

Herpetofauna: population estimates (using capture-mark-recapture data)

Version 1.0



This specification was prepared by Marieke Lettink in 2012.

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Synopsis

Population estimates, as described here, are estimates of abundance (i.e. population size) derived from the analysis of capture-mark-recapture (CMR) data. They are used to determine whether populations are declining, stable or increasing and thus inform conservation management. Population estimates can also be used to evaluate the impacts of threats, assess response to management actions designed to alleviate threats, and highlight areas where further research is needed (Lettink & Armstrong 2003). Population estimates are usually expressed as the number of individuals present within a defined area plus an associated measure of uncertainty (e.g. 95% confidence interval). This method requires that animals are sampled on multiple occasions using an appropriate field method, and that they are marked or identified from their natural markings (by photo-identification). For convenience, the term 'capture' is used throughout irrespective of whether animals are physically captured (as sightings represent 'visual captures').

The main advantage of using population estimates is that they account for variations in detection probability (hereafter 'detectability'), making them more accurate and robust than uncorrected counts (see 'Herpetofauna: indices of relative abundance'—docdm-493179). Consequently, response to management can be determined with greater certainty and within much shorter time frames. This may be crucial when working with critically endangered species that are undergoing rapid declines. For example, population estimates revealed that response to predator management (by mammal-exclusion fencing and intensive multi-species control) was evident within 3 years for grand (*Oligosoma grande*) and Otago (*O. otagense*) skinks at Macraes Flat in Otago (Reardon et al. in press.). The main disadvantages of population estimates is that they require greater sampling effort and more resources than uncorrected counts (Mazerolle et al. 2007), including access to a biometrician and/or person familiar with CMR data-analysis methods, and can cause more stress to animals (where they are marked and repeatedly captured).

A typical CMR analysis involves model building and selection using closed-population (Otis et al. 1978), open-population (Pollock et al. 1990) or robust design (Pollock 1982) estimators. The terms 'estimator' and 'model' are synonymous and may be defined as a mathematical simplification of reality that represents our understanding of how a system operates (Lettink & Armstrong 2003). A bewildering variety of models are available for estimating animal abundance from CMR data (reviewed by Schwarz & Seber 1999 and Amstrup et al. 2006) and there is now more than a century of literature on this topic (Cooch & White 2011).

Closed-population models are the main focus of this prescription. They were developed to estimate abundance and require sampling to be undertaken over short time intervals to ensure that the population does not change in size (i.e. no births, deaths, emigration or immigration over the sampling period). In contrast, open-population models were developed to estimate survival over longer time periods, and permit births, deaths, immigration or emigration. They are more complicated than closed-population models because extra parameters are needed to model recruitment, mortality and movements. While it is possible to use some open-population models to estimate abundance, the resulting estimates tend to be less precise and robust to variations in capture probability than those generated by closed-population models (Kendall 2001). Open-



population models are not discussed further. The robust design of Pollock (1982) combines the abundance estimation feature of closed-population models with the survival estimation component of open-population models. It is ideal for long-term monitoring where both abundance and survival are of interest.

The simplest model for estimating abundance is the Lincoln-Petersen estimator, which requires only two capture occasions (Lincoln 1930). On the first occasion, a portion of the population is captured, given generic marks (e.g. a spot of paint on the head) and released. The population is re-sampled on one other occasion, and the ratio of marked to unmarked animals is used to infer population size. Use of this estimator requires all animals to have the same probability of being caught, which rarely applies to herpetofauna (but see Moore et al. 2010). Additional capture occasions are highly recommended because this permits more flexible modelling (Mazerolle et al. 2007). Accordingly, most CMR studies of herpetofauna consist of one or more capture session(s), each containing a number of capture occasions (e.g. days or nights).

Prior to CMR analysis, capture data must be converted to encounter histories. These are strings of zeros and ones indicating whether an individual was captured (denoted '1') or not (denoted '0') on each capture occasion. For example, a frog with an encounter history of '11010' was captured on the first, second and fourth (but not on the third or fifth) nights of a capture session consisting of five capture occasions. A dataset containing the encounter histories for all animals caught is then analysed using an appropriate estimator and software program (e.g. program MARK; White & Burnham 1999). This generally requires access to a biometrician and/or person with considerable experience analysing CMR data, including a working knowledge of model selection procedures (Burnham & Anderson 2002).

Population estimates should not be used in any situation where it is possible to count all individuals present (i.e. detectability is 100% and animals are immobile with respect to the observer). This situation would not be expected for herpetofauna, but may apply when sampling plants or sessile marine animals (e.g. limpets on rocks). For New Zealand herpetofauna, population estimates (excluding minimum number alive (MNA) indices, which do not correct for detectability) have been obtained for tuatara (Cassey & Ussher 1999; Tyrrell et al. 2000; Nelson et al. 2002; Moore et al. 2010; Wilson 2010), frogs (Bell, Carver et al. 2004; Bell, Pledger et al. 2004; Pledger & Tocher 2004; Tocher & Pledger 2005; Haigh et al. 2007; Bell & Pledger 2010) and lizards (Towns 1994; Freeman 1997; Clark 2006; Tocher 2006; Hare et al. 2007; Wilson et al. 2007; Knox et al. (in press); Wilson 2010; Lettink et al. 2011).

Key issues in the design of a population estimation study are choice of marking method, number of capture sessions, their timing, and the number and arrangement of traps or other sampling devices. The amount of sampling effort required will depend on the aims of the study, and on the density and detectability of the target species. See ['Case studies'](#) for examples of potential uses of population estimates.



Assumptions

Generic assumptions:

- Observers are able to capture and/or photograph herpetofauna.
- Animals can be marked or identified from their natural markings.
- Marks do not influence the behaviour or survival of marked animals.
- The sampling area is representative of the wider habitat occupied by the target species (alternatively, inference is restricted to the sampling area).
- All relevant data (e.g. date, capture session, trap number, animal identification number) are recorded and used in subsequent analyses, where appropriate.

Statistical assumptions for closed-population models:

- The population is closed, i.e. no births, deaths, emigration or immigration during the study period.
- All animals have the same probability of being caught (alternatively, factors that underlie variations in capture probability are included in the analysis).
- Marks are not lost or overlooked.

A range of estimators can be used to obtain population estimates from CMR data, many of which allow the second statistical assumption (i.e. that of equal capture probability, which is commonly violated for animal populations) to be relaxed. With the exception of the Lincoln-Petersen estimator, animals must be given unique marks.

Advantages

- Population estimates can be very accurate provided assumptions are met and the sampling design is robust.
- Very useful for geographically well-defined populations of animals with restricted ranges (e.g. islands or discrete habitats).
- Unique and permanent marks permit rigorous long-term analysis of abundance and other population measures, including survival and longevity. This, in turn, allows for the development of population models that can be used to predict population responses to threats and/or alternative management scenarios.

Disadvantages

- Requires greater sampling effort and resources than other methods.
- Not suitable for highly mobile populations or populations with a large number of transient individuals.
- Each animal has to be uniquely marked or identifiable from natural markings (except for Lincoln-Petersen estimates, which require only generic or 'batch' marks to distinguish animals that were caught previously from those that have not, e.g. a spot on the head).
- Marking and repeatedly handling/capturing animals can be stressful for them.



- Lincoln-Petersen estimates are often low and inaccurate. This estimator should only be used if it is known to provide accurate estimates of population size without violating the statistical assumptions (e.g. Moore et al. 2010).
- A high percentage (ideally $\geq 40\%$) of the population needs to be marked within the defined sampling area to ensure accuracy and precision of estimates.
- If identification relies on natural markings, a significant time investment may be required to develop and test the accuracy of photo-identification methods.
- The number of capture occasions required is relatively high (usually, a minimum of 4–8) to ensure estimate accuracy and precision.
- Low or highly variable capture and recapture probabilities may give imprecise estimates (or in some cases, preclude the use of CMR analysis altogether).
- CMR analysis requires considerable training and/or a biometrician (except for Lincoln-Petersen estimates, which can be obtained using a calculator).

Suitability for inventory

Population estimates from CMR analysis are not suitable for inventory. This is because this method requires extensive resources (labour and time) and yields data that is beyond that required for inventory purposes.

Suitability for monitoring

Population estimates can potentially be used to monitor tuatara, frogs and lizards, given all assumptions can be met and sufficient resources are available. However, it is not recommended for low-density or highly mobile populations, species for which effective sampling methods have not been developed (e.g. striped skink *Oligosoma striatum*), species with low or highly variable detectability, and species that cannot be marked in an ethically acceptable manner or identified from their natural markings. Implications of the statistical assumptions required for monitoring are discussed below.

The assumption of population closure

The population closure assumption is generally met by restricting the length of the sampling interval so that births, deaths, immigration and emigration do not occur (or have negligible effects) over the study period. For short-term studies (i.e. one capture session with multiple capture occasions), the sampling interval is often less than 1 week and usually no more than a few weeks. For long-term monitoring, short capture sessions of a standardised length are repeated at regular (often annual) intervals. Births may occur if sampling coincides with the breeding season and deaths can occur at any time-of-year. Capture data for newborn animals or individuals that are known to have died during a sampling session should be omitted from the analysis.

Emigration and immigration are more difficult to deal with statistically, but tend to be less problematic for herpetofauna than other vertebrate groups (e.g. birds and fish), particularly in New



Zealand, because our amphibians and reptiles (with the exception of marine turtles and sea snakes) are non-migratory and adults tend to occupy fixed territories and/or home ranges. However, temporary emigration (movement into areas where they cannot be effectively sampled, e.g. underground or in the forest canopy) can be an issue for fossorial (burrowing) and arboreal species. Temporary emigration may require use of multi-state models or the robust design (e.g. Bailey et al. 2004). These are more complex than closed-population estimators and are not recommended for species with low and variable detection probabilities.

The assumption of equal capture probability

The assumption of equal capture probability is rarely met for herpetofauna. Animals may differ in their capture and recapture probabilities over time or due to weather conditions, inherent biological traits (e.g. age or sex), a behavioural response to previous capture and/or marking (usually trap-shyness), or for other reasons (Otis et al. 1978; White et al. 1982; Williams et al. 2002). This assumption may be relaxed by choosing an estimator that allows variation in capture probabilities (hereafter 'capture heterogeneity') to be factored into the analysis (Otis et al. 1978; Schwarz & Seber 1999; Borchers et al. 2002). Where capture heterogeneity is known to be related to measurable traits of individuals, the relevant data should be included in the analysis. This may be achieved by classifying the data into discrete groups (e.g. age and sex) or by including individual covariates for continuous data (e.g. snout-to-vent length).

Ultimately, the complexity of the analysis will depend on the nature of the CMR data. No estimator allows all possible effects to be examined simultaneously, even with a comprehensive dataset. Therefore, knowledge of the study species is crucial for developing a rigorous study design, including selection of an appropriate estimator. Two commonly used estimators are Huggins closed captures (Huggins 1989, 1991) and the robust design, which combines abundance with survival estimation (Pollock 1982). The main advantage of the Huggins closed-capture estimator is that it permits the inclusion of individual covariates. See ['Case studies'](#) for applications to herpetofaunal monitoring.

The assumption that marks are not lost or overlooked

The current lack of an ethically acceptable (i.e. DOC-approved) permanent marking method is a significant barrier to the use of population estimates for herpetofauna in New Zealand, particularly lizards (Table 1). Temporary marks may be acceptable for short studies in some cases (e.g. Jones & Bell 2010; Moore et al. 2010) but are easily lost, particularly in areas with dense vegetation or high rainfall. For example, pen marks (numbers written on the dorsum) of toe-clipped common skinks (*O. polychroma*) living in dense valley-floor grassland in the Eglinton Valley became illegible or were lost within 1–6 days following application (see ['Case study B'](#)).



Table 1. Methods used to mark or identify lizards in New Zealand and their suitability for population estimates (using capture-mark-recapture data). See Mellor et al. (2004) for a review of marking methods used for amphibians and reptiles.

Method	Description	Suitability for population estimates
Temporary pen marks	Application of an individual-specific mark (usually a number) to the dorsal or ventral surface of an animal with a fine-tipped non-toxic permanent marker. Ventral marks are recommended because dorsal marks can increase an animal's conspicuousness, putting it at greater risk of avian predation.	Not suitable for long-term studies because marks are temporary. Pen marks are lost when animals shed their skin or rub off, sometimes within a day of application.
Toe-clipping	The permanent removal of all or part of the distal phalange of no more than one toe per foot in individual-specific combinations.	Not currently approved by the DOC Animal Ethics Committee, other than in exceptional circumstances.
PIT-tagging	Subcutaneous injection of Passive Integrated Transponders (PITs)—small electronic units encased in biologically inert captures.	Can only be used on large species. To date, PITs have been used for one lizard species (Duvaucel's gecko <i>Hoplodactylus duvaucelii</i>) and tuatara.
Micro-branding	Application of small brands or tattoos to the skin with a custom-made branding iron in individual-specific patterns.	Not sufficiently accurate for long-term studies due to brand loss. Not a DOC-approved method.
Photo-identification	Taking digital photographs of animals or parts of animals with or without physical capture. Images are compared to a reference database by manual matching or use of pattern-recognition software (currently under development but not yet employed in NZ; requires development and testing of user-defined search algorithms).	Can only be used for species that have distinguishing marks that are stable over the lifetime of the animal. Manual matching is time-consuming and relies heavily on operator efficiency. Technical advances should improve future practicality.

Photo-identification can only be used for species that have distinguishing marks that are stable over the lifetime of the animal. It has been used in population estimates for Archey's frog *Leiopelma archeyi* (see '[Case study A](#)'), jewelled gecko *Naultinus gemmeus* (Knox et al. in press), and grand and Otago skinks (Reardon et al. in press). Considerable resources may be required to test the accuracy of photo-identification procedures (e.g. Bradfield 2004). It is fairly laborious and relies heavily on operator efficiency (Mellor et al. 2004). As currently used in New Zealand, it relies on manual matching of digital images, although use of pattern-recognition software is being investigated (N. Whitmore, pers. comm.). Technical advances, such as automated spot-recognition software that employs user-defined search algorithms (e.g. Speed et al. 2007) should improve its future practicality.



Skills

- The ability to capture, handle and mark or photograph herpetofauna.
- The ability to accurately read and record marks or use photo-identification.
- Experience with the field method employed (e.g. pitfall trapping).
- The ability to design a CMR sampling protocol, including an understanding of the relevant statistical assumptions and consequences of violating them.
- The ability to record and enter data (Microsoft Excel).
- The ability to convert capture data into binary encounter histories.
- Previous experience with CMR analysis (including familiarity with CMR software and model selection procedures) or access to a biometrician.
- See '[Full details of technique and best practice](#)' for more details.

Resources

Population estimates are expensive to obtain compared with indices of abundance. However, the expense may be justified by the increased accuracy and precision of population estimates, provided that all assumptions can be met and sufficient resources are available. This method requires the following resources:

- A pilot study and power analysis may be required to optimise the study design (e.g. Haigh et al. 2007; Wilson et al. 2007).
- Animal capture and marking equipment (or for photo-identification without capture, a high-quality digital camera and zoom lens is required).
- Field datasheets.
- Program MARK software; this can be downloaded (free of charge) from <http://warnercnr.colostate.edu/~gwhite/mark/mark.htm>. Alternatively, the RMark package in program R can be downloaded (free of charge) from <http://www.r-project.org/>. Novices should start with MARK, unless they have extensive experience with R programming.
- To run program MARK, you will need a computer with a 2 GHz (or more) CPU and at least 1 Gb of RAM (> 1 Gb is strongly recommended). MARK is a Windows program (requiring Windows XP or later versions), but can also be operated from a non-Windows platform (including Linux and Macintosh).
- The MARK help manual (Cooch & White 2011). This 900+ page electronic book can be downloaded (free of charge) in electronic format from <http://www.phidot.org/software/mark/docs/book/>.
- Access to the following books:
 - Burnham & Anderson (2002) for theory and best-practice guidelines on model selection
 - Borchers et al. (2002), Williams et al. (2002) or Amstrup et al. (2006) for reviews of statistical methods for estimating animal abundance
- Access to a biometrician and/or person experienced in CMR analysis (including familiarity with CMR software and model selection procedures).



Program MARK is the most comprehensive software package for CMR analysis. Alternatives (not covered here) are programs CAPTURE (Rexstad & Burnham, 1992), DENSITY (Efford 2004; Efford et al. 2004) and POPAN (Schwarz & Arnason 1996). Most of the estimators offered in these programs are now also available in program MARK. A comprehensive review of the statistical models available for estimating animal abundance was provided by Schwarz & Seber (1999).

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](#)'. It is recommended that novices obtain training in field methods from an expert herpetologist. Training in analysis methods may be obtained from a biometrician or suitably qualified person.

Essential attributes

At a minimum, the following should be documented:

- DOC staff must complete a 'Standard inventory and monitoring project plan' (docdm-146272).
- For all herpetofauna, New Zealand Amphibian/Reptile Distribution Scheme (ARDS) cards should be completed and forwarded to the Herpetofauna Administrator (address shown on ARDS card; Fig. 1).¹

At a minimum, the following data should be recorded:

- Observer and/or recorder.
- Date and time at the start and end of each sampling session.
- Location name/grid reference.
- Weather conditions, particularly ambient (shade air) temperatures recorded 1 m above the ground at the start and end of each sampling session. Alternatively, it may be possible to obtain this information retrospectively if weather records from a nearby weather station are accessible.
- For each animal captured, its location (e.g. trap number), species, unique identification number, whether it is a new capture or recapture, and all relevant attributes (e.g. age, sex of mature individuals, snout-vent length (SVL), reproductive status of females (pregnant/gravid or not gravid), and mass).

¹ The ARDS card is available online: <http://www.doc.govt.nz/conservation/native-animals/reptiles-and-frogs/reptiles-and-frogs-distribution-information/species-sightings-and-data-management/report-a-sighting/>



ARDS CARD		NEW ZEALAND AMPHIBIAN/REPTILE DISTRIBUTION SCHEME Herpetofauna Administrator, RD&I, Department of Conservation, P.O. Box 644, Napier.				Card No:	
Observer: J.O. Smith Initials Surname		Date: 6 Jan 10		Locality Name: Macraes flat			
Address: Conservation House 77 Lower Stuart St Dunedin 9016		Alt (m): 605m		GPS Easting: 1390575		Northing: 4972669	
Affiliation: DOC. Otago		Area Office: Coastal Otago		Conservancy: Otago		Ecol. District: ?	
Species name e.g. <i>Hoplostethus maculatus</i>		No.	Time	Habitat	Weather	Weather	
Woodworthia Otago/Southland		6	18:00	16, D, E	6,2,1	Light	
→		15	0830	18, H	2,3,2	1 Fine/Sunny 2 Part Cloudy 3 Overcast 4 Showers 5 Rain 6 Night 7 0-½ Moonlit 8 ½-1 Moonlit	
Voucher specimen(s)		Yes/No		Specify: 10 photos.			
Photograph(s)		Yes/No		→ Left & right (lateral sides)			
Extra notes on reverse side		Yes/No		from nose to foreleg			
Notes: Day 2 of 5 day funnel trapping session. g-minnow, single-ended traps • 6 traps per rock top; 5 for outcrops (30 traps total); all baited with pear							
Identified by: J.O. Smith. Authority used: Jewell 2008. Field Guide							
						Major Habitat Types	
						1 Beech forest 2 Podocarp forest 3 Broadleaf forest 4 Exotic forest 5 Scrub 6 Sub-alpine 7 Alpine 8 Undeveloped tussock land 9 Developed farmland 10 River terrace 11 Fresh water 12 Wet land 13 Coastal 14 Scree 15 Bare rocks 16 Beach 17 Urban 18 Developed 19 Tussock 20 grassland	
						Micro habitats A Foliage B Trunk C Branches D Under stones E Under wood F Open ground G Crevices H Rock tops	

Figure 1. Example of how to fill in a New Zealand Amphibian/Reptile Distribution Scheme (ARDS) card. Note that either a GPS location or a map series number is sufficient. Also, try not to leave blank spaces—instead leave an indication that those data were not available or collected. If further notes are collected these can be included under ‘Notes’, and continue on the back of the page if necessary.

Data storage

The following instructions should be followed when storing data obtained from this method. 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. 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. See Fig. 2 for an example.



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	date	obs	trap	ID	N/R	SVL	VT	regen	age	sex	repro	weight	notes
2	24-Jan-08	ML	36	1	0	88	147	c	A	M		14	
3	24-Jan-08	ML	7	2	0	103	78	0.5	A	M		20.4	
4	24-Jan-08	ML	10	3	0	84	111	0.5	J			12.4	
5	24-Jan-08	ML	32	4	0	103	103	90	A	F	NP	22.6	obvious throat scar
6	24-Jan-08	ML	29	5	0	85	107	c	J	F?		14.8	
7	24-Jan-08	JVH	28	6	0	107	118	35	A	F	P	25.6	
8	24-Jan-08	JVH	27	7	0	87	110	15	J	F?		13.2	
9	24-Jan-08	JVH	25	8	0	83	129	c	J			12.8	
10	24-Jan-08	JVH	18	9	0	103	137	c	A	M		22.6	
11	24-Jan-08	JVH	17	10	0	105	158	c	A	M		22.8	
12	24-Jan-08	ML	35	11	0	93	103	30	A	M		17.6	
13	24-Jan-08	ML	38	12	0	85	103	3?	J			12.9	tail end bitten?
14	25-Jan-08	ML	2	13	0	88	132	c	A	F?	NP	16.6	hard lump in abdomen
15	25-Jan-08	WS	7	14	0	102	113	28	A	M		21.3	
16	25-Jan-08	WS	14	15	0	97	141	c	A	M		20.1	
17	25-Jan-08	WS	14	16	0	97	109	51	A	F	NP	20.1	
18	25-Jan-08	WS	15	17	0	96	133	c	A	F	NP	16.7	
19	25-Jan-08	WS	32	18	0	96	86	23	A	F	P	21.3	visibly pregnant
20	26-Jan-08	ML	35	1	1								
21	27-Jan-08	ML	37	12	1								
22	27-Jan-08	ML	5	19	0	97	145	c	A	M		16.8	
23	27-Jan-08	ML	22	20	0	113	88	52	A	F	P	25.6	
24	28-Jan-08	ML	27	5	1								
25	28-Jan-08	ML	14	16	1								
26	28-Jan-08	MI	32	18	1								

Figure 2. Excel spreadsheet containing capture-mark-capture data. Column headings: obs = observer, ID = individual identification number, N/R = newly-captured (0) or recaptured (1), SVL = snout-vent length, VT = vent-tail length, regen = length of any tail regeneration (or 'c' if complete), age = adult (A) or juvenile (J), sex = sex of mature individuals, repro = reproductive status of females where P = pregnant and NP = not pregnant. The 'Notes' column is used to record any interesting observations. For recaptures, only the trap number and identification number need to be recorded.

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.

For CMR analysis conducted in program MARK, capture data will need to be converted into encounter histories and the specific format required by MARK (see ['Full details of technique and best practice'](#)).

Analysis, interpretation and reporting

Standardised analysis and interpretation allows comparisons to be made at different sites and at different times. Follow these instructions when analysing and interpreting data:

- Seek statistical advice from a biometrician or suitably experienced person prior to designing a population estimation study and undertaking any analysis.
- For each new site, fill out an ARDS card with the total number of individuals caught of each species and submit this to the Herpetofauna Administrator.²
- Summarise the number of individuals caught at each site, separated into newly-captured, recaptured and total numbers of individuals caught. It is useful to present this information both in table and graph formats (Fig. 3).
- The proportion of recaptures relative to total captures (i.e. number of recaptures divided by the total number of captures) provides a rough indication of the suitability of the data for CMR analysis. As this ratio increases, so will the accuracy and precision of population estimates.
- Convert the capture data to the encounter history format required by program MARK and save this as an 'inp' file (refer to '[Full details of technique and best practice](#)'). This enables the file to be opened from within MARK and analysed using an appropriate estimator (Cooch & White 2011).
- If CMR analysis is not possible for some reason (e.g. due to insufficient captures and/or recaptures), simply report the number of animals caught.
- Report results in a timely manner (usually within a year of the data collection).

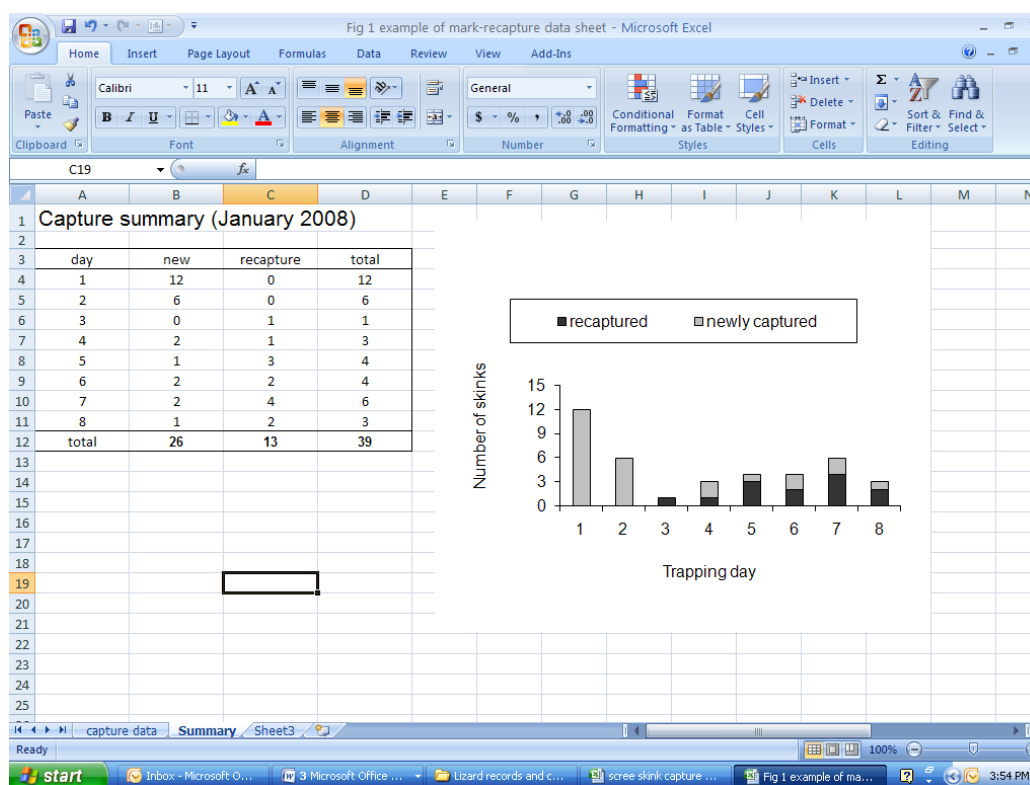


Figure 3. Simple summary and graph for capture-mark-recapture (CMR) data from a single capture session with 8 capture occasions (days), showing several features typical of CMR pitfall trapping studies of lizards: (1) higher capture numbers in the first few days of the study (suggesting a negative behavioural response to capture); (2) variability in the numbers of animals caught over time (most likely to be weather-related); and (3) an increase in the ratio of recaptures to new captures over time. If (3) does not occur, there could be a problem with the study design (e.g. excessive trap spacing or an insufficient number of capture occasions).

² The ARDS card is available online: <http://www.doc.govt.nz/conservation/native-animals/reptiles-and-frogs/reptiles-and-frogs-distribution-information/species-sightings-and-data-management/report-a-sighting/>

Case study A

Case study A: population estimates of Archey's frog (*Leiopelma archeyi*) based on photo-identification combined with CMR analysis

Synopsis

Concerns over possible declines in a significant mainland population of Archey's frog (Nationally Critical) highlighted the need for a robust population monitoring program. Population estimates were required because uncorrected counts were too variable. Haigh et al. (2007) provided recommendations for a monitoring protocol based on the results from CMR pilot studies. Five capture sessions with 2–4 capture occasions (nights) each were carried out in 2004–2005. Frogs were captured by hand and identified from their unique natural markings using a single digital photograph of the frog on a custom-built stage surrounded by mirrors. A previous study (Bradfield 2004) revealed that photo-identification was highly accurate (99.2% success rate). Preliminary estimates of abundance and capture probabilities were used in power analyses to determine the number and size of sampling grids needed, and the number of sampling occasions required to detect small population declines with confidence.

Objectives

- To monitor long-term trends in abundance and other parameters
- To design a monitoring protocol capable of detecting a specified decline in abundance (20% or greater within 2–8 months of the decline occurring)
- To test the influence of environmental variables on frog emergence

Sampling design and methods

Sampling was conducted on two grids containing representative habitat known to support high densities of frogs in Whareorino Forest, Waikato. The two grids were nested with a 'small' (5 m × 7 m) grid embedded in a 'large' (10 m × 10 m) grid. The grid was divided into 2-m wide lanes and searched at night by torchlight for emerged frogs, starting 1 h after sunset for 2–4 consecutive nights in January, February and November 2004, and in February and March 2005. One search of either grid was completed each night. Weather conditions (relative humidity, ambient temperature, cloud cover, rainfall and wind speed) were recorded at the start and finish. Frogs were captured by hand and placed in re-sealable plastic bags for processing. Observers wore disposable gloves to minimise the spread of amphibian disease. Upon capture of each frog, the time of capture, capture location, habitat, height above ground and age class were noted. Each frog was then photographed on a stage surrounded by mirrors that allowed four views (both sides, front and back) to be captured in one digital image (Wallace 2004; Smale et al. 2005). Frogs were then released at their capture locations. Subsequent photo-identification followed the methods of Bradfield (2004).

Two datasets were generated (i.e. one for each grid) and analysed in program MARK using the robust design. Capture data was structured into primary (month) and secondary (night within each



month) periods. The robust design estimates survival between primary periods and abundance for each secondary period, meaning that population closure needs only to be satisfied for each secondary period. Modelling considered the potential effects of time (denoted t) and a behavioural response to capture (b), but not capture heterogeneity (h). This was likely to be present but could not be modelled because it required at least four nights of sampling per month (this was achieved only in February 2004, but there were insufficient captures that month for clear results). A range of weather covariates were also specified. A likelihood version of the Jolly-Seber model (an open-population model) was also used but is not discussed here. Model selection was based on Akaike's Information Criterion (AIC), following the guidelines of Burnham & Anderson (2002). Results from the CMR analysis were used in a power analysis (Lebreton et al. 1992) to determine the probability of detecting a specified decline in abundance under various scenarios.

Results

A total of 55 and 116 different frogs were caught on the small and large grid, respectively (all sampling sessions combined; $n = 9$ nights per grid). The robust design analysis failed for the small dataset due to insufficient captures and recaptures. For the large dataset, the top-ranking (most parsimonious) model included time-dependent survival and capture probabilities. According to this model, mean nightly capture probability (p) was 0.31 ± 0.11 (SE) (range 0.16–0.67). Abundance (\hat{N}) ranged from 63.5 ± 9.7 (SE) in November 2004 to 103.7 ± 16.5 (SE) in March 2005. The power analysis revealed that four 10 m \times 10 m grids (two per treatment area for a planned future study testing the effect of rodent control on frog numbers) would give good power for detecting a change in abundance, assuming high frog density (c. 100 frogs per grid). Two or three capture sessions per year, each consisting of 4 or 5 nights, were recommended. It was suggested that capture sessions be extended if there are < 40 captures per session (c. 25 first captures and 15 within-session recaptures).

Limitations and points to consider

Limitations:

- Sampling was limited to one location and two nested grids.
- Only one of the two datasets contained sufficient captures and recaptures.
- Capture sessions were too short to allow the effects of capture heterogeneity to be evaluated. Consequently, a full robust design analysis was not possible.
- The study provided useful guidelines regarding the minimum numbers of captures and recaptures required for CMR analysis. However, since photo-identification is not done in the field, it would be impossible to know how many animals had been recaptured within a given capture session. This would make it impossible to know whether sampling needed to be extended or not.

Point to consider:

- Some flexibility is required to ensure that capture sessions coincide with a favourable forecast (e.g. in this case, not continuing dry weather).



- The recommended monitoring protocol requires high frog densities (c. 100 animals per 10 m x 10 m grid or 10,000 frogs per hectare). When setting up monitoring in a new area, a pilot study (capture session) may be required to determine whether densities are sufficiently high. If densities are too low, the sampling area (grid size) could be extended or another sampling area chosen.
- A substantial decline in abundance, as seen for Archey's frog in the Coromandel ranges (Bell et al. 2004) could cause populations to decrease to a point where CMR analysis is no longer possible due to insufficient data.
- Because sampling was conducted on only one grid where frog density was known to be high (versus multiple sites that were randomly selected), inference about abundance cannot be extrapolated beyond the sampling grid.
- The CMR analysis included 13 weather covariates, many of which were correlated (e.g. start and finish temperatures). None were selected in the top-ranking models, but could easily have been confounded with time variation. Key variables could have been identified *a priori* through exploratory data analysis (e.g. by generating a correlation matrix or lattice graphs examining relationships between weather variables and uncorrected count data).

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Case study B

Case study B: population estimates from CMR pitfall trapping of common skinks (*Oligosoma polychroma*)

Synopsis

Lettink et al. (2011) used CMR pitfall trapping to obtain population estimates for common skinks (*O. polychroma*) in a valley-floor grassland in the Eglinton Valley, Fiordland. These estimates were then used to test the accuracy and precision of skink counts from artificial retreats (this part of the study is not described here; see Lettink et al. 2011). Skinks were sampled on eight paired grids containing either 25 pitfall traps or 25 artificial retreats spaced 2 m apart in a 5 × 5 pattern. Skinks caught on the pitfall-trapping grids were marked by toe-clipping. Pitfall trapping was done for 8 (December 2007) or 9 (November–December 2009) consecutive days. Huggins closed-capture models were used to estimate population size (\hat{N}) for each grid. The top-ranking model included time, grid, body size (i.e. snout-vent length; SVL) and behavioural effects on capture (p) and recapture (c) probabilities. \hat{N} ranged from 47 (34–89; 95% CI) to 120 (82–214; 95% CI) skinks per grid in 2007. The 2009 mark-recapture data could not be analysed due to insufficient capture and recaptures. Common skink abundance in the Eglinton Valley was high compared to other sites.

Objectives

- To use population size estimates (\hat{N}) obtained by CMR pitfall-trapping to test the accuracy of single-day skink counts from artificial retreats
- To compare population estimates from the Eglinton Valley with other common skink populations

Sampling design and methods

At each of eight randomly selected sites (separated by at least 200 m to ensure their independence), one grid of 25 pitfall traps and one grid of 25 artificial retreats were deployed in identical layouts (spaced 2 m apart in a 5 × 5 pattern, with a 5-m buffer between grids). It was assumed that population sizes did not differ between adjacent grid pairs. Pitfall traps were baited with small (1 cm³) pieces of canned pear (replaced every second day) and checked daily for 8 (December 2007) or 9 (November–December 2009) consecutive days. On their first capture, skinks were uniquely marked by toe-clipping. Any natural toe loss was integrated into the marking system to prevent the unnecessary removal of toes. Toe-clipping was used because temporary pen marks (numbers written on the dorsum) became illegible within 1–6 days of their application in a previous study conducted at the same site.

CMR data were analysed with Huggins closed-capture models (Huggins 1989, 1991) in program MARK (White & Burnham 1999). This estimator allows inclusion of individual covariates, in this case SVL, which had been identified as having a positive influence on capture rate in previous pitfall-trapping studies (i.e. larger lizards had higher capture probabilities than smaller lizards;



Whitaker 1982, Lettink et al. 2010). Capture data from newborn skinks were excluded to avoid violating the assumption of population closure. Factors considered likely to influence capture probabilities of skinks were included in the global or starting model. Briefly, these were time (t), grid (g), body size (SVL) and trap-shyness (denoted b and built into the model by allowing $c < p$ by a constant). The fit of the global model was then compared with alternative models representing less complex parameterisations of p and c , using the model selection guidelines of Burnham & Anderson (2002). Population estimates from the top-ranking model were converted to density estimates (see Lettink et al. 2011 for methods) to enable comparison with data from other common skink populations.

Results

Pitfall trapping yielded 542 captures of 365 individual skinks in 2007 and 148 captures of 141 skinks in 2009. For data from 2007, \hat{N} ranged from 47 (34–89; 95% CI) to 120 (82–214; 95% CI) skinks per grid (Table 2). Density estimates ranged from 3639 (2591–6827; 95% CI) to 9245 (6346–16431) skinks per hectare, which was high compared with other common skink populations nationwide. Low capture and recapture rates in 2009 precluded the use of CMR analysis for data from that year.

Table 2. Numbers of individuals caught, recaptures, total captures and population size estimates (\hat{N}) for common skinks (*Oligosoma polychroma*) on eight pitfall-trapping grids, December 2007, Eglinton Valley. Modified from Lettink et al. (2011).

Grid	Individuals	Recaptures	Total captures	\hat{N}	95% CI (\hat{N})
1	46	10	56	85	61–149
2	46	32	78	53	48–67
3	45	12	57	75	56–122
4	57	10	67	120	82–214
5	38	8	46	69	49–127
6	41	32	73	48	43–62
7	65	66	131	71	67–83
8	27	7	34	47	34–89
Total	365	177	542		

Limitations and points to consider

Limitations:

- Only one of five attempts to obtain population estimates by CMR pitfall trapping was ultimately successful. In this and previous studies conducted at the same site, attempts to increase recapture rates by reducing the trap spacing (from 4 m to 2 m) and by sampling at a warmer time-of-year (mid-summer) failed to yield sufficient data for CMR analysis (Lettink et al. 2011).
- This study did not consider potential differences in c and p between sexes.



Points to consider:

- This study could not have been done without toe-clipping because temporary pen marks became illegible within 1–6 days following their application.
- Without knowing the ecological reasons for the low recapture rates, it is difficult to know how to address this problem, which has also been observed by other researchers conducting CMR studies on terrestrial skinks (e.g. Freeman 1997; Wilson et al. 2007; Jones & Bell 2010). Increasing the size of the sampling grids and/or the number of capture occasions should increase capture numbers, but may not improve recapture rates. A further reduction in trap spacing (from 2 m to 1 m) could increase recapture rates, but this would require a substantial increase in sampling effort without guaranteed results.
- Because the activity of skinks and other herpetofauna is weather-dependent, CMR models should always include a term that allows this variation to be captured (this is usually done by allowing c and p to vary with time).
- Within species, optimal trap spacing may vary as a function of population density and/or habitat structure. A trap spacing of 4–5 m is often used in CMR studies of small terrestrial skinks, but appears to be excessive in some areas (e.g. Wilson et al. 2007; Jones & Bell 2010; Lettink et al. 2011).

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Full details of technique and best practice

There is no generic best-practice approach for obtaining population estimates for herpetofauna, as each species will have its own set of optimal design and analysis parameters. Monitoring protocols (using CMR methods but not necessarily restricted to abundance estimation) have been developed for some species in some areas, e.g. Archey's frog in Whareorino Forest (see '[Case study A](#)'), Hamilton's frog *Leiopelma hamiltoni* on Stephens Island (Pledger 1998), grand and Otago skinks (Reardon et al. in press) and small populations of tuatara on remote islands (Moore et al. 2010). For other species and populations, the following best-practice points apply when designing a study to obtain population estimates:

- Use of the Lincoln-Petersen estimator is not generally recommended.
- Closed-population estimators should be used if abundance is the only parameter of interest (see '[Closed-capture models available in program MARK](#)' below). Although estimation of abundance is possible with some open-population models, such estimates tend to be less precise than those derived from closed-population models because of the extra parameters required to model changes in population size. Also, the abundance estimation component of open-population models is not robust to capture heterogeneity, whereas closed-population estimators are (Kendall 2001).
- The robust design should be used where survival estimates are required in addition to population estimates. This will require greater sampling effort (more capture sessions) than the use of closed-population models. The smallest practical design recommended by Pollock (1982) was three primary periods (e.g. years), each containing five secondary periods (e.g. successive days/nights on which sampling is undertaken). For an example, see Reardon et al. (in press), who used the robust design to monitor grand and Otago skinks.
- The sampling area must be well-defined and remain constant during the study.
- A pilot study and power analysis may be appropriate to determine whether: (a) a sufficient proportion of the target population can be captured and marked; (b) capture and recapture probabilities are sufficient for CMR analysis; and (c) the statistical assumptions are satisfied. In addition, where photo-identification is used, the method will need to be tested prior to use to ensure its accuracy.
- Potential sources of bias and violations of the statistical assumptions must be identified and addressed in the study design (see '[Assumptions of closed-population models](#)' below). This requires a thorough understanding of the ecology of the target species.
- The marking method must not alter the behaviour or survival of marked animals. DOC Animal Ethics Committee (AEC) approval will be required for any study where animals need to be permanently marked (e.g. by toe-clipping or PIT-tagging), unless a standard operating



procedure is developed. AEC approval should be sought at least 6 months prior to the start of the intended study, as the AEC meet infrequently.

- Adequate resources must be made available to allow a sufficient number of individuals to be captured, marked and recaptured over an appropriate time period. For monitoring herpetofauna, Thompson et al. (1998) suggested that the study area should contain at least 100 individuals with capture probabilities of at least 0.3 but preferably above 0.5. It is possible to obtain population estimates for small populations (as few as 20–30 individuals), but this will require higher capture probabilities and/or more sampling periods.
- CMR practitioners should be sufficiently flexible to reschedule sampling to avoid unfavourable forecasts or extend capture sessions if need be.
- Program MARK (White & Burnham 1999) is recommended for analysis of CMR data. Data must be imported in a specific format (see '[Converting capture data to the format required by MARK](#)' below).

Assumptions of closed-population models

Population estimation using closed-population models requires that:

- The population remains closed during the sampling period
- All animals have the same probability of being caught (or factors that underlie variations in capture probability are included in the analysis)
- Marks are not lost or overlooked

Violations to these assumptions will result in biased population estimates. For the first assumption, births are easily dealt with by omitting data for animals born during the sampling period from the analysis. Movement of animals to-and-from the sampling area is more difficult to detect, but can be tested for with program CloseTest (Stanley & Burnham 1999). This Windows program can be downloaded (free of charge) from <http://www.mesc.usgs.gov/Products/Software/ClosTest/>. The test returns a Chi-square statistic that can identify permanent or temporary emigration and immigration. If this is present, closed-population estimators should not be used to estimate abundance. Alternative options are to report count-based indices (e.g. minimum number alive) or to use more complex multi-state models (see Mazerolle et al. 2007 for examples).

The second assumption is commonly violated due to capture heterogeneity, a feature of most animal populations. This has been the driving force behind the development of many different models that allow this assumption to be relaxed (Schwarz & Seber 1999). Models that allow capture probability to vary with time (denoted M_t , where 'M' stands for model), a behavioral (or trap) response (M_b), and heterogeneity (M_h) have been available for some time (Otis et al. 1978). For M_h , heterogeneity is generic (i.e. acknowledged to be present but not attributed to any particular source). Where capture heterogeneity is associated with factors (e.g. age, sex or size class), data should be stratified by attribute group. Inclusion of individual covariates (e.g. SVL or mass) will require the use of Huggins closed-capture models. Data for the same trait measured in a different way (e.g. SVL and body size class) will be correlated and therefore should not be used in the same analysis. In general, it is best to assume that capture heterogeneity will be present and measure potentially relevant variables (e.g. sex, age and/or size) for inclusion in the analysis. Goodness-of-fit



testing is possible within program MARK for models with attribute groups, but not for models that include individual covariates (Cooch & White 2011).

The third assumption is rarely tested. Uncertainties about this assumption can be addressed by double-marking animals (to check whether marks are lost) or by using multiple observers (to check whether marks are overlooked) in a pilot study. Double marking will require use of a permanent marking method. For example, concomitant use of temporary pen marks and toe-clipping revealed that common skinks living in dense grassland in the Eglinton Valley did not retain temporary marks for long enough to enable population estimation by CMR pitfall trapping (Lettink et al. 2011).

Recommended software: program MARK

The most comprehensive software for analysis of CMR data is program MARK (or RMark, which uses a formula-based interface that requires some experience with the R programming language; Cooch & White 2011). It is recommended that novices start with MARK (rather than RMark) because of its intuitive Windows-based interface. While it is possible to teach yourself the basics of CMR modelling by following the comprehensive (900+ page) MARK manual (Cooch & White 2011), intending users will benefit immensely from attending a MARK training course. There is also an on-line help forum (www.phidot.org) that can be searched by topic. If the topic of interest is not already represented among the 5000+ posts on this site, users may submit queries to be answered by other forum members (c. 1500 members at the time of writing), including some of the people that developed the software.

Closed-capture models available in program MARK

MARK currently offers 12 closed-population models that estimate a user-defined combination of the following 'nuisance' parameters (which are considered nuisance parameters because it is usually \hat{N} that is of interest): initial capture probability (p), recapture probability (c), and the proportion of the population with a particular mixture of p 's and c 's (denoted π). The simplest models are the 'closed captures' models of Otis et al. (1978), which estimate only p and c . The most complicated models estimate all three parameters and are known as the heterogeneity mixture models (Pledger 2000). Users also have a choice between models that have abundance in the likelihood (i.e. \hat{N} is a direct output of the model) and those that have abundance conditioned out of the likelihood (the Huggins models, for which \hat{N} is estimated as a derived parameter). The two groups of models are not directly comparable with standard (AIC-based) model selection techniques. Inclusion of individual covariates is only possible with the Huggins closed-capture models (Huggins 1989, 1991).

Converting capture data to the format required by MARK

Program MARK requires data to be imported in a specific format (see also Pryde 2003; Cooch & White 2011). The steps required to create this file are as follows:

1. In an Excel spreadsheet, convert the capture data into binary encounter histories (using one row per animal) denoting whether each animal was caught (1) or not (0) on each capture occasion within a



capture session. For example, a skink with a toe-clip combination of 3530 that was caught on the first, third and last days of a 7-day capture session would have the following encounter history:

3530	101001						
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2. Add any attribute groups, e.g. grid that an animal was caught on. For example, if there were two sampling grids and the skink was caught on grid 1, this would be:

3530	101001	1	0				
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Alternatively, if the skink was caught on the second grid, this would be:

3530	101001	0	1				
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3. Add any individual covariates, scaled between 0 and 1. Individual covariates are used for continuous data, such as body condition indices or size measurements (but not sex or age, which are entered as attribute groups). Snout-vent length (SVL) measurements can be entered as centimetres if there are no individuals with SVLs > 100 mm, otherwise scaling will be required. For example, skink 3530 caught on grid 1 with an SVL of 71 mm would have the encounter history:

3530	101001	1	0	0.71			
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4. Once you have done this for all animals in the dataset, insert columns containing the punctuation required by program MARK (it will not be able to read the data if it is not in this strict format). Your data should now look something like this:

/*	3530	*/	101001	1	0	0.71	;
/*	3525	*/	110010	1	0	0.56	;
/*	3524	*/	100010	0	1	0.68	;

5. You are now ready to save the file in the 'inp' format required by MARK. The easiest way to do this is by saving it as 'NAME OF FILE.INP' and as file type 'FORMATTED TEXT (SPACE DELIMITED)'. The file can now be opened, viewed and analysed in MARK (for further details, refer to Cooch & White 2011).

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Appendix A

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

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| docdm-493179 | Herpetofauna: indices of relative abundance |
| docdm-146272 | Standard inventory and monitoring project plan |