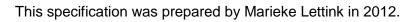
# Herpetofauna: photo-identification Version 1.0



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#### Disclaimer

This document contains supporting material for the Inventory and Monitoring Toolbox, which contains DOC's biodiversity inventory and monitoring standards. It is being made available to external groups and organisations to demonstrate current departmental best practice. DOC has used its best endeavours to ensure the accuracy of the information at the date of publication. As these standards have been prepared for the use of DOC staff, other users may require authorisation or caveats may apply. Any use by members of the public is at their own risk and DOC disclaims any liability that may arise from its use. For further information, please email biodiversitymonitoring@doc.govt.nz

Inventory and monitoring toolbox: herpetofauna DOCDM-1023668

Department of Conservation Te Papa Atawbai

# Synopsis

Photo-identification is a method for identifying individual animals from natural markings (e.g. ornamentation patterns; Bradfield 2004; Kenyon et al. 2009; Lama et al. 2011) or other features (e.g. the shape and size of scales; Sacchi et al. 2010) present on one or more parts of the body. Although photo-identification is less-commonly applied to herpetofauna than other vertebrate groups (Plummer & Ferner 2012), it is increasingly used as a non-invasive alternative to permanent marking methods, which require the application of individual-specific marks or tags (e.g. toe clips, microbrands, Visible Implant Alphanumeric (VIA) and Passive Integrated Transponder (PIT) tags; Mellor et al. 2004; Langkilde & Shine 2006; Clemas et al. 2009; Hitchmough et al. 2012). Identifying individuals in a population, whether by photo-identification or by other means, is desirable because it enables researchers to determine longevity, reproductive output, movements, and population size and viability (Hitchmough et al. 2012).

Photo-identification involves taking high-resolution, standardised photographs of a pre-defined region (e.g. dorsal surface) of all animals encountered during a sampling session and comparing these to photographs taken on previous occasions to determine their identities. To permit analysis of the data, each newly-encountered individual is assigned a unique identification number. The best photos (in-focus, correct exposure and clearly showing the features of interest) of each individual are kept as reference photos and archived in a photo library (usually in electronic format). Photo matching is traditionally done by eye. Depending on sample size and ease of identification, it can be done in the field by comparing live animals to digital or hard copy photographs of animals caught previously (e.g. Kenyon et al. 2009) or in the office once field work has been completed (e.g. Bradfield 2004; <u>Case study A</u>). Pattern-recognition software has recently been developed to improve its efficiency and to facilitate monitoring of large populations by photo-mark-recapture (Speed et al. 2007; Gamble et al. 2008; Bolger et al. 2012).

The international literature contains different terms for studies that use photo-identification to identify individuals. The term 'photo-identification' is generally used where there is no formal analysis of the data. For example, landowners who regularly encounter jewelled geckos on their land may wish to know whether these geckos are all different animals or the same individuals seen on multiple occasions. The term 'photo-mark-recapture' may be used for any study that combines photo-identification with capture-mark-recapture (CMR) analysis, irrespective of whether animals are physically captured or not (as sightings represent visual captures). The term 'photo-resight' (or 'photo-mark-resight') is an alternative term that applies only to studies where animals are photographed remotely (e.g. <u>Case study A</u>).

Photo-identification can potentially be used to monitor any wildlife species with distinguishable natural markings or other features that remain constant through time. Animals may be photographed with or without physical capture, depending on whether the features of interest can be accurately recorded from a distance (e.g. dorsal fin markings of Hector's dolphins (*Cephalorhynchus hectori*) are routinely photographed remotely by observers positioned on small boats; Slooten et al. 1992). In some studies, the treatment of animals varies depending on whether they have been encountered in a previous sampling session or not. For example, animals may be

captured on their first encounter to allow standardised reference photographs to be taken and identified remotely on subsequent encounters (note that this will only be possible where there is a fast and accurate means of identifying animals in the field). Because amphibians and reptiles in New Zealand tend to be small-to-medium-sized and cryptic in their behaviour and/or colouration, physical capture is likely to be required for most species (but see '<u>Case study A</u>'; Gebauer 2009).

In New Zealand, photo-identification has been trialled for various herpetofaunal species, including:

- Archey's frog (Leiopelma archeyi, Bradfield 2004)
- Chevron skink (Oligosoma homalonotum; Barr 2009)
- Grand skink (O. grande) and Otago skink (O. otagense) (Case study A)
- Hamilton's frog (*L. hamiltonii*; Newman 1982; Avi Holzapfel, DOC Hamilton, pers. comm. June 2012)
- Harlequin gecko (*Tukutuku rakiurae*; Mandy Tocher, Wildlands Consultants Ltd., Dunedin, pers. comm. June 2012)
- Maud Island frog (*L. pakeka*; Germano 2006; Lukis 2010)
- Small-scaled skink (O. microlepis; Gebauer 2009)
- Jewelled gecko (Naultinus gemmeus; Knox et al. in press)
- Scree skink (O. waimatense; Lettink & Lange 2010)
- Southern forest gecko (Mokopirirakau 'Southern forest'; Hoare et al. in press)
- Whistling frog (*Litoria ewingii*; <u>Case study B</u>)

Reported accuracy rates (i.e. the proportion of correctly identified individuals) vary from 88.1% (Gebauer 2009) to 100% (Knox et al. in press).

Before photo-identification can be used to monitor new species (i.e. any species for which its usefulness has not been demonstrated), a pilot study may be required to determine which regions of the body to use, whether the features of interest are sufficiently variable (i.e. individual-specific), and the accuracy and/or speed with which observers are able to identify recaptures (Bradfield 2004; Kenyon et al. 2009; Knox et al. in press). To date, all photo-identification conducted in New Zealand has been manual (by eye; sometimes referred to as manual matching). Use of pattern recognition software (I<sup>3</sup>S: <u>http://www.reijns.com/i3s/</u>) was tested for grand and Otago skinks but proved to be inefficient (James Reardon, DOC Te Anau, pers. comm. July 2012). The software package SLOOP is currently being customised for photo-resight monitoring of grand and Otago skinks (Andy Hutcheon, Grand and Otago Recovery Programme Manager, DOC Dunedin, pers. comm. June 2012).

The main advantage of photo-identification is that it is a completely non-invasive method for identifying animals in long-term studies. This makes it ethically acceptable to groups of people who are opposed to the use of permanent marking methods, particularly toe-clipping (e.g. institutional Animal Ethics committees and iwi; Perry et al. 2011; Hitchmough et al. 2012). The ability to develop a monitoring database that is not compromised by the effects of direct disturbance or capture on subsequent capture probabilities is of special value when considering estimation of survival or abundance estimates for a population. Photo-identification is most appropriate for monitoring small populations that occupy well-defined areas (Beausoleil et al. 2004).

The main disadvantages of photo-identification are that: (1) it cannot be used for species that lack distinguishing marks; (2) animal handling and/or identification times are typically longer than those reported for commonly used permanent marking methods (e.g. <u>Case study B</u>; Kenyon et al. 2009); and (3) accuracy may be less than 100%, particularly where some animals in the population lack distinguishing features and/or where natural markings change over time (e.g. Kenyon et al. 2009). See Figures 1 & 2 and the case studies within this method for examples of use of this method.



Figure 1. Six jewelled geckos (*Naultinus gemmeus*) from Canterbury, showing variability in their natural markings and colouration. Photos of the dorsal markings of jewelled geckos can be used to accurately identify individuals of this species in Canterbury (M. Lettink, pers. obs.) and Otago (Knox et al. in press) (photos: Marieke Lettink).





Figure 2. Photograph of an Archey's frog (*Leiopelma archeyi*) taken on a custom-built, portable photo stage that allows frontal, lateral and dorsal views of each frog to be combined in one digital image. After identification of this individual, the photograph was labelled and archived in a photo library (photo: DOC).

## Assumptions

- The target species has variable natural markings or other features (hereafter 'natural marks') that remain constant or distinguishable over time.
- All observers are able to capture and/or photograph natural marks.
- All observers are able to correctly identify individuals by photo-matching.
- The sampling area is representative of the wider habitat occupied by the target species.
- All relevant data (e.g. date, weather conditions, artificial retreat number and site) are recorded and used in subsequent analyses, where appropriate.

A range of analytical methods can be used to analyse data collected from photo-identification studies. Therefore, additional assumptions may apply depending on the aims of the study. For example, data may be converted to encounter histories and analysed using mark-recapture software to estimate population size (see 'Herpetofauna: population estimates'—docdm-833600 for a list of additional assumptions required to estimate population size).

## **Advantages**

- The only non-invasive method for identifying animals in long-term studies.
- Causes little stress to animals, particularly if done without physical capture.

- For the above reasons, it is ethically acceptable to groups of people who are concerned about the use of permanent marking methods (particularly toe-clipping), such as iwi and institutional Animal Ethics Committees.
- Low material costs after the initial purchase of a digital camera.
- Analysis of count data (e.g. number of individuals photographed on each sampling occasion) requires little statistical training.

## **Disadvantages**

- Can only be used for species with distinguishable natural marks that remain constant over time. Even in species that have such marks, they may be lacking or poorly-developed in the pre-adult life stages (e.g. Gamble et al. 2008).
- May require longer animal handling and/or identification times than some commonly used permanent marking methods (particularly toe-clipping).
- May not be 100% accurate (varies with target species and observer skill level).
- Image quality will depend on the camera used and observer skill level.
- Photo-matching is sensitive to subjective operator error.

# Suitability for inventory

Photo-identification is not suitable for inventory because it requires resources (labour and time) beyond those required for inventory purposes. Inventory reporting only requires a summary of the number of animals found of each species at a given point in time (i.e. there is usually no repeat sampling). In contrast, photo-identification is used to identify individuals of the same species over time in studies with multiple sampling sessions. It is acknowledged that high-resolution, clear photographs of animals taken on an opportunistic basis (e.g. during systematic searches) can be extremely useful for identifying herpetofauna to species level; however, this is photography, not photo-identification.

# Suitability for monitoring

This method can potentially be used to monitor any herpetofaunal species with natural marks that remain constant over time. It is most easily applied to small populations that occupy well-defined areas (Beausoleil et al. 2004). Photo-identification needs to be validated before it is applied to new species to determine whether it is sufficiently accurate and cost-effective compared to permanent marking methods (<u>Case study B</u>; Kenyon et al. 2009; Knox et al. in press). Where ethical concerns prohibit the use of permanent marking methods (e.g. Perry et al. 2011; Hitchmough et al. 2012), photo-identification may be the only method available for identification of individuals in long-term studies.

Manual photo-matching can be very time-consuming, particularly for large populations (hundredsto-thousands of individuals), species with subtle marks, and for long-term studies with many sampling sessions and/or species with high detection probabilities (as each sampling session will generate many photographs to identify). In such cases, it may be worth investing in patternrecognition software. There is no generic software for photo-identification: instead, species-specific algorithms need to be written by computer experts and validated using a large number of photographs (e.g. Speed et al. 2007; Gamble et al. 2008; Bolger et al. 2012). It is not necessary for these algorithms to be 100% accurate: their value lies in restricting the number of potential matches to a small number that is easily handled by human observers.

## Skills

- The ability to capture and/or photograph herpetofauna
- The ability to identify individuals by photo-matching
- The ability to record and enter data (Microsoft Excel or other statistical software)
- A basic understanding of statistics

See 'Full details of technique and best practice' for more details.

## Resources

- One or more skilled observer(s)
- A digital camera capable of taking clear, high-resolution photographs
- A spare camera battery and Secure Digital (SD) card
- Software for downloading photos onto a computer (or an electronic card reader)
- A computer with sufficient RAM to maintain a photo library
- Datasheets/notebooks and pencils
- GPS to record site locations

Additional resources may be required, depending on the nature of the study:

- Printer
- Photo-album for storage of (hard copy) photographs
- Zoom lens (for photo-resight studies where animals are not captured)
- Custom-built, portable photo stage (this is currently being used to identify individual Archey's frogs; it uses mirrors to allow images of the front, back and sides of the frog to be combined in a single digital photograph)
- Image-manipulation and/or pattern-recognition software
- Disposable gloves to prevent spread of chytridiomycosis (native frogs only)
- Temporary holding bags (e.g. thin calico/cotton bags)
- Hand sanitiser (to prevent spread of Salmonella and other pathogens)

## Minimum attributes

Consistent measurement and recording of these attributes is critical for the implementation of the method. Other attributes may be optional depending on your objective. For more information, refer to 'Full details of technique and best practice'.

DOC staff must complete a 'Standard inventory and monitoring project plan' (docdm-146272).

Minimum attributes to record:

- Observer
- Date and time
- Location name/grid reference
- For each site, record the number of animals that were encountered and photographed. It may also be useful to record the number of photographs taken and their reference numbers (as given by the digital camera).
- Photos must be clear and in focus (take multiple photos if there is any doubt).
- Weather conditions, particularly ambient (shade air) temperatures recorded 1 m above the ground at the start and end of each sampling sessions. Alternatively, it may be possible to obtain this information retrospectively if there is access to weather records from a nearby weather station.

Depending on the aims of the study, it may be useful to record other information:

- Habitat and micro-habitat characteristics (e.g. altitude, aspect, vegetation cover and description) at locations where animals are encountered.
- Additional weather variables (e.g. relative humidity, overnight minimum temperature, day-time maximum temperature, precipitation, cloud cover, wind direction and strength).
- Where animals are physically captured for photo-mark-recapture monitoring, additional data should be collected (e.g. snout-vent length, mass (g), sex of mature individuals and the reproductive status of females (pregnant/gravid or not gravid)). Such data can be included as individual covariates or grouping variables during mark-recapture analysis (see 'Herpetofauna: population estimates'—docdm-833600).

## Data storage

Forward copies of completed survey sheets to the survey administrator, or enter data into an appropriate spreadsheet as soon as possible. Collate, consolidate and store survey information securely, also as soon as possible, and preferably immediately on return from the field. The key steps here are data entry, storage and maintenance for later analysis, followed by copying and data backup for security.

Photographs should be downloaded, viewed and archived on a computer after each sampling session or field trip. Out-of-focus or poor-quality photographs should be discarded (unless they are the only images available for a particular individual). The best images of each individual should be archived in a photo library. Images must be labelled correctly (e.g. species, animal identification number and date on which the photograph was taken). For security, the photo library should also be backed up on a second computer and/or portable electronic device (e.g. external hard drive, DVD or USB flash drive) stored at another location.

Summarise the results in a spreadsheet or equivalent. Arrange data as 'column variables'; i.e. arrange data from each field on the data sheet (date, time, location, plot designation, number seen,

identity, etc.) in columns, with each row representing the occasion on which a given survey plot was sampled.

If data storage is designed well at the outset, it will make the job of analysis and interpretation much easier. Before storing data, check for missing information and errors, and ensure metadata are recorded.

Storage tools can be either manual or electronic systems (or both, preferably). They will usually be summary sheets, other physical filing systems, or electronic spreadsheets and databases. Use appropriate file formats such as .xls, .txt, .dbf or specific analysis software formats. Copy and/or backup all data, whether electronic, data sheets, metadata or site access descriptions, preferably off-line if the primary storage location is part of a networked system. Store the copy at a separate location for security purposes.

# Analysis, interpretation and reporting

- Summarise the number of individuals encountered in each sampling session in an Excel spreadsheet.
- For each individual, use photo-matching to determine whether the animal has been encountered previously. Photo-matching can be done using hard (printed photographs) or electronic copies of photographs (e.g. JPEG images).
- If the animal is new (i.e. does not match any images in the photo library), it should be assigned a unique identification number, usually the next available number in a numeric series. For example, if the photo library has images of 26 individual harlequin geckos, a logical label for the new animal would be 'Tr27' (where 'Tr' represents an abbreviation of *Tukutuku rakiurae*). The best photo or photos (depending on the number of areas of the body that are photographed) of each new animal should be retained as reference photos.
- If an animal is a visual recapture (i.e. matches photo(s) in the photo library), it should be assigned the unique identification number that has already been assigned to the individual whose photos it matches. It may be useful to retain photos of recaptures to provide a visual record that can be checked if subsequent data analysis reveals inconstancies and to confirm that natural marks remain stable over an individual's lifespan. Photos of the same individual taken on different days can be identified by labelling the image accordingly (e.g. 'Tr27, 1 Oct 09').
- Photos of animals from different populations should be stored in separate folders to minimise the number of photos that need to be checked. An alphabetic site identifier can be added (e.g. 'TrA27, 1 Oct 09').
- Enter the animal identification numbers and any other relevant data (e.g. trap number, snoutvent length or size class, mass, sex of mature individuals, reproductive status of adult females and any distinguishing features such as natural toe loss of tail regeneration) in the Excel spreadsheet.
- Report results in a timely manner (usually within a year of the data collection).
- Analytical protocols are not covered in this section. If using photo-mark-recapture for estimating population parameters (e.g. abundance, survival or population growth rates), the process is

identical to that used for conventional capture-mark-recapture analysis. Seek statistical advice from a biometrician or suitably experienced person prior to undertaking any analysis.

## Case study A

#### Case study A: lizards recover after predator control

## Synopsis

Reardon et al. (2012) used photo-resight methods (i.e. photo-mark-recapture without physical capture) to test the effectiveness of two conservation tools for the protection of grand and Otago skinks, two of New Zealand's largest and rarest lizard species. Both species were assigned the highest threat status possible (Nationally Critical; Hitchmough et al. 2010) and failed to respond to mammalian predator control (targeting cats and ferrets) in a previous research-by-management study (Tocher 2006). Translocation to pest-free offshore islands is not considered feasible for these species because there is no equivalent island habitat. This left two potential options for in-situ management: landscape-scale predator trapping networks and use of mammal-proof fencing. The overall aim of this study was to test whether these mainland-specific conservation tools would allow population recovery in grand and Otago skink populations at Macraes Flat in north-eastern Otago.

## **Objectives**

- To test whether the eradication of five predator species within a mammal-proof fence would allow population recovery in grand and Otago skinks.
- To test whether predator suppression through landscape-scale predator trapping could achieve similar results.

## Sampling design and methods

The study was conducted over three field seasons (2006–2008) on conservation land near the township of Macraes Flat. Grand and Otago skink populations at six sites (described by Tocher 2006) were subjected to three predator management treatments: (1) near-eradication of predators within a mammal-proof fence (one site); (2) predator suppression by large-scale trapping networks with various layouts (three sites); and (3) no management of mammalian predators (two sites). Only two out of the six sites had both skink species present and management of one site changed partway through the study (from predator trapping to fencing). Predator eradication and suppression targeted five species of introduced predators (cat, ferret, stoat, weasel and hedgehog) using various trap and bait combinations. Rabbit and mouse management was also undertaken.

Skinks at each site were surveyed 3–9 times per field season using photo-resight methods to minimise potential adverse effects of handling on skink behaviour and to improve the robustness of population models. Surveys were carried out in standardised conditions (warm and sunny with little wind). Once skinks were located, observers attempted to photograph both sides of each skink, targeting the nose-to-foreleg (lateral) region (Fig. 3). This area contains distinctive blotches and/or

curvilinear black and gold markings in both skink species. The best images of the left- and righthand sides of each skink were coded and archived in a photo library. Subsequent images were visually compared to the images in this library. Photo-identification software was tested (I<sup>3</sup>S Interactive individual identification systems: <u>http://www.reijns.com/i3s/</u>) but probability ranking of images proved equal or less time-efficient than manual matching (James Reardon, pers. comm. July 2012).



Figure 3. Archived photos of grand (top) and Otago (bottom) skinks (photos: DOC).

Photo-resight data were converted to encounter histories and analysed using the robust design with the Huggins closed population estimator in Program MARK. For further details of the statistical analysis methods, see Reardon et al. (2012).

#### Results

Populations of both skink species increased in the mammal-proof fence and at the site with the most extensive predator-trapping network. In contrast, there was a catastrophic (85%) decline in the number of grand skinks at the unmanaged site (from 76 to 11 skinks over the 3-year study), but no evidence for a decline or increase in Otago skink numbers. Photo-identification was an accurate identification method for both species. Skinks had exceptionally stable patterning over time, with only minor changes observed in stippling (which tended to develop with age) and scale colouration.

#### Limitations and points to consider

This study had the following limitations:

- Treatments were not replicated and site allocation was not random. However, designing the perfect experiment is not always possible or advisable for endangered species because of their rarity, a pressing need for conservation management and/or other constraints (e.g. the cost of mammal-proof fencing).
- Management of one of the sites changed part-way through the study (a fence was built around one of the predator trapping sites).
- Sampling effort was not standardised (sites were sampled 3–9 times per season). Poor weather conditions restricted the number of surveys conducted in the second year (2007). Sampling effort increased at one of the fenced sites in 2008 because preliminary analysis revealed variable detection probabilities.
- The upward trend in survival and uncertainty associated with point estimates (i.e. large confidence intervals) suggests that the results would have been less equivocal if data for subsequent years had been included in the analysis.

Points to consider:

- This study generated thousands of photographs, including many photographs of the same individuals (i.e. resightings). When using artificial permanent marking methods, identification and processing of recaptures is usually much faster than marking and processing times for newlyencountered animals. There is no such time saving for resightings when using photoidentification.
- It currently takes staff working on the Grand and Otago skink Recovery Programme more time to process, identify and archive images collected during the field season than it does to complete the field work (Andy Hutcheon, pers. comm. June 2012). It is anticipated that patternrecognition software (currently under development) will produce significant time savings for the Programme.
- Despite similar habitat requirements, grand and Otago skinks appear to have different survival and population growth rates following predator removal. This raises the question of whether they should be treated as a single management unit, as is currently the case (Reardon et al. 2012).

## References for case study A

- Hitchmough, R.A.; Hoare, J.M.; Jamieson, H.; Newman, D.; Tocher, M.D.; Anderson, P.J.; Lettink, M.; Whitaker, A.H. 2010: Conservation status of New Zealand reptiles, 2009. New Zealand Journal of Zoology 37: 203–224.
- Reardon, J.T.; Whitmore, N.; Holmes, K.M.; Judd, L.M.; Hutcheon, A.D.; Norbury, G.; Mackenzie, D.I. 2012: Predator control allows critically endangered lizards to recover on mainland New Zealand. *New Zealand Journal of Ecology 36(2)*: 141–150.
- Tocher, M.D. 2006: Survival of grand and Otago skinks following predator control. *The Journal of Wildlife Management 70:* 31–42.

# Case study B

#### Case study B: comparison of frog marking methods

## Synopsis

Clemas et al. (2009) compared three methods (photo-identification, toe-clipping and fluorescent Visible Implant Alphanumeric (VIA) tags) for individual identification of whistling frogs living in three ponds in Dunedin. Although this species is not of conservation concern (being an Australian introduction), it is of similar size to native frogs and can therefore be used as a model to trial the effectiveness of new marking methods. The study was motivated by the need to find an ethical and effective alternative to toe-clipping for research and conservation management of amphibians.

## **Objectives**

- To assess the suitability of fluorescent VIA tags for use in whistling and Southern bell (*L. raniformis*) frogs.
- To compare handling and processing times required for photo-identification, VIA tags and toeclipping in whistling frogs.

## Sampling design and methods

An initial trial tested the suitability of fluorescent VIA tags in whistling frogs (n = 3) and Southern bell frogs (n = 4). The VIA tags measured 1.0 mm (width) × 2.5 mm (length) and contained alphanumeric characters (e.g. 'A84') that could be read in normal light and fluoresced green under blue light. Tags were inserted in subcutaneous lymph sacs (various locations on the body) that were sufficiently large to accommodate the tags but were small enough to prevent them from moving around and flipping over. Sterile cuticle scissors were used to make a 3-mm long incision in the skin, into which the tag was inserted using the needle-like injector provided by the manufacturer. The cut was then sealed with Liquid Bandaid®. Frogs were kept in captivity for a 3-week observation period prior to the start of the main study.

In the main study, a total of 110 whistling frogs were caught from three areas in Dunedin. Frogs were randomly assigned to be marked by one of the three methods and the time taken to process each frog was recorded. VIA tags were inserted in the interfemoral sac (inside of the left hind thigh), as described above. Toe-clipping entailed the removal of no more than two toes per frog in individual-specific combinations using sterilised scissors. Photo-identification involved taking several photographs of each frog from different angles. Processing times for this method included the time required to acquire, download, format and archive the images. Frogs were then released back into the wild and re-sampled on subsequent visits. Analysis of variance (ANOVA) was used to compare processing times among methods.

#### Results

All frogs involved in the initial trial of the fluorescent VIA tags had 100% survival in captivity and showed no negative health effects (e.g. infection) post-tagging. However, VIA tags proved unsuitable for use in Southern bell frogs because the tags were obscured by the pigmentation and thickness of the skin. While the outline of the tags could be seen (when viewed under a blue light), the characters on the tags were only partially readable or illegible. There were also problems with tag movement inside the subcutaneous sacs, which are larger in this species than in whistling frogs.

Toe-clipping was the most time-efficient and least expensive method used in the main study, but VIA tags had significantly faster handling times for recaptured animals. Photo-identification was the slowest method, both for newly-captured and recaptured individuals. Photo-matching was time-consuming and difficult because most whistling frogs had similar markings. For these reasons, use of photo-identification was not recommended for this species. The researchers noted that photo-identification is dependent on the camera type and skill of the observer, and that photo-matching is open to subjective operator error.

#### Limitations and points to consider

This study had the following limitations:

- Sample size in the initial trial was very low (*n* = 7; both species combined).
- The number of recaptures was low (but evenly distributed among methods).
- Although the researchers concluded that toe-clipping was the least expensive method, they did not provide materials or labour costs for any of the methods used. Such information would be useful when selecting a marking method.

Points to consider:

- Photo-identification was slow and difficult for whistling frogs because most individuals had very similar markings and colouration. Because natural marks vary among species, comparisons of photo-identification with other methods will always yield species-specific results. In contrast, the time taken to apply permanent marks would be expected to be similar across most species.
- Some studies have reported the migration and occasional disappearance of tags (e.g. Davis & Ovaska 2001). A 3-week captive observation period may be insufficient to determine whether there is excessive tag movement, particularly for field use in native frogs because many species are long-lived (e.g. 23 years in Maud Island frog; Bell 1994).
- The ideal marking method should have minimal impact at the individual level. Considerations should include the invasiveness of the method as well as the impact of repeated capture and handling times on animals, all of which have been shown to cause stress in herpetofauna (Langkilde & Shine 2006).

#### References for case study B

- Bell, B.D. 1994: A review of the status of New Zealand Leiopelma species (Anura: Leiopelmatidae), including a summary of demographic studies in Coromandel and on Maud Island. New Zealand Journal of Zoology 21: 341–349.
- Clemas, J.R.; Germano, J.M.; Speare, R.; Bishop, P.J. 2009: Use of three individual marking methods in Australian frogs (Genus: *Litoria*) with notes on placement of Visible Implant Alphanumeric tags. *New Zealand Natural Sciences* 34: 1–7.
- Davis, T.M.; Ovaska, K. 2001: Individual recognition of amphibians: effects of toe-clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. *Journal of Herpetology* 35: 217– 225.
- Langkilde, T.; Shine, R. 2006: How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei. Journal of Experimental Biology 209:* 1035–1043.

## Full details of technique and best practice

#### Background preparation

Depending on the methods used, field work may require DOC Animal Ethics Committee (AEC) approval and a Wildlife Act permit. DOC employees are permitted to capture, handle, measure and tail-tip (take a genetic sample from) herpetofauna under the Wildlife Act, but externals are required obtain an appropriate permit from DOC. For the above activities, a 'Low Impact Research & Collection' permit is sufficient. The application form can be downloaded from the DOC website<sup>1</sup> and is usually processed within 6 weeks. The processing fee may be waived if the work is of benefit to DOC. People with no experience handling herpetofauna will require training by an experienced practitioner if required for the study. Training should be completed before the start of any field work. A digital camera and accessories (e.g. spare camera battery, charger and SD cards) must be purchased before the start of field work. Camera operators should ensure they are familiar with the controls (particularly for single-lens reflex (SLR) cameras used with high-magnification zoom lenses).

#### Pilot study

A pilot study may be required to determine whether photo-identification is suitable for new species. This involves photographing a range of individuals of different ages and sexes to document variability in natural marks present on one or more regions of the body. Regions of the body that can be used for photo-identification will vary depending on whether physical capture is required and

<sup>&</sup>lt;sup>1</sup> <u>http://www.doc.govt.nz/about-doc/concessions-and-permits/research-collection-and-wildlife-permits/application-forms/</u>

on the natural marks present. It may be necessary to initially photograph multiple regions of the body (Fig. 4), particularly where there are few distinguishable natural marks. While photo-resight methods have the obvious advantage of eliminating stress caused by marking and the repeated capture and handling of individuals, only visible regions on the bodies of emerged animals are able to be photographed. To date, photo-resight studies of New Zealand skinks have utilised the nose-to-foreleg region (Case study A; Gebauer 2009). For photo-mark-recapture studies of species with subtle natural marks, it will be useful to apply a permanent mark (e.g. toe clip) at the time of capture for validation of individual identity (e.g. Denton & Beebee 1993; Kenyon et al. 2009).



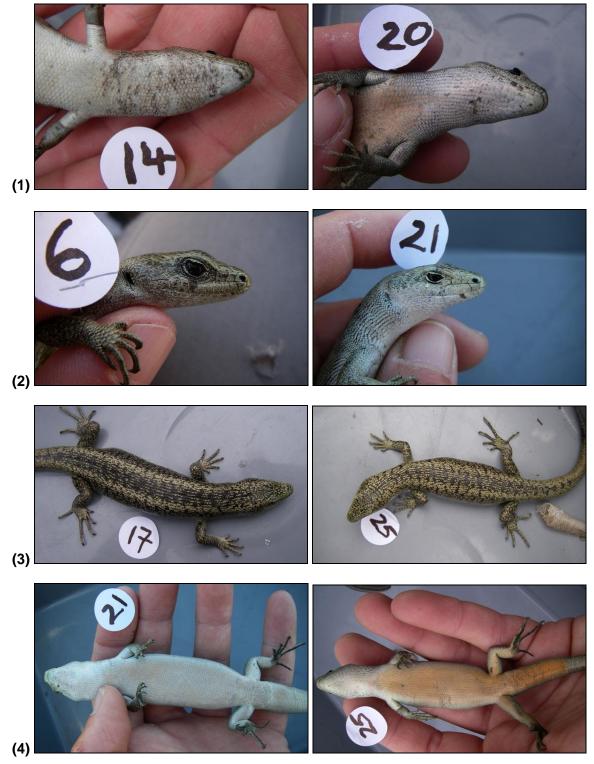


Figure 4. Regions of the body that were initially considered for photo-mark-recapture monitoring of scree skinks (*Oligosoma waimatense*) captured in pitfall traps in Canterbury, in order of appearance: (1) throat speckling and scarring patterns; (2) lateral views of the head; (3) dorsal markings; and (4) ventral markings and colouration. Throat scarring and speckling patterns changed over time. Close-up lateral views of both sides of the head in combination with dorsal markings were used for individual identification in subsequent monitoring (photos: M. Lettink).

#### Increasing the efficiency of photo matching

Photo-matching can be a very time-consuming process. Time taken to identify individuals will increase with the size of the photo library. Several steps can be taken to reduce the amount of time required to make a match. Firstly, photographs of animals from different sites (i.e. independent populations that do not mix) should be stored in separate folders. Secondly, it may help to divide animals into subgroups to reduce the number of images that have to be visually assessed. For example, Gill (1978) grouped individual red-spotted newts (Notophythalmus viridescens) by the number of spots on each side of the dorsal surface. This facilitated rapid identification ( $\leq 30$ seconds per individual) of newts from a large photo-library containing images of more than 8500 individuals. The development of a key is useful where multiple characteristics and/or regions of the body are used for photo-identification (Bradfield 2004). Finally, for photo-mark-recapture studies, additional data collected at the time of capture (e.g. capture location, snout-vent length or size class, sex, incidence of tail loss, length of any tail regeneration, natural or researcher-induced toe loss) can be used to narrow down potential candidate animals. For example, a sub-adult grand skink with tail loss and regeneration can only have been a juvenile or sub-adult in previous seasons and if the tail loss is fully regenerated then a note of tail loss would have been recorded in the previous season. Hence, all adult animals with entire tails from the location being surveyed can be removed from the search when trying to match current images of this individual with photos from the previous season(s).

#### Considerations when choosing a digital camera

Although photo-identification is possible with conventional cameras, it is assumed here that operators will want to use digital cameras. These should be capable of taking high-resolution images and be sufficiently robust for field use (ideally, water-resistant and shock-proof). Two main designs are available: compact (also known as point-and-shoot) cameras and digital single-lens reflex (DSLR) cameras. Compact cameras are small, portable and easy to use, making them well-suited for casual and 'snapshot' uses. They have an inbuilt lens and many semi- or fully-automated functions. Images are usually stored as JPEG files. Compact cameras are suitable for photographing animals in the hand and from short distances (e.g. Knox et al. in press.). Cameras used for close-up photography must have a good macro lens (DSLR) or macro function (compact camera).

Compared with compact cameras, DSLR cameras are larger and more expensive, produce images of better quality overall in a range of formats, and permit greater user input (e.g. ability to control depth-of-field and shutter speeds). They are constructed with reflex mirrors that allow operators to see exactly what is shot through the lens (this means that the focus on the subject can be adjusted as if looking through binoculars). DSLR cameras can be used with a variety of lenses (e.g. macro, zoom, telephoto, wide angle) to focus over a wide range of distances, making them well-suited for close-up photography and photo-resight studies alike.

DSLR cameras used for photo-resight studies should be of a reputable brand (e.g. Canon or Nikon). It is worth investing in 'semi-pro' (semi-professional) camera bodies because they have

better componentry and are usually more robust (but may weigh more) than 'amateur' (standard) camera bodies. Examples of semi-professional cameras are the Canon EOS 60D and Nikon D7000/D300s. Essential camera features for photo-resight studies are instant shutter release and versatile light metering, including spot metering. Spot metering allows users to measure light levels of a very small area of the scene (typically 1–5% of the viewfinder area; e.g. for animals hiding in rock cracks) and is commonly used to shoot scenes with very high contrast.

Most zoom lenses have brand-specific fittings (e.g. a Nikon lens will not fit a Canon camera body). They are typically described by the ratio of their longest to shortest focal lengths (e.g. a zoom lens with focal lengths ranging from 100 mm to 300 mm may be described as a 3:1 or 3× zoom). Zoom lenses used for photo-resight studies should have a reasonable (but not excessive) magnification, a small minimum-focusing distance (ideally, less than 1.5–2 m), image stabilisation (IS) to reduce camera shake, and not be too large or heavy for field use. For example, field staff employed by the Grand and Otago Recovery Programme mostly use 70–300 mm Canon IS zoom lenses on Canon EOS (20D to 50D series) semi-pro camera bodies. Skinks are typically photographed from distances of 3–4 m, but this camera and lens combination provides workable identification photos from a distance of up to 10 m (Andy Hutcheon, pers. comm. June 2012; see also <u>Case study A</u>).

#### Photographing animals from a distance

To date, photo-resight studies in New Zealand have only focused on diurnal species. Binoculars and/or the naked eye can be used to scan an area for emerged (basking or foraging) individuals, ideally under optimal weather conditions with the sun directly behind the observer. Once an animal has been located, the observer should approach slowly and quietly. Care should be taken to avoid making rapid and jerky movements, cast ones' shadow across the animal and/or accidentally brush the vegetation that animal is sitting on (for arboreal species). If an animal is spooked in the process and disappears from view, the observer should step back a few meters and wait quietly for the animal to re-emerge. Individuals naturally vary in their degree of vigilance.

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# Appendix A

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

- docdm-833600 Herpetofauna: population estimates
- docdm-146272 Standard inventory and monitoring project plan