples can be collected either randomly or systematically. The leaf litter samples are taken by gathering up everything within a measured area or collecting a measured volume. Hence both soil cores and leaf litter samples can provide quantitative data—the number of specimens per area, volume or the dry weight of material that was collected. Once collected, many of the invertebrates within the soil or litter samples may be extracted by placing them on wide mesh in a Berlese or Tullgren funnel. The invertebrates are extracted by slowly and progressively either drying out or heating up the sample from the top down so that the invertebrates move downward and eventually either drop through a funnel into a collection container containing either ethanol or water.

Invertebrate diversity and abundance can vary at a fine scale (even tens of centimetres), so search and extraction methods yield results that are extremely site-specific. However, generalisations can be made for larger areas if sufficient sampling is done over the area. Most invertebrates collected using Berlese or Tullgren funnels will be less than 2–3 mm long and will require identification by specialists using a microscope. The soft-bodied invertebrates collected such as slugs, flatworms and earthworms may require specialised preservation.

When manual search methods are used for general inventory they reflect the habitat type, the species that were active at the time and location(s) searched, the ability of the researcher to detect the target species, and what species can be caught using the methods used. When these methods are used to target specific species, it is necessary to know the specific habitat requirements of the species being sought.

Regardless of the collection method, it is critically important to keep full details of where the specimens were collected (see 'Minimum attributes'). Specimens without collection data are absolutely useless.

The following general principles apply to all manual collection methods:

- They do not provide a comprehensive species list of invertebrates at a given location.
- A species is not necessarily absent if it is not found.
- There will be several common species but most species will only be represented by a few individuals.

- The number of sampling points will be determined by the extent of the area being sampled and the point at which you are confident that no new species have been collected in the samples. This is an arbitrary point.

Assumptions

When search and extraction methods are used opportunistically for inventory purposes, then the assumptions are:

- Not all species will be detected.
- The number of species collected is proportional to collection effort.

When search and extraction methods are used for obtaining quantitative data in a standardised way, the assumptions common to all methods are:

- Each individual has an equal chance of being caught.
- The capture of one animal will not interfere with the capture of another individual of the same species (or possibly other species). Note: if the species is rare this interaction may well occur.
- There is a direct (but unknown) proportional relationship between the numbers of a particular invertebrate species caught and the abundance of that species that are active in the environment.

Advantages

- Search methods can be used to target particular species so that fewer non-target specimens are collected. This also reduces sorting and identification costs.
- Minimal equipment is required, and the equipment is cheap and easily operated by almost anyone. The full range of equipment may be easily transported to almost any location and, when used together, may provide a reasonably good survey at a given time.
- In some cases it is possible to customise a quantitative sampling design for a specific survey or monitoring (usually where the habitat preferences of the target species is known and the methods which are available are efficient at sampling it).

Disadvantages

- Generally, collection data from manual methods are unsuitable for statistical analysis except where sampling has been standardised for quantitative work.
- Sampling targeted species requires knowledge of their habitat requirements (where and when they are likely to be found).
- Collection may be poor if conditions are not suitable or if a species is cryptic.

- Usually, only qualitative conclusions can be made from the information gathered using these methods.
- Manual search methods generally only give presence/absence information at the instant and the location where the sample was taken. Not finding a species does not mean that it is absent.
- Some of the chemicals used to preserve soft-bodied invertebrates are toxic and may be difficult to acquire.

Suitability for inventory

Manual search and extraction methods are the most frequently used methods for inventory. They commonly provide information about which species are present but should be used with the understanding that a species is not necessarily absent if it is not found.

The effectiveness of manual searching may be improved with experience and knowledge of the species targeted. Using them in repeated surveys and assessing the number of new species found each time will give some idea of how comprehensive an inventory is by using statistical programmes such as Estimate-S and PC-ORD. These can predict the total number of species that could be caught in the area with the methods used from your data—provided you have repeated the searches a sufficient number of times.

Suitability for monitoring

The methods can be useful for monitoring, particularly if a species is not very active (and therefore unlikely to be collected by passive traps). Specific knowledge of the habitats and behaviour of the targeted species is required. When search and extraction methods are used for monitoring, the sampling design needs to be thoroughly understood so that errors are not made and sampling bias is not introduced.

Skills required

- An understanding of the habitat use and basic biology of the target species
- Training in the use of sweep nets, soil corers and Berlese or Tullgren funnels
- Use of GPS units
- Knowledge about how to store the specimens correctly (curation) until they can be identified

For target studies, the collector needs to be thoroughly familiar with how to identify the species and how to distinguish it from any other similar species that may be present at the location.

Resources

Resources required for hand searching

- Personnel—ideally at least two people required for safety, collecting invertebrates and to record data.

- Notebook and pencil to record date, position and data associated with the collection of invertebrate specimens.

- Accurate map of area to identify the geographical boundaries of land and habitats.

- Spotlight, torches and/or headlamps to assist with night searches.

- Watch or stopwatch to record time and duration of search time.

- GPS to record the position of study site or area searched.

- Camera to record information on vegetation or habitat type and to take photos for presentations or reports.

- Invertebrate collecting equipment—including vials, tubes, soft-touch forceps, fine paint brush, pooter, sweep nets, chilly bin, zip-lock bags, marker pens, 99% ethanol, and labels— to collect and store invertebrates for identification on subsequent sampling occasion/s.

- Safety equipment—such as cell phone, VHF radio and first aid kit—to contact emergency services if necessary or administer basic first aid.

- Identification guide (see 'Invertebrate identification aids'—doccm-388198) to determine which groups of insects are being collected.
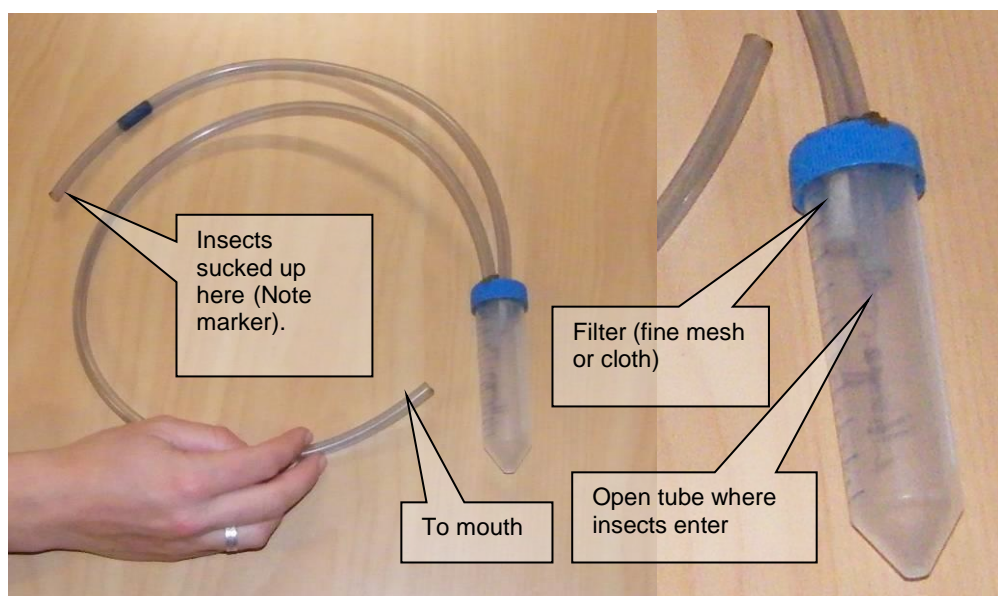
Figure 1. A pooter or aspirator. This can be made using any small, clear, elongate container that can be modified many ways to suit specific requirements. It allows specimens (especially fragile ones) to be collected without actually touching them. Insects are sucked up with a short, sharp inhalation after applying the mouth to the filtered tube. The opening through which insects enter is marked with tape to avoid accidentally sucking the wrong tube. Care is needed with delicate insects, which can be smashed against the end of the container is too much suction is applied.

Resources required for foliage beating

- Personnel—ideally at least two people required for safety, collecting invertebrates and to record data.
- Notebook and pencil to record date, position and data associated with the collection of invertebrate specimens.
- Accurate map of area to identify the geographical boundaries of land and habitats.
- A white tray or sheet to collect the dislodged invertebrates.
- Watch or stopwatch to record time and duration of collection time.
- GPS to record the position of study site or area searched.
- Camera to record information on vegetation or habitat type and to take photos for presentations or reports.
- Invertebrate collecting equipment—including vials, tubes, soft-touch forceps, fine paint brush, pooter, sweep nets, chilly bin, zip-lock bags, marker pens, 99% ethanol, and labels—to collect and store invertebrates for identification.
- Safety equipment—such as cell phone, VHF radio and first aid kit—to contact emergency services if necessary or administer basic first aid.
- Identification guide (see 'Invertebrate identification aids'—doccm-388198) to determine which groups of insects are being collected.
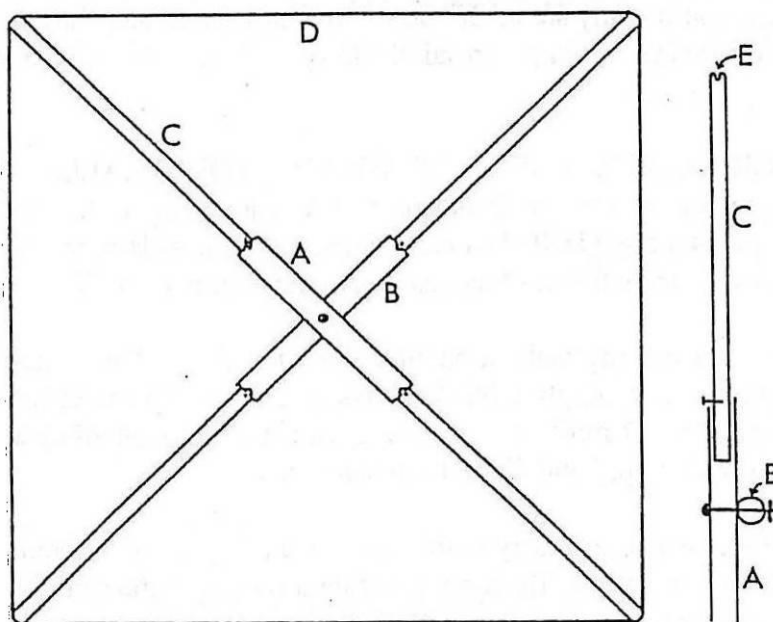

Figure 2. A beating tray or sheet. A and B are aluminium tubes fastened together with a bolt, and 50–70 cm lengths of dowelling, C, fit into the tubes. A cord is stretched around the ends of the dowels and is held in place by notches at the ends, E. White cotton cloth (or parka nylon is good) is sewn over the cord to form the sheet. Diagram by Liz Grant, with permission, Massey University.

Resources required for sweep netting

- Personnel—ideally at least two people required for safety, collecting invertebrates and to record data.
- Notebook and pencil to record date, position and data associated with the collection of invertebrate specimens.
- Accurate map of area to identify the geographical boundaries of land and habitats.
- Spotlight, torches and/or headlamps to assist with night searches.
- Watch or stopwatch to record time and duration of search time.
- GPS to record the position of study site or area searched.
- Camera to record information on vegetation or habitat type and to take photos for presentations or reports.
- Invertebrate collecting equipment—including vials, tubes, soft-touch forceps, fine paint brush, pooter, sweep nets, chilly bin, zip-lock bags, marker pens, 99% ethanol, and labels—to collect and store invertebrates for identification.
- Safety equipment—such as cell phone, VHF radio and first aid kit—to contact emergency services if necessary or administer basic first aid.
- Identification guide (see 'Invertebrate identification aids'—doccm-388198) to determine which groups of insects are being collected.

Resources required for soil and litter extraction

- Personnel—ideally at least two people required for safety, collecting invertebrates and to record data.
- Notebook and pencil to record date, position and data associated with the collection of invertebrate specimens.
- Accurate map of area to identify the geographical boundaries of land and habitats.
- Soil corer to collect standardised core samples.
- Plastic zip-lock bags to collect soil samples and thick paper bags to collect leaf litter samples.
- A quadrat or tape measure and pegs to standardise the area from which the sample was collected.
- GPS to record the position of study site or area searched.
- Camera to record information on vegetation or habitat type and to take photos for presentations or reports.
- Invertebrate collecting equipment—including vials, tubes, soft-touch forceps, fine paint brush, pooter, sweep nets, chilly bin, zip-lock bags, marker pens, 99% ethanol, and labels—to collect and store invertebrates for identification.
- Safety equipment—such as cell phone, VHF radio and first aid kit—to contact emergency services if necessary or administer basic first aid.

- Identification guide (see 'Invertebrate identification aids'—doccm-388198) to determine which groups of insects are being collected.
- Tullgren or Berlese funnel and suitably sized collection containers to extract invertebrates from samples.

Figure 3. Soil core sampler. The handle is used to push and twist the corer into the soil. Near the base of the handle is a yoke attached to an internal disc (shown in Figure 4) which is used to push the soil sample out afterwards.

Figure 4. End of a soil core sampler. The notch in the cylinder helps dig the instrument into the soil when it is twisted while the disc (attached to the yoke shown in Figure 3) is used to push the sample out of the corer.

Figure 5. Two different designs of Tullgren funnel. Both have light bulbs in the lid (Figure 6) which progressively dry material suspended on coarse mesh inside (Figure 7). Invertebrates move downward as the material dries and fall into the collecting containers below.

Figure 6. Tullgren funnel with lid lifted to show the lightbulb.

Inventory and monitoring toolbox: invertebrates

Figure 7. View inside a Tullgren funnel showing leaf litter suspended on a coarse metal mesh.

Minimum attributes

These attributes are critical for the implementation of the search and extraction methods. Other attributes may be required depending on your objectives.

DOC staff must complete a 'Standard inventory and monitoring project plan' (doccm-146272).[1]

Minimum attributes to record:

- GPS coordinates of the collection locality (usually 8-figure coordinates)
- The date of collection
- The collector's name and address or institutional affiliation
- Method of capture
- Authority identifying the specimen
- For quantitative work, as appropriate, precise measurements of the area or volume searched (m^2), and/or the time taken to search for each sample unit (per minute)

It is essential to write out a small label on laundry tag paper or goatskin parchment at the time of collection giving the location, GPS position, collection date and collector's name for each sample and keep it with each sample as it is processed.

Additional attributes (record where appropriate):

- The time when the specimens were collected
- Where the specimen is lodged

---

[1] http://www.doc.govt.nz/Documents/science-and-technical/inventory-monitoring/im-toolbox-standard-inventory-and-monitoring-project-plan.doc

- Photographs of the location

- Brief description of habitat

- Meteorological conditions when caught, especially the temperature, humidity if available, and rainfall. In some situations, such as sweep netting, manual searching, and use of beating sheets, description of weather conditions such as sunshine and temperature are extremely useful.

Data storage

Forward copies of completed survey sheets to the survey administrator, or enter data into an appropriate spreadsheet as soon as possible. Write up the fieldwork, preferably as the work is done in the field or immediately on return from the field, and make an electronic copy. Entering the data in the field allows you to check for missing information and errors and correct them, whereas it is often impossible to correct or add missing data once you have left the field. The key steps here are data entry, metadata entry (these are data associated with the sample such as location, GPS position, date and collector as in 'Minimum attributes' above, plus your write-up of the field work) followed by checking for errors and missing information, and finally secure storage.

If data storage is designed well at the outset, it will make the job of analysis and interpretation much easier. Storage can be either on paper or electronic (preferably both). Paper storage will usually be collection or summary sheets, whereas spreadsheets should be saved electronically.

Once the sample has been sorted, data for each species or recognisable taxonomic unit (RTU) are entered into an Excel spreadsheet using the following suggested fields:[2]

- Person who collected the sample

- Location of trap

- GPS coordinates (eastings and northings in separate columns)

- GPS estimated accuracy (if available)

- Altitude (from GPS)

- Sample number (individual identification for each quadrat or search area).

- Order

- Family

- Species/RTU

- Number of individuals

- Sampling series

- Date the sample was collected

---

[2] For an example spreadsheet, see 'Example data sheet invertebrate monitoring' (doccm-428226) http://www.doc.govt.nz/documents/science-and-technical/inventory-monitoring/invertebrate-monitoring-datasheet-example.pdf

Note: considerable taxonomic revision will occur over time because taxonomic revision is a standard part of all entomological studies. The creation of a 'look-up' table in Excel may ease this process.

Ultimately invertebrates collected for inventory should be deposited in a museum or in the National Arthropod Collection administered by Landcare Research.[3] Institutions should be contacted first to find out their requirements. Collectors are encouraged to lodge unusual or rare specimens in the New Zealand Arthropod Collection (NZAC, maintained by Landcare Research) or in museum collections such as Te Papa if possible. Sometimes everything collected from an unusual or remote location will be accepted by such institutions. Contact the institution to establish if they will take the specimens because to manage the ongoing work required to maintain collections of specimens, they have acquisition plans stipulating what they will accept. Such institutions require specific standards for labelling and the information recorded, as well as specific methods for preserving the specimens. For more information on depositing specimens and taxonomic expertise, see 'Invertebrates: advice and diagnostic support' (doccm-2686377)[4] and 'Preliminary sorting of invertebrate samples' (doccm-388193).

DOC staff should enter location records (GPS coordinates) of identified species into the BioWeb database.

Analysis, interpretation and reporting

Seek statistical advice from a biometrician or suitably experienced person prior to undertaking any analysis. Many analytical approaches may be used depending on your monitoring questions and study design (see 'Introduction to statistical analysis of invertebrate monitoring data'—doccm-525907)[5].

Introduction

The following outline is intended to highlight some of the practical considerations of dealing with the data resulting from search and extraction techniques. Once the data have been collected and the invertebrates sorted into species or RTUs, it is recommended that the data are summarised and presented either in a table or graphically. This can be done in an Excel spreadsheet or in R. Basic data summary statistics and an overview of the common types of analysis used for invertebrate data (with worked examples) are provided in 'Introduction to statistical analysis of invertebrate monitoring data' (doccm-525907). However, extensive training in statistical methods is required before attempting most statistical analyses. The information provided here is intended to familiarise staff with some of the options available so they can be discussed with a statistician. The information is not intended to be a comprehensive guide to data analysis.

---

[3] http://www.landcareresearch.co.nz/resources/collections/nzac

[4] www.doc.govt.nz/documents/science-and-technical/inventory-monitoring/im-toolbox-invertebrates-advice-diagnostic-support.pdf

[5] http://www.doc.govt.nz/documents/science-and-technical/inventory-monitoring/im-toolbox-statistical-analysis-of-invertebrate-monitoring-data.pdf

Analysis of search and extraction data

Most of the search and extraction methods described are only suitable for an inventory of the species occupying a habitat and will simply require a list of the species found in each trap, the dates when the samples were collected and the locations of the traps (a GPS position). Systematic studies involving hypothesis testing and modelling invertebrate communities are more complex and are designed to answer a specific ecological question. The statistical methods used with search and extraction techniques depend on the hypothesis being tested (the questions that you want answered) and the design of the study. Once the data have been collected and summarised, there may be trends apparent that warrant further investigation. Statistical tests for unreplicated studies (e.g. one treatment group and one control group) will be limited to testing whether one group of data differs from another, whereas replicated studies (e.g. three treatment groups and three control groups) allow for more sophisticated tests that enable you to determine whether treatments differ from each other and to what extent they differ. If information regarding associated environmental variables has also been collected quantitatively, it will be possible to explore the data for relationships with environmental variables using multivariate analysis techniques (see the 'Exploring data for relationships' section in 'Introduction to statistical analysis of invertebrate monitoring data'—doccm-525907)[6]. This might include determining whether certain species are indicative of specific environmental variables using a program such as PC-Ord or using ordination techniques to determine whether the species collected are responding to environmental gradients. If the results suggest that there are obvious associations between particular species or groups of species with the key environmental variables or gradients that have been measured, it is important to present these results in the context of the biology of those species.

In summary, the following issues should be addressed as part of your study design but may need to be considered when analysing the data as well:

- Care should be taken to minimise the influence of confounding variables at the time a study is designed, and where possible, results should be interpreted in the context of these.

- As with many other collection methods, the results are a reflection of insect activity and the relative abundance of the species present at the time that the sample was taken.

- At the time studies are designed, decisions need to be made regarding which key groups are going to be analysed. Some invertebrates, such as ants, are aggregated in groups (or colonies), and this may influence how you search for target species and how the study is designed.

If the study has assessed the beetle fauna occupying an area, it may be possible to summarise the data in terms of the functional groups. This can provide important information about the role of the beetle community in the local environment.

The data can be only be compared between sites or sampling occasions if a standard sampling unit has been defined and can be easily quantified, e.g. number of individuals observed in 1 m^2 during a 10-minute search.

---

[6] http://www.doc.govt.nz/documents/science-and-technical/inventory-monitoring/im-toolbox-statistical-analysis-of-invertebrate-monitoring-data.pdf

It may be important to accurately quantify environmental variables (e.g. associated richness of plant communities, distance from forest edges or other gradients such as altitude) so that they can be incorporated into multivariate analyses.

Case study A

Case study A: monitoring flax snails (*Placostylus hongii*) at Peach Cove, Whangarei Heads: an example where searching is used to monitor population density

Synopsis

This example shows how manual searching can be used effectively to follow how a small population of flax snails, *Placostylus hongii* (Gastropoda: Bulimulidae; Figure 8), at Peach Cove, Whangarei Heads, changed while intensive rodent poisoning was carried out. It also provides direct estimates of changes in their density (number of snails per area).

Placostylus hongii grows to about 85 mm in shell height. Hatchlings feed on the algae that grow on leaves of broadleaf trees and shrubs such as karaka, kohekohe and hangehange, but older juveniles and adults live on the ground where they feed on recently fallen leaves from these trees and shrubs. During the daytime they hide by burrowing under the leaf litter, thereby also reducing water loss. These snails are particularly vulnerable to predation by rodents, which break pieces of shell away, starting from the thin edge around the aperture, until they reach the snail. This leaves characteristic damage of a jagged-edged corkscrew opening that spirals around the whorls of the shell. When mature, the snail shell ceases enlarging and the snail produces a thick lip around the aperture, which protects the snail from most rodent attacks. They are still vulnerable to larger mammals such as hedgehogs and pigs.

Prior to the arrival of humans, *P. hongii* occurred along the east coast of Northland between Whāngārei and Whangaroa and on some of the eastern islands. Peach Cove is now one of the last remaining locations where it occurs on the mainland.

The original monitoring procedure used in 1996 was seriously flawed because it did not follow a fully randomised design. The procedure was changed in 2005 to stratified random sampling, which is a recommended method. The initial data are still included here, despite being dubious, because they are the only information available when snail abundance was very low.

Here we explain only the methods and give results of live snail numbers. A full account will be published in future as an example of how effective mammal control at Peach Cove influenced the recovery of the snails. This will include corresponding details of mammal control, how the mammals were monitored and changes in the numbers and sizes of both live and empty shells, including snails that were preyed on.

Figure 8: An adult flax snail or pūpūharakeke (*Placostylus hongii*) with a partly uncovered nest of its eggs. Adults have a thickened lip around the aperture of the shell, which can be seen in this photograph.

Objective

The initial objective was to follow the fate of the snail population while rodents were intensively controlled with poison. Rodent control was instigated because fears were held for the viability of the snail population. The snails were subsequently monitored to check how they responded to a reduction in rodent abundance.

Sampling design and method

Monitoring site

Monitoring was done at Peach Cove, Whangarei Heads.

Search method

Samples were taken by counting the number of snails found by manually searching 40 or 50 circular plots. Each plot was delimited by first driving a thin stake into the ground at the centre of the plot and then slipping a noose at the end of a thin rope over it. Initially, this rope extended 1.0 m from the stake, but after 2004 it was 1.26 m long. All the ground and leaf-litter around the stake was then carefully searched, and any snails reached by the rope were recorded after first being examined to determine if they were alive or empty shells, and if they were juveniles or adults.

Monitoring procedure

Monitoring was done annually or biennially in summer.

Initial design: 1996–1998 (Do not use this method)

A 150 m baseline was first established about 5 m in from the edge of the bush and parallel to the coast by permanently marking every tree approximately 10 m apart. These trees were numbered 1 to 16. On each sample occasion, 10 of these trees were chosen randomly and a tape measure was run inland from them, at right angles to the baseline. Five plots, each 5 m apart, were then sampled starting 5 m from the trees. If a plot fell where a sample could not be taken (e.g. centred within a tree trunk or on a large rock), then the stake was moved to the right (facing away from the sea) until it could be pushed into the soil. The area searched for each sample was 3.14 m^2 (i.e. the rope reached 1 m from the stake).

Modified initial design: 1999–2004 (Not recommended)

The same procedure was followed as in the initial design except that the five sample positions from each tree were chosen at random distances from the trees.

Final design: 2005–2008 (Recommended)

This method was restricted to 100 m of the baseline adjacent to where snails were known to occur. Four samples were then taken at random every 10 m along this baseline and up to 30 m inland. The position of each sample was located by taking a random distance (in metres) between 1 and 10 along the baseline and another random distance between 0 and 30 at right angles from the baseline. The result was stratified random sampling with four random samples being taken within each of the 10 m long regions (total 40 samples). If a plot was centred where a sample could not be taken, then the sample was moved on a further 2 m, but if it reached 30 m then it was returned to the baseline. The area searched for each sample was 5 m^2 (a rope 1.26 m long was used).

Analysis

Excel was used to calculate the mean and standard errors (SEs) from the raw results, and these were then graphed. The amount of overlap between SE bars was used to indicate the approximate significance of differences visually as follows: means are not different when SE bars overlap, but they are different if imaginary bars of twice the length of SE bars (i.e. 95% CI bars) do *not* overlap. When SE bars do not overlap but 95% CI bars do overlap, then the means *might* be significantly different, but it is best to consult a statistician at this point. We can't be sure because we have used the SEs for each mean when we should use the SEs of the difference between each mean, which are larger and also harder to calculate.

Results and interpretation

The numbers of snails found between 1996 and 2008 are shown in Figure 9. The numbers of live adult snails fluctuated between 1996 and 2001, but none of these changes showed any sign of being possibly significant until numbers decreased to a minimum in 2002. Numbers of live juveniles also fluctuated and remained at low levels between 1996 and 2002. Following 2002 both the numbers of live adults and juveniles certainly look like they increased (although we cannot be sure by looking at the SE bars in most cases). How can you test this, if you needed to? All samples were taken from the same population, so the samples involve what statisticians call *positional*

pseudoreplication, so you cannot use the normal statistical tests such as a *t*-test between results from two occasions, or multiple comparison tests between more than two occasions. Such tests require that the data being compared are independent of each other, and they are not. There are specialised ways to analyse the sort of data we have here—time series analyses and mixed effects models—that is why it is best to consult your statistician at this stage.
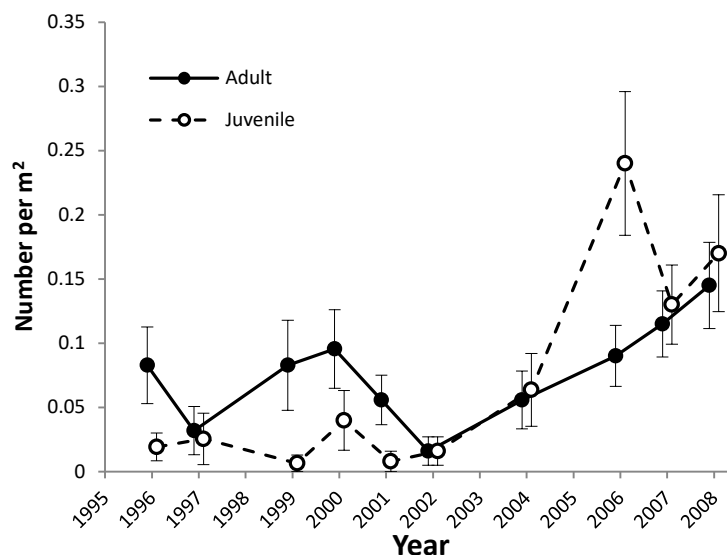

Figure 9: Change in density of live *Placostylus hongii* snails at Peach Cove. SE bars are shown. Adults and juveniles have been jittered (slightly offset from each other) for clarity.

Limitations and points to consider

This example shows that manual search methods can be used effectively for monitoring. However, it does depend on the situation. In this case the adult snails live only on the ground, so it was relatively easy to find them because they are large and easily located by searching through leaf litter.

The method of searching the area delimited by everything reached by a thin rope or twine rotated around a central stake is much faster than pegging out large square quadrats accurately. In addition, several people can search different sectors of the same circular plot working in from the outer circumference if several ropes are used. Accumulating samples quickly also provides searchers with an increasing sense of accomplishment, which helps retain their enthusiasm for searching.

This example also shows that the data produced are actual numbers per area (densities) and that they are quite suitable for statistical analysis, despite the simplicity of the method. A statistician would infinitely prefer that a fully randomised and if necessary also stratified design was employed for locating the positions where the samples were taken from. There is no doubt that the results from 1996 to 2004 are suspect despite what they apparently show. The reasons why the initial sampling procedures were set up as they were was to minimise the potential damage to the habitat and snails from trampling by leaving most areas untouched (this snail species is protected by New

Zealand law (Wildlife Act 1953). There is no excuse for not initially randomising the sample positions from the baseline.

An essential point that this example illustrates is that you should consult a statistician *at the start* to ensure that you use the best sampling design to start with. You should then keep sampling the same way, consistently, for the entire period. If you change the method during the study, as was done here, then you may not know if the results that follow are truly comparable. It is sometimes greatly tempting to alter or adjust a method, even slightly, to 'improve' it, but this should normally be avoided. If you change the method then you should start again—but this (as here) is not always possible. We have included the initial data while declaring that the initial sampling method has serious flaws so the reader can look at those data with as much scepticism as they like.

Variation in the fluctuations of juvenile numbers was mostly related to the numbers of hatchling snails present, but no analysis of any changes that may have occurred in juvenile sizes was presented. Often adults are the essential interest for such monitoring because they represent the essential reproducing portion of the population. Changes in the numbers of juveniles in different size classes would undoubtedly be analysed if the recruitment rate for juveniles to adults was low because this could indicate if juveniles of some age (size) had higher than normal mortality. If so, then we might then try to design an appropriate management intervention to decrease the mortality at that stage and so increase recruitment to adults. Sherley et al. (1998) provides an example where juvenile snails' sizes are analysed, indicating when they are sensitive to bird and rodent predators.

References for case study A

Sherley, G.H.; Stringer, I.A.N.; Parrish, G.R.; Flux, I. 1998: Demography of two landsnail populations (*Placostylus ambagiosus*, Pulmonata: Bulimulidae) in relation to predator control in the Far North of New Zealand. *Biological Conservation* 84: 83–88.

Full details of technique and best practice

This section describes in detail the four common search and extraction methods, which are often used together: hand searching, foliage beating, sweep netting, and extraction from soil cores and leaf litter samples using Berlese or Tullgren funnels.

Generally, invertebrates are most easily sampled as adults and when they are active. Thus environmental conditions such as temperature, humidity and diurnal/nocturnal behaviour need to be considered to optimise catch. Generally, warm, humid conditions stimulate insect activity and different invertebrates usually have different activity periods, which may be for different times during the day, change of light or night-time.

Practical considerations

The data collected from search and extraction methods are usually qualitative rather than quantitative. Search and extraction techniques are more commonly used for inventory work as it is difficult to replicate studies using these methods. However, if the study has been designed carefully and includes replication and randomisation, then it may be possible to complete some statistical analysis. Results from search and extraction methods are often confounded (compromised) by variables such as weather, seasonal conditions and the skill of the searcher. Make a careful budget for the study. Of particular importance are the additional time and resources needed for sorting and identifying the specimens. To reduce the scale of the collection, consider targeting collections to species or a range of species.

Practical considerations when hand searching

Hand searching can be relatively non-destructive but, if repeated samples are taken in an area, there may be disturbance which can influence the results. Data collected along transects also have some inherent problems involving disturbance, particularly if the area to be searched is walked over while establishing the transects. As with any transect data, care needs to be taken that the invertebrates are not moving further along the transect while they are being counted. If quantitative studies are being undertaken, randomisation can be introduced by choosing a random start point along each transect, e.g. 2 m from the start. Hand searching is susceptible to observer bias so that any long-term studies for cryptic species, such as grasshoppers, are best completed by the same person. The results from hand searching are best summarised as the number of individuals per unit of search effort. It is often better to search working uphill because there is more control when turning over logs or rocks, and you are closer to the targets making the searching easier physically—hence the search is likely to be more thorough. It may be advantageous to search with one or more colleagues—often it is easier if one person lifts or rolls an object while another spots and captures the specimens. Some speed is often required as animals make their escape. Also, with two or more people searching uphill close at hand to each other, it is possible to conduct a semi-quantitative search by timing the search to produce data in the form of number found per unit effort (number found divided by the number of searchers and the time taken). Bear in mind that searching by hand is often destructive and one should consider this carefully if searching in sensitive and fragile habitats. Similarly, if parasitic insects are being collected from birds, there are specialist techniques and permissions that are needed.

Practical considerations when foliage beating

Foliage beating is a commonly used inventory technique which can be useful for some invertebrates but not others. It is biased towards collecting spiders, beetles, weevils, bugs, and Lepidoptera larvae. The results are biased towards collecting sedentary species, and species that can move away quickly are less likely to be collected.

Practical considerations when sweep netting

Sweep netting is mostly used for inventory studies as it is difficult to standardise the method. As with other methods, it is important not to assume that a species is absent if it is not collected. The success of sweep netting can be dependent on the weather conditions at the time.

Practical considerations when collecting soil core samples or leaf litter samples

Soil core samples are often used to systematically sample beetle larvae, e.g. Argentine stem weevil and Cromwell chafer beetle. This is a destructive method of sampling and not necessarily very efficient for rare species (unless a large number of samples are taken) or for species that are aggregated. Litter samples that are used to sample litter-dwelling invertebrates can be standardised by taking litter from a specific area, e.g. 0.25 m^2. Be aware that the litter depth may vary considerably and may need to be recorded or standardised between samples. It is also possible to collect a set weight of leaf litter but there is considerable variability in the moisture levels. It may be necessary to retain the litter sample and get a dry weight once the invertebrates have been extracted. This will enable the data to be summarised per unit weight. Samples need to be placed in Tullgren or Berlese funnels as soon after they are collected as possible (as the method relies on the invertebrates being alive) and for a similar length of time (at least 5 days). This requires a large number of funnels being available at once, otherwise the fresher samples may produce more invertebrates than the older ones, significantly influencing the results.

Hand searching

There is no prescription for searching habitat – the best hand searchers are observant, inquisitive, thorough and can conceptualise what 'ideal' habitat for sheltering the target species looks like, find it and search it (this comes with experience from searching for target species). In practice, searching involves sifting through litter; looking under rotting logs (and inside them), on and under bark and aggregations of dead litter (such as under epiphytes and tree ferns); looking under stones, boulders and banks; and scanning likely surfaces that invertebrates might be sitting on. Most invertebrates living under sheltering habitat are photophobic (avoid light) and will take evasive action as soon as they are disturbed. Some species (such as amphipods) are even sensitive to vibrations (such as approaching footsteps or thumps on the ground such as from over-turning logs) and will try to hide. Understanding these reactions to search efforts and where diversity and high abundance occurs will increase the chances of successful searching. Areas of high abundance often involve the interface between different types of habitats (the ecotone) such as lake, stream and river edges, transition zones between different vegetation types (e.g. clearings in forests), between soil, rotting wood and intact timber, and changes in environmental conditions (e.g. transition to dusk, temperature and humidity changes or static gradients).

Hand searching can be quite successful for surveying diversity and abundance of particular groups (such as Carabids/ground beetles) if used to complement other methods such as pitfall trapping. This is because hand searching may be more effective than trapping methods for invertebrates that have low mobility and/or are cryptic. Quantitative results can be obtained by searching measured quadrats or transects.

Logs and other material which is disturbed during searching should be returned as much as possible to their original positions/states.

If the target species is nocturnal, spotlighting in known habitat may be required.

Spotlight searching is often used to search for spiders and wētā, but it is not suitable for collecting quantitative information about populations. Some spider families can be easily detected using eye-shine from a spotlight, e.g. Lycosidae and Oxyopidae. Spotlighting may be useful for finding nocturnal insects (e.g. wētā). Selecting the appropriate spotlight is a trade-off between wanting to see as well as possible over the greatest distance appropriate for detecting the target species, and not over-illuminating the habitat. Beams of light that are too wide may frighten off insects or cause them to hide before they come into view. This is especially so if the observer's focus is in the middle of the light beam. The type of light is also an important consideration. Different bulbs and light systems emit in different regions of the light spectrum (colours) and these influence how well the human eye and brain can identify the target as well as the reactions of the target species. For example, insects rarely see red but it is tiring for humans to search well using a red light. Some experimentation is required. In the past, incandescent bulbs have delivered the best quality light (broadest range of light frequencies) and the most illumination. However, these bulbs also produce a lot of heat (and are therefore inefficient) and typically drain batteries relatively quickly. Light-emitting diode (LED) based light systems are now available that produce good quality light (meaning colours are relatively well illuminated) and provide more than adequate illumination for invertebrate work (e.g. see the 'nightling' range). These lights have the added benefit that they use little power so smaller and lighter batteries can be used.

Artificial habitats (e.g. wētā motels, wooden discs, Ondaline covers,[7] portable cast cement refugia) are useful to determine the extent of populations or presence/absence data, but the results are confounded by the availability of natural habitat. A summary of the results should include an index of the amount and quality of suitable habitat in the immediate vicinity.

To collect mites and lice from either feathers or from birds, the following techniques are recommended:

1. Methods for collecting feather mites from individual feathers:

   a. Non-destructive—simply use gentle brushing or picking of mites from the feather and then fix them in ethanol. This method is likely to be biased and not very efficient.

   b. Destructive—treat the feather with potassium hydroxide (KOH), which dissolves the keratin in the feather but not the cuticle of the mite, so that the exoskeletons can be removed for microscopic examination. This method is not suitable if DNA analysis is required.

2. Methods for collecting feather mites from birds:

---

[7] Other materials such as portable cast cement are now being used in preference to Ondaline because Ondaline has a tendency to bend and twist after being exposed to environmental elements.

a. Dust ruffling with insecticide—this method was developed primarily for collecting feather lice from live birds in the field but has been used to sample feather mites (this method has not been thoroughly evaluated). Anesthetising the host can increase the sampling efficiency (as the mites move when the bird is distressed), but this is only recommended if the host can be brought into a laboratory. NB: lice collected in this way can hinder DNA analysis.

b. Washing with water-detergent solution (1–2% soap), with or without mechanical shaking in a paint mixer—this is only recommended for dead hosts.

Feather mite species can show extreme microhabitat specificity on the host, preferring particular feathers or even particular regions of an individual feather (Clayton & Walther 1997). It is recommended that careful notes are made (or a reference collection) of where feather mites are found on each of the flight feathers (and even different regions of the same feather). Unlike mites, lice are not likely to move around the host as much since they glue their eggs to the host's feathers in areas that the bird cannot preen, e.g. on the head or under the wings.

Foliage beating

This method was successfully used to quantify invertebrates occupying broom *Cytisus scoparius,* L. (Link) (Memmott et al. 2000). In their study, the efficiency of the method was tested by beating five branches on five bushes and this accounted for 87% of invertebrate abundance. The results obtained from beating foliage are influenced by the state of the vegetation being sampled, e.g. flowering branches will have a different fauna from branches with mainly leafy vegetation.

Foliage beating can be done over beating trays made from sheets of usually white material stretched taut between the corners of a square or rectangle made of a light frame of poles (usually made of alloy, wood or cane) hinged in the middle so that the tray may be collapsed when not in use (Figure 2). The material, such as nylon tent material, is sewn to a cord that goes around the ends of the frame where it is secured in notches at the ends of the poles. The material may also have gussets sewn in to create a slightly bulging surface to direct falling invertebrates towards the centre. Theoretically, any size of beating tray may be used providing it relates to the general size of the vegetation being sampled. Typically, the beating tray is about 1.5 m square. However, different sizes may be justified depending on practical considerations such as portability and ease of moving it into position without unduly disturbing the vegetation that is about to be sampled.

When the tray is in position, a suitably solid stick is used like a club to hit the supporting branch(es) of the vegetation and produce a short, sharp shock through the finer branches, branchlets, twigs and leaves to dislodge invertebrates on the foliage, branches and twigs. Anything that falls is collected in the tray held below. The white material usually provides sufficient contrast to see the collected specimens. These can be collected directly into vials or using tweezers or a pooter as appropriate.

The size of beating trays and how they are used (e.g. number of strikes on the vegetation) may be standardised to allow systematic sampling of the habitat. Care must be taken to remove all invertebrates after each use to avoid carry-over between samples. Modifications to the basic design

could include stretchable sides to allow the board to conform better to the contours of trunks or branches. Some entomologists simply use an umbrella, usually with black or white material so that specimens are more easily seen.

Sweep netting

The most obvious way of using a net is to 'chase down' flying insects (e.g. butterflies, dragonflies, wasps). Fast flying insects can sometimes be caught more easily by swinging the net up underneath them rather that over them or from the side. The other method of using a net is to sweep it over or through vegetation to capture insects hovering or flying close to the vegetation as well as some of those that are on it. In both cases, when the sweep is completed, the net should be twisted to close off the bag, as shown in Figure 10. It may be necessary to place the folded net on the ground and slowly unfold it to retrieve the captured invertebrates. Captures may need to be placed in a killing jar immediately to prevent them damaging themselves, and butterflies and moths might need to be pinched first (see 'Killing and preserving insects'—doccm-436527).

Figure 10. Closing off the bag to contain specimens after completing a sweep with a net.

A net is usually used in combination with other manual methods when surveying and making an inventory of invertebrates at a location. Alternatively, it may be used to target specific species of interest. Sampling by using a net has the limitation that only whatever is flying on the day (or time of day) is sampled. This does not necessarily include all the winged species that live in the habitat. It is also difficult, and perhaps impossible, to standardise how a net is used in order to yield catch per unit effort data. Hence it is likely that only qualitative information is obtained, which is at best presence (not absence) data.

Most nets used for sweeping and ambushing individuals are about 50 cm in diameter with a fine white or green gauze (about 1 mm mesh size) material 'sock' attached to the hoop. The hoop is best made of flexible material such as high-tensile steel that is relatively stiff but will bend and return to shape if knocked sufficiently hard. The sock should be about 1 m long and should taper to

a rounded end about 5 cm in diameter. A rounded end allows easy access to insects, whereas insects are difficult to retrieve if the end of the net tapers to a point. Ideally, the net should be inter-changeable rather than sewn directly onto the hoop to allow it to be easily removed for repair or for a new one to be fitted when necessary. This can be done by sewing on a hem with strips of Velcro that is wide enough to enclose the hoop. White netting material assists the user to see captured insects, but many insects, especially fast flying ones, are more easily caught if the material is green. The handle length on the nets may be quite variable depending on the user's preference and confidence on having control over it. Typically the handle length is about 1500 mm.

Successful netting is about gauging conditions and locations—some flowers such as *Buddlia* and *Hebe* attract many different insects, and sunny or otherwise warm sites are often also good. Knowledge of host species for targeted taxa can be an advantage.

When sweep netting, the net is 'brushed' past or through the vegetation (usually the outer leaves)—usually with a degree of mechanical disturbance. This helps to dislodge the specimens. The net may also be used in the same manner as a beating board and invertebrates shaken into it as it is held below vegetation and hit with a stick.

Extraction from soil core samples and leaf litter samples

Soil cores

Soil core (typically up to 10 cm deep) samples can be searched manually to extract at least molluscs and earthworms. It is possible other taxa are better extracted this way; some specialist advice on the merits of this or using Berlese or Tullgren funnels is warranted. The number of soil core samples required will depend on the density of invertebrates in the substrate and the degree of aggregation that the particular species of interest has.

Litter samples

Litter samples for collecting micro land snails and other litter-dwelling invertebrates may be separated by hand by spreading the sample out on a white sheet of paper (or something similar—as long as the contrast is sufficient), sorting through the material and individually picking out the specimens one at a time. The process may be accelerated by sifting the sample through sieves of different mesh sizes to extract the larger pieces of litter first. This method is commonly used for separating micro land snails especially and has the added advantage of yielding the empty shells and other dead invertebrates (see 'Collecting micro land snails in terrestrial and freshwater habitats'—doccm-598715)[8]. NB: Berlese funnels are not recommended for micro snails.

Using Berlese and Tullgren funnels

Berlese and Tullgren funnels can be used to extract invertebrates from soil or litter samples. The method uses the animals' aversion to heat and negative phototaxis from incandescent lights

---

[8] www.doc.govt.nz/documents/science-and-technical/inventory-monitoring/im-toolbox-collecting-micro-land-snails-terrestrial-freshwater-habitats.pdf

overhead. The sample is held below a lamp in a funnel or sieve usually of about 3 mm (depending on the size of specimens sought). The funnel directs anything that falls down away from the light into a container of 70% or 95% alcohol. Any number of funnels may be arranged on a grid to process multiple samples at once. Steps can be taken to increase the temperature gradient which should make the animals move out of the sample faster (e.g. placing the funnels in a refrigerated room). Quantifiable samples are possible, such as soil cores taken over a set area and depth. The soil core is inverted over the funnel for extraction. Depending on the type of sample (litter or soil) and the taxa sought, extraction time can vary from 12 hours to 2 weeks. Some species will take longer to emerge from the sample than others. Variations in the design of Berlese and Tullgren funnels can be made to extract different taxa.

After checking that the size of the mesh and the wattage of the bulbs is the same in each funnel, place the sample into the sieve and turn on the lights. Samples may be kept in the fridge for a few days if necessary but storing them in paper bags is preferable.

References and further reading

Clayton, D.H.; Moore, J. (Eds). 1997: Host-parasite evolution: general principles and avian models. Oxford University Press, Oxford. 473 p.

Memmott, J.; Fowler, S.V.; Paynter, Q.; Sheppard, A.W.; Syrett, P. 2000: The invertebrate fauna on broom, *Cytisus scoparius*, in two native and two exotic habitats. *Acta Oecologica 21*: 213–222.

Sherley, G. 1996: Field notes from Te Paki *Placostylus* research 7 to 15 October 1996. Unpublished report, Department of Conservation. 16 p.

Sherley, G.H.; Stringer, I.A.N.; Parrish, G.R.; Flux, I. 1998: Demography of two landsnail populations (*Placostylus ambagiosus*, Pulmonata: Bulimulidae) in relation to predator control in the Far North of New Zealand. *Biological Conservation* 84: 83–88.

Southwood, T.R.E.; Henderson, P.A. 2000: Ecological methods. 3rd edition. Blackwell Science, Oxford. 575 p.

Appendix A

The following Department of Conservation documents are referred to in this method:

doccm-598715   Collecting micro land snails in terrestrial and freshwater habitats

doccm-428226   Example data sheet invertebrate monitoring

doccm-525907   Introduction to statistical analysis of invertebrate monitoring data

doccm-388198   Invertebrate identification aids

doccm-2686377   Invertebrates: advice and diagnostic support

doccm-436527   Killing and preserving insects

doccm-388193   Preliminary sorting of invertebrate samples

doccm-146272   Standard inventory and monitoring project plan