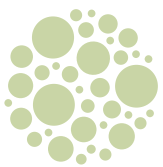

NEW ZEALAND JOURNAL OF ECOLOGY

RESEARCH

Small swabs, small frogs: buccal swabbing for minimally-invasive genetic sampling of *Leiopelma* species

Hadley P. Muller[1]*, Joanne M. Monks[1], Tania M. King[1], Ben D. Bell[2], Nicolas J. Rawlence[1] and Ludovic Dutoit[1]

[1]Department of Zoology, University of Otago, Dunedin, Aotearoa New Zealand
[2]Centre for Biodiversity and Restoration Ecology, Victoria University of Wellington, Wellington, Aotearoa New Zealand
*Author for correspondence (Email: hadleymuller7@gmail.com)

Published online: 12 May 2026

Abstract: Genomics can provide conservation-relevant insights into population size, genetic diversity, and connectivity with relatively little sampling effort. However, for frogs and other small vertebrates, invasive genetic sampling (e.g. toe-clipping) can impact animals' long-term survival and welfare. Minimally-invasive genetic sampling methods may be required to facilitate robust conservation genomic studies of small vertebrates. In Aotearoa | New Zealand, previous genetic studies of the three native *Leiopelma* spp. frogs have relied almost entirely on toe-clips or whole specimens, creating an ethical barrier to genomic studies. Here, we trialled duplicate buccal swabbing as a minimally-invasive genetic sampling method on the small-bodied pepeketua, Hamilton's frog (*Leiopelma hamiltoni*) and Hochstetter's frog (*Leiopelma hochstetteri*). DNA yield was primarily influenced by our duplicate swabbing method rather than by frog body size, condition, or species. The second swab had a significantly higher DNA yield (721 ± 104 ng), more than twice that of the first swab (317 ± 49 ng). We compared the utility of buccal swabs and toe clips for genotyping-by-sequencing (GBS) in Hamilton's frogs and assessed the suitability of buccal swabs for long-range PCR in Hochstetter's frogs. Buccal swabs were as effective as toe-clips for GBS, with the proportion of missing single-nucleotide polymorphisms not significantly different between sampling methods. However, only 7/20 buccal swabs amplified successfully with long-range PCR. Our results indicate that genetic sampling of *Leiopelma* spp. could move towards buccal swabbing as a standard method. Toe-clipping and euthanasia of individuals should be limited to situations where high molecular weight DNA is crucial for analysis (e.g. genome assembly) or whole voucher specimens are needed for taxonomy.

Keywords: amphibians, conservation, conservation genetics, genomics, *Leiopelma hamiltoni*, *Leiopelma hochstetteri*, small vertebrates

Introduction

Small vertebrates often display behavioural crypsis and can be long-lived, making field study and long-term population monitoring difficult (Ferreira et al. 2018; Martin et al. 2022; Stock et al. 2023; Johnson et al. 2024). Compared to field studies, conservation genomics requires relatively little sampling effort, and can provide complementary insights into population structure, dispersal, genetic diversity, and demography (Shafer et al. 2015; Ambu & Dufresnes 2023; Stock et al. 2023; Theissinger et al. 2023; Martin et al. 2024). Conservation genomics of small vertebrates has traditionally relied on invasive genetic sampling methods (e.g. toe-clipping or euthanasia; Zemanova 2019, 2020). Invasive sampling methods likely endure because scientists may perceive an increased reliability for downstream analysis compared to minimally-invasive methods (e.g. buccal swabs or faecal sampling) and may assume that the conservation benefits of the study outweigh any ethical concerns (Zemanova 2019, 2020). However, invasive sampling methods can impart significant stress to animals and impact their welfare or long-term survival (Narayan et al. 2011; Ferreira et al. 2018). Invasive sampling may be particularly inappropriate for endangered or sensitive species, because the negative consequences of invasive sampling can be exacerbated at the population and/or individual level (Ferreira et al. 2018; Zemanova 2019, 2020; Ambu & Dufresnes 2023). Developing efficient, minimally-invasive genetic sampling methods is therefore needed to minimise welfare impacts and permit robust conservation genomic studies of small vertebrates (Shafer et al. 2015; Ferreira et al. 2018; Ambu & Dufresnes 2023; Theissinger et al. 2023).

Frogs are among the most threatened and sensitive of small-vertebrates (Luedtke et al. 2023). Genetic sampling methods for adult frogs include euthanasia, toe-clipping, and buccal or skin swabbing (Germano 2023; Rainey et al. 2024). As for other small vertebrates, invasive sampling is predominant (Zemanova 2019; Ambu & Dufresnes 2023). The effect of toe-clipping on frog survival and movement is

DOI: https://doi.org/10.20417/nzjecol.50.3631

debated. Studies report both neutral and negative long-term effects (Bell & Pledger 2010; Narayan et al. 2011; but see Zamora-Camacho et al. 2023). In the short term, toe-clipping can elicit a significant hormonal stress response (Narayan et al. 2011). Zamora-Camacho et al. (2023) reported no negative effects on jumping distance. Yet, despite the existence of specialised toe-pads in some species (like *Leiopelma* spp.), the long-term effect on other movements, such as walking, climbing, or swimming, has not been evaluated (Waddle et al. 2008; Easton 2018; Zamora-Camacho et al. 2023).

Swabbing methods are minimally invasive (Ambu & Dufresnes 2023; Martin et al. 2024). Skin swabbing involves minimal handling but is almost solely useful for salamanders, with a lower efficacy for frogs (Prunier et al. 2012; Pichlmüller et al. 2013; Easton 2018; Ringler 2018). Frogs' sloughed skin can be dry and covered in secretions which inhibit PCR-based analyses (Melzer et al. 2011; Easton 2018; Ringler 2018). Conversely, buccal swabbing has found wide efficacy as a minimally-invasive genetic sampling method across many frog species (Goldberg et al. 2003; Pidancier et al. 2003; Poschadel & Möller 2004; Gallardo et al. 2012; Easton 2018; Ambu & Dufresnes 2023; Martin et al. 2024; Rainey et al. 2024).

Buccal swabbing was first developed for larger frog species (Goldberg et al. 2003; Pidancier et al. 2003). Its use for small frogs raises ethical questions and doubts about its utility for genomic approaches (Pidancier et al. 2003; Ringler 2018; Rainey et al. 2024). There is a risk of bleeding or injury to the mouth, which may be exacerbated in small anurans (Pidancier et al. 2003; Ringler 2018). Although recent studies have indicated buccal swabbing small anurans is possible (Mudke 2020; Rainey et al. 2024), no specific, practical protocol for swabbing small anurans has been published. A practical guide is especially pertinent for frogs which resist opening their mouths because there is a heightened risk of injury to the lips, tongue, and jaw (Ambu & Dufresnes 2023; Rainey et al. 2024). Furthermore, published studies on small anurans only quantify the efficacy of buccal swabs for PCR-based genetic techniques (e.g. microsatellites, mitochondrial DNA) and quantify DNA yield using spectrophotometry, which can overestimate DNA yield at lower concentrations (Gallardo et al. 2012; Nakayama et al. 2016; Mudke 2020; Martin et al. 2024; Rainey et al. 2024). Case studies of buccal swabbing and its application in genomic studies of small anurans are needed.

Aotearoa | New Zealand has three endemic frog species, all in the genus *Leiopelma* (Burns et al. 2025). Conservation genetic studies of *Leiopelma* have lagged behind those of other endemic herpetofauna, in part because minimally-invasive sampling techniques have not been developed. For New Zealand's endemic lizards, mitochondrial-based phylogenies have provided a basis for modern population genomic studies, largely using tail-tip samples (Chapple et al. 2009; Nielsen et al. 2011; Adams & Angus 2022). Lizard tail tips regenerate, so tail-tipping is generally viewed to be more ethical than toe-clipping (Adams & Angus 2022; Germano 2023). Previous genetic studies of *Leiopelma* almost wholly involve taking toe-clips, which do not regenerate (e.g. Gleeson et al. 2010), or whole specimens (e.g. Fouquet et al. 2010). A single study trialled skin swabbing on kurī peke | Archey's frog (*Leiopelma archeyi*) and pepeketua | Hamilton's frog (*Leiopelma hamiltoni*) but could not reliably amplify nuclear microsatellite markers (Easton 2018). Toe-clipping is permitted by the New Zealand Department of Conservation and is still used for individual identification in mark-recapture of select *Leiopelma* populations in order to maintain long-term population studies (Bell et al. 2004; Bell & Pledger 2010, 2023). A long-term monitoring study has not reported any negative effect of toe-clipping (Bell & Pledger 2010). Nevertheless, within the New Zealand context, the ethical concerns around genetic sampling of *Leiopelma* are particularly pertinent. *Leiopelma* frogs are considered taonga (treasured) species by Māori, the indigenous people of Aotearoa (Bishop et al. 2013; Cisternas et al. 2019). Many iwi (tribes) have an intimate relationship with *Leiopelma* frogs, are heavily involved in their conservation as kaitiaki (guardians) (Cisternas et al. 2019) and are not supportive of invasive genetic sampling.

In the current study, we aimed to establish the efficacy of buccal swabbing as a minimally-invasive genetic sampling method for small anurans, using the terrestrial Hamilton's frog (snout-vent-length [SVL] ≤ 52 mm) and the semi-aquatic pepeketua | Hochstetter's frog (*Leiopelma hochstetteri*; SVL ≤ 47 mm) as a model system (Bell 1978; Bell & Pledger 2023). Specifically, we sought to (1) understand the influence of sampling technique and frog species, body size, and condition on total DNA yield, (2) compare the utility of buccal swabs to toe clips for genotyping-by-sequencing (GBS), and (3) assess the suitability of buccal swabs for long-range PCR of the mitochondrial genome, which presents a useful paradigm to compare buccal swabs to toe-clips. Long-range PCR is considered a more traditional genetic method, and typically requires input fragment lengths of ≥ 10kb, while GBS, a genomic method, has a much shorter input length. However, because mitochondria are present in multiple copies per cell, we expect greater efficacy from long-range PCR for mitochondrial DNA compared with GBS which targets variation across the whole genome (Elshire et al. 2011; Horn 2012).

Methods

Buccal swabbing

Hamilton's frogs were sampled from a translocated population on Motuara Island in the Marlborough Sounds during June 2024 as part of a separate study investigating the outcome of the translocation to Motuara Island (HPM unpubl. data). We searched for emerged Hamilton's frogs by spotlighting a standardised site-occupancy monitoring grid. Twenty-nine juveniles (SVL < 20 mm) and subadults (SVL 20–35 mm) were buccal swabbed.

Twenty Hochstetter's frogs were sampled from four wild, relict populations (five per population), one at Sanctuary Mountain Maungatautari in the Waikato, and three at field sites across Auckland's Rodney District: Moir's Hill, Tamahunga, and Totara Peak, during July 2024. We hand searched potential daytime retreat sites near streams and seepages.

We followed standard protocol for *Leiopelma* frog surveys in New Zealand (Nájera-Hillman et al. 2009). All objects moved during surveys were carefully replaced in the same position as found to reduce habitat disturbance. Frogs were captured with gloved hands and placed into new, partially closed resealable bags. We weighed frogs to the nearest tenth of a gram using a 10 g Pesola balance (Pesola®, Chur, Switzerland). Snout-vent-length was measured to the nearest millimetre by holding a ruler above the frog's body, extended flat against the palm. To minimise the potential spread of disease, new gloves were used for each frog. All equipment was cleaned with F10 veterinary disinfectant (F10® Products, Gauteng, South Africa) between study sites.

We modified published buccal swabbing protocols (Ambu & Dufresnes 2023; Martin et al. 2024) for *Leiopelma* frogs (see Supplementary Material for more detail). Frogs were removed from resealable bags and gently held in one hand, so that the head exited between the thumb and the pointer and middle fingers, with the thumb on the belly. This hold ensured the head did not move during buccal swabbing and prevented Hochstetter's frogs from pushing loose against the palm; in contrast, Hamilton's frogs are placid in-hand. Using the other hand, a sterile 200 μL pipette tip was used to open the mouth. The pipette tip was manoeuvred under the top lip, in the corner of the mouth, to get the frog to chew before inserting the pipette tip further and moving towards the middle of the mouth. Once the mouth was open, the pipette tip was inserted slightly further and gently pressed down to keep the mouth ajar.

A second person then inserted the swab, avoiding the lips as much as possible. We used Copan FLOQSwabs® 516C (flocked swabs) with plastic shafts and a nylon ultra-mini tip to sample small-bodied *Leiopelma* frogs (SVL < 50 mm) (Bell 1978). Swabs were rubbed around the inside of the mouth, focusing on the tongue and cheeks, for 15–30 seconds or until wet. The first swab was then removed before inserting a second swab and collecting another replicate sample for downstream analyses. The 200 μL pipette tip was removed from the mouth after inserting the second swab or once swabbing was complete. Whenever there were any concerns for a frog's well-being, only a single swab was taken. After swabbing, frogs were briefly (about two minutes) returned to resealable bags to check for any injury or aberrant movements. We broke the swabs off into 1.5 mL microcentrifuge tubes containing 70% (Hochstetter's frogs) or 100% (Hamilton's frogs) ethanol. We used ethanol concentrations available in the field at the time but recommend 70% as a standard method. Swabs were stored at ambient temperatures in the field for up to three days and then refrigerated at 4 °C for up to three months before DNA extraction. Research carried out after our sampling has since shown that storage at −20 or −80 °C are preferable options for mid to long-term storage (Martin et al. 2024).

In addition, 55 toe-clip samples were obtained from long-term monitoring studies of three populations of Hamilton's frog: Takapourewa | Stephen's Island (n = 15), Te Pākeka | Maud Island, including the relict population (n = 20) and the translocated population in restored coastal forest at Boat Bay (n = 20). Toe-clips were collected between 2018 and 2024. Toe-clips were frozen and stored in 70% ethanol until extraction.

DNA extraction and quantification

DNA was extracted using a Qiagen™ DNeasy Blood and Tissue Kit. Toe-clips were extracted following the protocol for animal tissue. The following modifications were made for buccal swabs: to remove excess alcohol, swabs were transferred from collection tubes to fresh 1.5 mL microcentrifuge tubes and dried for five minutes at 30 °C in an Eppendorf Concentrator plus™. Swabs were vigorously vortexed with buffer ATL and Proteinase K to aid the release of mucosal cells from flocked swabs. Following overnight incubation, we removed swabs from the digest and took care to recover the digest supernatant from swabs by pressing hard against the walls of the microcentrifuge tube. After the addition of buffer AL, samples were incubated for a further 10 minutes in a MS-100 Thermoshaker Incubator set to 56 °C at 600 rpm. To maximise DNA yield, two separate DNA elutions were performed using buffer AE warmed to 56 °C.

We quantified the DNA yield of buccal swabs using a Qubit™ dsDNA Quantification High Sensitivity Assay Kit following the manufacturer's instructions. Samples below the detection level of the kit (< 0.005 ng μL^{-1}) were treated as zero values and were not used for further genetic analysis. To obtain total yield for each swab, Qubit™ concentrations were multiplied by elution volume (200 μL).

Genotyping by sequencing

Hamilton's frog buccal swabs were prepared for GBS analysis alongside toe-clips. Library preparation followed Elshire et al. (2011), with modifications from Dussex et al. (2016). Samples were assigned unique DNA indexes, and the pooled library was size-selected (200–600 bp) and sequenced at AgResearch Invermay on an Illumina Novaseq with a lane of an S4 flow cell alongside other libraries.

We called single-nucleotide polymorphisms (SNPs) using Stacks v. 2.61 (Catchen et al. 2013; Rochette et al. 2019). In short, adapter sequences and low-quality reads were identified using FASTQC v. 0.12.1 (Andrews 2010) and removed by CUTADAPT v. 4.4 (Martin 2011). Stacks module *process_radtags* was used to demultiplex sequence data. To assemble loci, and call SNPs de novo (i.e. without a reference genome), we used the *denovo_map.pl* pipeline. To address computational constraints of clustering a large number of sequences, we limited each sample to a maximum of 5 million reads. The number of reads per sample was a reflection of the amount of DNA that was sent to sequencing after concentration was normalised across samples, not a reflection of sample quality. Parameters controlling *denovo_map.pl* were optimised for the highest number of polymorphic loci across 80% of samples (r80 rule): n = M = 2 and m = 3 (Paris et al. 2017). A single frog (sampled via buccal sampling) was excluded from further analysis with > 60% missing data. For subsequent analyses, only the first SNP was kept from each locus, and the minimum coverage depth was set to five.

Long-range PCR of mitogenomes

We attempted to amplify the whole-mitogenome of Hochstetter's frog from buccal swabs using long-range PCR in two overlapping fragments (Table 1). Long-range PCR was performed using PrimeSTAR® GXL DNA Polymerase (*TaKaRa*). Each 25 μL PCR reaction contained 1X PrimeSTAR® GXL buffer, 0.2 mM of each dNTP, 0.625 U of PrimeSTAR® GXL DNA Polymerase, 0.2 μM of each primer, and 50–100 ng of genomic DNA. Following PCR, we visualised amplification products using agarose gel electrophoresis. Each sample was run on a 1% agarose gel stained with SYBR safe (Invitrogen), alongside GeneRuler DNA ladder Mix (Thermo Fisher).

Statistical analysis

All analyses, graphing, and model checking were completed in R v. 4.5.0 (R Core Team 2025). To understand the effect of frog biology and sampling methods on DNA yield (ng), we ran a linear mixed model, using the package "lme4" (Bates et al. 2015). A fourth root transformation of DNA yield was utilised to improve normality because the DNA yield (ng) was highly left-skewed and contained zero values. Fixed predictor variables included species, snout-vent-length, body condition (see below), swab number (taken first or second), and time in days from collection until extraction. Not all buccal swabs

Table 1. List of long-range PCR primer pairs used to amplify the Hochstetter's frog (*Leiopelma hochstetteri*) mitogenome, including fragment length (bp) and cycling conditions (″ = minutes, ′ = seconds).

Primer	Primer sequence	Fragment length (bp)	Cycling conditions
ATP_F	AGTTAAACCCAGGCCCATGAC	c. 7000	(98 °C for 15″ → 62 °C for 15″ → 68 °C for 12′) *27 cycles
Thr_NC	GGCTTACAAGGYCGGTGC		
Cytb_F	CAAATTGCCTCAATYACCTACTTCTC	c. 10 000	(98 °C for 15″ → 60 °C for 15″ → 68 °C for 12′) *40 cycles
ATP_R	GGGCTCATTAATKGGYTGGTG		

were extracted at the same time; therefore, time until extraction was included to understand whether buccal swab DNA was degrading in storage. 'Frog ID' was included as a random effect to address non-independence between first and second swabs from the same individual.

Body condition was measured as the scaled mass index (SMI) to account for allometric growth (Peig & Green 2009) and because it accurately predicts individual health and fitness in frogs (Brodeur et al. 2020). SMI was calculated using equation (1):

$$SMI\colon \widehat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^b \qquad (1)$$

where $\widehat{M}_i$ is the body condition (scaled weight) for individual i, M_i and L_i are the measured body mass and SVL of individual i respectively, L_0 is the mean SVL value of the study population and b is the scaling exponent which determines the dimensional balance between mass and SVL (Peig & Green 2009; Brodeur et al. 2020). We calculated b as the slope of a non-linear regression of mass and SVL. For herpetofauna, this method ensures that SMI is independent of body size (Brodeur et al. 2020). L_0 and b were determined independently for Hamilton's and Hochstetter's frogs to account for different growth patterns between species (Peig & Green 2010).

Model validation was completed by checking residual plots and using the "car" and "lmtest" packages (Zeileis & Hothorn 2002; Fox & Weisberg 2019). Residual plots and the Breusch-Pagan and Shapiro tests indicated a good fit for the model. P-values, confidence intervals, standard errors, and pseudo-R^2 values were calculated using the "lmtest" and "MuMIn" packages (Zeileis & Hothorn 2002; Barton 2009).

Additionally, we compared GBS sequence data from Hamilton's frog buccal swabs and toe-clips. VCFtools v. 0.1.15 (Danecek et al. 2011) output option *–depth* provided the total number of sites and *--missing-indv* the proportion of missing SNPs for each individual. Mean values of the total number of loci and proportion of missing sites for buccal swabs and toe-clips were compared via a Welch's t-test accounting for unequal variance. Graphs were produced with the package "ggplot2" (Wickham 2016).

Results

We buccal swabbed a total of 29 Hamilton's frogs (mean SVL = 27.1 mm) and 20 Hochstetter's frogs (mean SVL = 35.8 mm), resulting in 53 individual swabs from Hamilton's frogs, and 39 from Hochstetter's frogs. Singular swabs were taken from five Hamilton's and one Hochstetter's frog. *Leiopelma* frogs are known to have a stress response to overheating, which causes them to lose their righting reflex (J. Germano, Department of Conservation, Nelson, pers. comm.). This overheating can occur with extensive handling by researchers with warm hands and was observed in two Hamilton's frogs and one Hochstetter's frog. After regaining their righting response, we continued to observe frogs for a few minutes to ensure they were active before release.

DNA yield per swab was highly variable. Swab number was the only variable with a significant effect, with the second swab having more than twice the DNA yield of the first swab (Fig. 1). Swab one had a DNA yield (mean ± standard error) of 317 ± 49 ng and swab two of 721 ± 104 ng, a mean percentage difference of 127%. The fixed effects alone explained a relatively low proportion of the variance in the data ($R^2_{marginal} = 0.252$), while the inclusion of both fixed and random effects increased the explained variance ($R^2_{conditional} = 0.365$). Frog characteristics, including SVL (Appendix S2 in the Supplementary Material), SMI, and species had little effect on DNA yield, with confidence intervals overlapping with zero (Table 2).

DNA yields were sufficient for downstream genetic analysis, including both long-range PCR, and high-throughput GBS techniques. DNA yield per swab was 389 ± 63 ng (mean ± standard error) for Hamilton's frogs and 663 ± 105 ng for Hochstetter's frog. During GBS library preparation of Hamilton's frog swabs, it was often necessary to pool first elutions extracted from both swabs, and sometimes all elutions (taken from a single frog), to reach (or approach) the 500 ng of DNA called for by these laboratory methods. However, frogs with yields as low as 100 ng still produced sufficient SNP data after sequencing. Our results indicate that buccal swabs from *Leiopelma* were as effective as toe-clips for GBS analyses (Fig. 2). The GBS dataset resulted in a total of 21 250 variant sites for downstream analyses. The total number of sites was not significantly different between toe-clips and buccal swabs ($t = −0.35$, $df = 45.44$, $p = 0.72$) and nor was the proportion of missing SNPs ($t = 0.59$, $df = 49.42$, $p = 0.56$).

Buccal swabs were only partially successful for long-range PCR. Despite the buccal swabs appearing to have sufficient DNA concentration, mitogenome amplification was successful for only 7/20 Hochstetter's frogs. Amplification success was correlated with concentration, working only for those seven frogs with the highest concentration from a single swab.

Discussion

Although buccal swabbing has become a popular genetic sampling method for frogs, concerns persist surrounding its application to genomics and the ethics of swabbing smaller

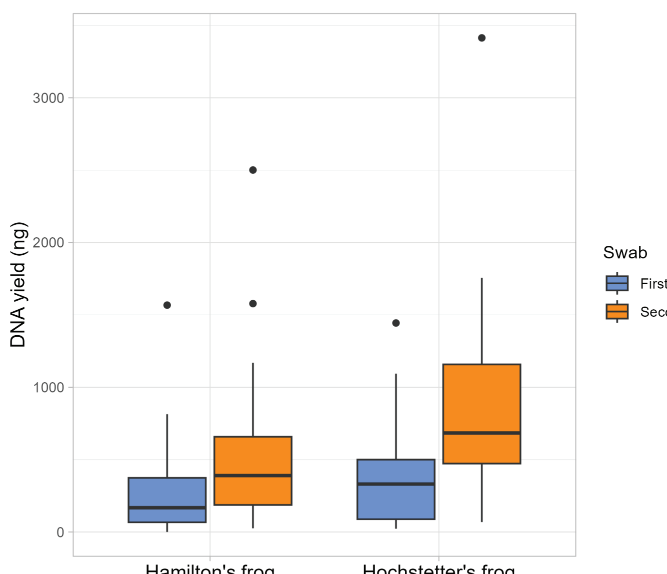

Figure 1. Boxplot showing total DNA yield in ng from the first and second swabs collected from Hamilton's (*Leiopelma hamiltoni*) and Hochstetter's (*L. hochstetteri*) frogs. Boxes encompass the middle 50% of data, with the middle line representing the median; whiskers show minimum and maximum values, excluding the outliers shown as dots. The second swab had a significantly higher DNA yield than the first swab ($p < 0.001$).

Table 2. Model coefficients of the fixed effects in the linear mixed model, with the fourth root of the total DNA yield as the response variable. Hochstetter's frog (*Leiopelma hochstetteri*) is compared to Hamilton's frog (*L. hamiltoni*), and the second swab to the first.

Fixed Effect	Coefficient	Standard Error	2.5% Interval	97.5% Interval	T-value	P-value
Species Hochstetter's	0.595	0.709	−0.759	1.103	0.839	0.406
Scaled Mass Index	0.143	0.201	−0.24	0.528	0.714	0.480
Snout-vent-length	−0.029	0.027	−0.081	0.022	−1.095	0.278
Swab number	1.148	0.294	0.565	1.72	3.909	< 0.001
Time to extraction	−0.002	0.005	−0.012	0.008	−0.359	0.721

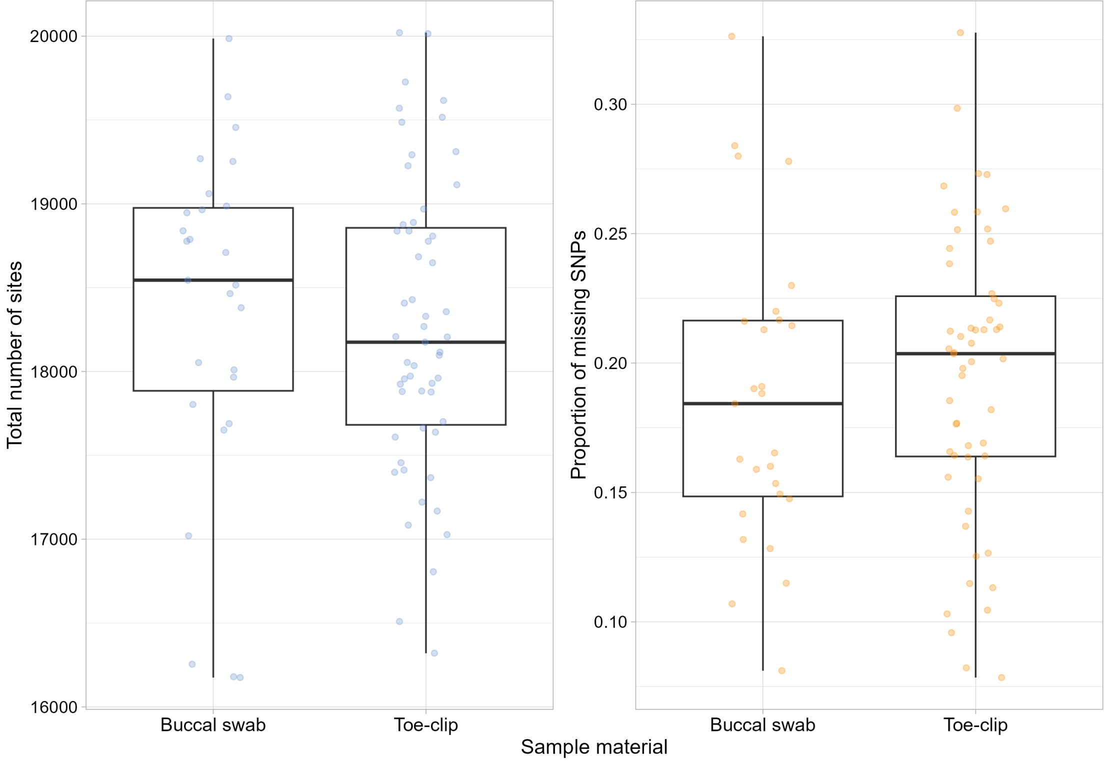

Figure 2. Boxplots showing the total number of variable sites (left) and the proportion of missing single-nucleotide-polymorphisms (SNPs; right) of individual Hamilton's frogs (*Leiopelma hamiltoni*), compared between buccal swab ($n = 28$) and toe-clip ($n = 55$) samples. Neither the total number of sites ($p = 0.72$) or the proportion of missing SNPs ($p = 0.56$) were significantly different. Boxes encompass the middle 50% of data, with the middle line representing the median; whiskers show minimum and maximum values.

species (Pidancier et al. 2003; Ringler 2018; Ambu & Dufresnes 2023; Rainey et al. 2024). Previous studies on small frogs have trialled buccal swabbing for PCR-based genetic analyses and quantified DNA yield using spectrophotometry, a method known to be inaccurate at low concentrations (Gallardo et al. 2012; Nakayama et al. 2016; Mudke 2020; Martin et al. 2024; Rainey et al. 2024). To encourage the uptake of, and collaboration on, conservation genomic projects by practitioners, researchers, and indigenous communities, we have shown buccal swabs are effective for population genomic analysis using GBS, and as such, have included a clear buccal swabbing protocol for *Leiopelma* frogs in Appendix S1 of the Supplementary Material.

Buccal swabs had a low yet highly variable DNA yield, which was similar to that of larger frog species (Martin et al. 2024). DNA yield was seemingly not influenced by frog species, body size, or body condition. Instead, variation in DNA yield was driven by swab order, with the second swab having more than twice the DNA yield of the first swab. Yet, both swabs were collected using the same standardised technique. The higher DNA yield of the second swab probably reflects the removal of surface moisture and mucus by the first swab, thereby increasing friction and promoting epithelial cell detachment during the second swabbing, rather than differences in handling or technique (Poschadel & Möller 2004). Swabs were only in the mouth for 15–30 seconds, whereas for larger Anurans swabs can be in the mouth for up to a minute (Goldberg et al. 2003). Either a single longer swab (Goldberg et al. 2003) or a second duplicate swab may produce higher DNA yields. Previous research has already revealed swabbing technique can drive variation in DNA yield; "gentle swabbing", whereby inexperienced handlers are overly cautious, results in low DNA yield (Ambu & Dufresnes 2023; Rainey et al. 2024). Although a single swab left in the mouth for up to a minute may be efficient for larger anurans (Goldberg et al. 2003), duplicate swabbing is useful for *Leiopelma*, in spite of the lower yields of the first swab. *Leiopelma* are prone to heat stress during handling (Germano 2023), and given the small size of their mouths, swabs can become contaminated by touching the skin. Duplicate swabbing allows frogs to be returned to a partially closed resealable bag if concerns for their well-being arise, or if the frog grabs (thus contaminating) the swab, allowing the frog to recover before deciding whether to take a second swab.

Our SNP dataset had ample variable sites for downstream population genomic analysis (Shafer et al. 2015; Theissinger et al. 2023). Buccal swabs from *Leiopelma* were as effective as toe-clips for GBS with no significant difference in the total number of sites or proportion of missing SNPs (Fig. 2). Comparatively, buccal swabs were only partially successful for long-range PCR of the mitogenome. The specific long-range PCR method used here required DNA fragments 7 kb or longer for successful amplification, and the primer pair Cytb-F / ATP- R (Table 1.) spans a large duplication in the Hochstetter's frog mitogenome (Carr et al. 2015). Conversely, GBS requires a shorter input length. Our results indicate that DNA from buccal swabs is representative of the entire genome but is somewhat fragmented, particularly for samples with lower DNA yields. Compared to the current study, more careful preservation, i.e. storing swab samples at −20 °C or −80 °C (Martin et al. 2024), amplifying the mitochondrial genome over several shorter fragments (Irisarri et al. 2010), or using hybridisation-capture techniques (Horn 2012) would potentially enable recovery of the mitogenome. Our DNA extraction method could also be improved. Additional tests in the lab demonstrated that, following overnight incubation, swabs can be removed from the digest and transferred to a 0.5 mL tube with a small hole pierced in the bottom using an 18-gauge needle, nested within a 1.5 mL microcentrifuge tube. After centrifuging at 8000 rpm for 2 minutes, an additional c. 20 μL of digestion supernatant can be recovered. We also vortexed swabs vigorously to aid the release of cells, but overnight incubation may be sufficient alone. Replacing any further vortexing steps with gentle pipetting may also help to minimise shearing of high molecular weight. Regardless, we recovered sufficient DNA yield from buccal swabs of small frogs for modern conservation nuclear genomic protocols.

Levering open the mouth is the most delicate step in the field (Rainey et al. 2024). Special care needs to be taken for smaller frogs, especially for *Leiopelma* that are resistant to opening their mouth. Because the buccal cavity is so small, care needs to be taken not to aggravate the lips or inadvertently touch the underside of the eyes with the pipette tip. For juvenile frogs, incomplete ossification of the jaw exacerbates these issues (Easton 2018). However, once the mouth is open, using a medical, flocked swab with a small nylon tip makes swabbing simple (Ambu & Dufresnes 2023; Martin et al. 2024; Rainey et al. 2024). We observed Hamilton's frogs as small as 18 mm SVL moving immediately after swabbing and showing no visible signs of stress or discomfort. Buccal swabbing such small frogs without injury is relatively easy, but researchers should explicitly justify the need to swab juvenile *Leiopelma* to reduce the risk of harm.

We did, however, record three frogs that became limp during the swabbing procedure. *Leiopelma* frogs are susceptible to heat-stress and going limp is a common response to any extended periods of handling, particularly with warm hands (Germano 2023). *Leiopelma* usually recover their righting response quickly when immediately placed in a cool (≤ 16 °C) environment before being released (Germano 2023). To avoid heat stress, we strongly suggest that any handlers (1) utilise cool river stones or ice packs to cool their hands before swabbing *Leiopelma*, and (2) practice several times. In New Zealand, the invasive brown tree frogs (*Rawlinsonia ewingii*) are common, and practical for learning buccal swabbing of *Leiopelma* given their small size, active temperament in the hand (compared to *Leiopelma*) and semi-aquatic habitat (slippery in hand). Furthermore, practice on brown tree frogs would avoid so called "gentle swabbing", improving DNA yield (Ambu & Dufresnes 2023).

Genomics could be a crucial facet of *Leiopelma* conservation management and overcome the difficulties faced by traditional field-based studies (Shafer et al. 2015; Stock et al. 2023). *Leiopelma* frogs are sit-and-wait predators, which display behavioural crypsis (Bell 1978; Bishop et al. 2013; Reilly et al. 2015). Field studies of *Leiopelma* frogs can struggle to accurately monitor populations beyond single count surveys (Nájera–Hillman et al. 2009; Johnson et al. 2024). Such monitoring difficulties are especially apparent for the widespread Hochstetter's frog (Bishop et al. 2013; Johnson et al. 2024), for which mark-recapture has been unsuccessful and long-term monitoring datasets fail to confidently estimate population trends (Bell & Pledger 2023; Johnson et al. 2024). Conservation genomic methods would require only a simple daytime survey of Hochstetter's frog habitat, but provide crucial information on effective population size, genetic diversity, and connectivity (Bell 1996; Shafer et al. 2015; Stock et al. 2023; Theissinger et al. 2023). Hochstetter's frogs are thought to be in decline across New Zealand, and it is unknown whether they

disperse overland between catchments (Bishop et al. 2013; Burns et al. 2025). Effective conservation prioritisation across Hochstetter's frog's eleven evolutionary-significant-units, without an adequate understanding of their rate of decline or population connectivity, is near impossible (Gleeson et al. 2010; Johnson et al. 2024). To date, large conservation genomic studies on *Leiopelma* have, at least in part, been prevented by a lack of minimally-invasive sampling techniques, making ethical permissions and iwi approval complex (Cisternas et al. 2023). The fact that buccal swabbing is adequate for population genomic research on *Leiopelma* frogs should encourage its uptake in future conservation management (Taylor et al. 2017; Zemanova 2020; Ambu & Dufresnes 2023).

Currently, toe-clipping is the standard for genetic sampling (Germano 2023), but this presents ethical issues for *Leiopelma*. No effects on survival have been detected from mark-recapture studies using toe-clipping to date (Waddle et al. 2008; Bell & Pledger 2010; Zamora-Camacho et al. 2023). However, the effects of toe-clipping on *Leiopelma* locomotion, especially the arboreal behaviour observed in Archey's and Hamilton's frog, are yet to be assessed (Altobelli et al. 2021; Cisternas et al. 2023; Zamora-Camacho et al. 2023). Hamilton's frogs have mushroom-like expansions on their phalanges for climbing (Clarke 2007; Easton 2018). Further, toe-clipping protocols in New Zealand do not restrict clipping of the longest fourth toe, despite its involvement in frog behaviours such as moulting (Germano 2023; Zamora-Camacho et al. 2023). Because toe-clipping could impede movement or moulting, future research should investigate whether toe-clipping has any long-term behavioural or movement effects in *Leiopelma* or other walking and/or arboreal frogs (Waddle et al. 2008).

We suggest that scientists move towards buccal swabbing as a standard method for genetic sampling of frogs. Toe-clipping and euthanasia should be limited to situations where long DNA fragments (and high concentrations) are key to successful analysis (e.g. genome assembly) or voucher/type specimens are needed for taxonomy. Where possible, within the research questions being addressed, the use of ethanol-preserved legacy collections housed in museums and universities should be investigated as well. Unless researchers can explicitly justify the need for toe-clipping, buccal swabbing should be preferred (Zemanova 2019; Martin et al. 2024). Buccal swabbing can provide genome-wide insights suitable for population genomic questions that have remained unresolved using PCR-based genetic approaches in *Leiopelma* frogs (Fouquet et al. 2010; Gleeson et al. 2010; Shafer et al. 2015; Easton 2018; Theissinger et al. 2023).

Acknowledgements

We acknowledge the support of the following Māori iwi: Ngāti Koata, Ngāti Kuia, Te Ātiawa, Rangitāne, Ngāti Apa, and Ngāti Manuhiri. Also, thank you to Johan Schuerch for guidance with the sampling protocol. We are grateful to Tobia Dale, Angus Sonny Davis, Harriet Wills, Sabine Melzer, Janelle Ward, and especially Luke Easton, for field assistance. Lastly, we thank the anonymous reviewer for their comments and advice.

Additional information and declarations

Conflicts of interest: The authors declare no conflicts of interest.

Funding: Funding for this study was provided by the University of Otago and a Society for Research of Amphibians and Reptiles New Zealand Herpetological Research Award.

Ethics: Buccal swabbing was carried out under ethical approval from the University of Otago Animal Ethics Committee under permit number AUP-23-110. Victoria University of Wellington authorised toe-clipping as part of a long-term mark recapture study under approval 30172.

Data availability: Data and code are available from the corresponding authors upon request.

Author contributions: HM and LD conceptualised the project and developed the methods. HM, TK, and BB conducted data collection. HM, LD, and JM worked on the analysis. HM wrote the original manuscript. NR, TK, BB, LD, JM and HM reviewed and edited the final manuscript.

References

Adams L, Angus I 2022. Tissue sampling for reptiles SOP. doc-6743426. Wellington, Department of Conservation.

Altobelli JT, Lamar SK, Bishop PJ 2021. Archaic, terrestrial Hamilton's frogs *Leiopelma hamiltoni* display arboreal behaviours. New Zealand Journal of Ecology 45(2): 3442.

Ambu J, Dufresnes C 2023. Buccal swabs for amphibian genomics. Amphibia-Reptilia 44(2): 249–255.

Andrews S 2010. FastQC: A quality control tool for high throughput sequence data. Version 0.12.0 https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Barton K 2009. MuMIn: multi-model inference. R package version 1.48.4 http://r-forge.r-project.org/projects/mumin/.

Bates D, Mächler M, Bolker B, Walker S 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1): 1–48.

Bell BD 1978. Observations on the ecology and reproduction of the New Zealand Leiopelmid frogs. Herpetologica 34(4): 340–354.

Bell BD 1996. Aspects of the ecological management of New Zealand frogs: conservation status, location, identification, examination and survey techniques. Ecological Management 4: 91–111.

Bell BD, Pledger SA 2010. How has the remnant population of the threatened frog *Leiopelma pakeka* (Anura: Leiopelmatidae) fared on Maud Island, New Zealand, over the past 25 years? Austral Ecology 35(3): 241–256.

Bell BD, Pledger SA 2023. Post-metamorphic body growth and remarkable longevity in Archey's frog and Hamilton's frog in New Zealand. New Zealand Journal of Ecology 47(2): 3583.

Bell BD, Carver S, Mitchell NJ, Pledger S 2004. The recent decline of a New Zealand endemic: how and why did populations of Archey's frog *Leiopelma archeyi* crash over 1996–2001? Biological Conservation 120(2): 189–199.

Bishop PJ, Daglish LA, Haigh A, Marshall LJ, Tocher M, McKenzie KL 2013. Native frog (*Leiopelma* spp.) recovery plan, 2013–2018. Wellington, Department of Conservation.

Brodeur JC, Damonte MJ, Candioti JV, Poliserpi MB, D'Andrea MF, Bahl MF 2020. Frog body condition: basic assumptions, comparison of methods and characterization of natural variability with field data from *Leptodactylus*

latrans. Ecological Indicators 112: 106098.

Burns RJ, Armstrong DP, Bell BD, Haigh A, Germano JM, Rawlence NJ, Thurley T, Hitchmough RA, Makan T, Michel P 2025. Conservation status of amphibians in Aotearoa New Zealand, 2024. Wellington, Department of Conservation.

Carr LM, McLenachan PA, Waddell PJ, Gemmel NJ, Penny D 2015. Analyses of the mitochondrial genome of *Leiopelma hochstetteri* argues against the full drowning of New Zealand. Journal of Biogeography 42(6): 1066–1076.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA 2013. Stacks: an analysis tool set for population genomics. Molecular Ecology 22(11): 3124–3140.

Chapple DG, Ritchie PA, Daugherty CH 2009. Origin, diversification, and systematics of the New Zealand skink fauna (Reptilia: Scincidae). Molecular Phylogenetics and Evolution 52(2): 470–487.

Cisternas J, Wehi PM, Haupokia N, Hughes F, Hughes M, Germano JM, Longnecker N, Bishop PJ 2019. 'Get together, work together, write together': a novel framework for conservation of New Zealand frogs. New Zealand Journal of Ecology 43(3): 3392.

Cisternas J, Easton LJ, Bishop PJ 2023. Use of dead tree-fern trunks as oviposition sites by the terrestrial breeding frog *Leiopelma archeyi*. New Zealand Journal of Ecology 47(2): 3533.

Clarke B 2007. Comparative morphology and amphibian taxonomy: an example from the osteology of Discoglossoid frogs. In: Heatwole H, Tyler M eds. Amphibian biology: systematics. NSW, Australia, Surrey Beatty & Sons. Pp. 2465–2603.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST 2011. The variant call format and VCFtools. Bioinformatics 27(15): 2156–2158.

Dussex N, Chuah A, Waters JM 2016. Genome-wide SNPs reveal fine-scale differentiation among wingless alpine stonefly populations and introgression between winged and wingless forms. Evolution 70(1): 38–47.

Easton L 2018. Taxonomy and genetic management of New Zealand's *Leiopelma* frogs. Unpublished PhD thesis, University of Otago, Dunedin, New Zealand.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6(5): e19379.

Ferreira CM, Sabino-Marques H, Barbosa S, Costa P, Encarnação C, Alpizar-Jara R, Pita R, Beja P, Mira A, Searle JB 2018. Genetic non-invasive sampling (gNIS) as a cost-effective tool for monitoring elusive small mammals. European Journal of Wildlife Research 64(4): 46.

Fouquet A, Green DM, Waldman B, Bowsher JH, McBride KP, Gemmell NJ 2010. Phylogeography of *Leiopelma hochstetteri* reveals strong genetic structure and suggests new conservation priorities. Conservation Genetics 11(3): 907–919.

Fox J, Weisberg S 2019. An R companion to applied regression 3rd edition. R package version 3.1-3 https://www.john-fox.ca/Companion/.

Gallardo C, Correa C, Morales P, Sáez P, Pastenes L, Méndez M 2012. Validation of a cheap and simple non-destructive method for obtaining AFLPs and DNA sequences (mitochondrial and nuclear) in amphibians. Molecular Ecology Resources 12(6): 1090–1096.

Germano JM 2023. Toe clipping for native frogs SOP. doc-7277708. Wellington, Department of Conservation.

Gleeson D, Clay C, Gemmell N, Howitt R, Haigh A 2010. Summary report: *Leiopelma hochstetteri* population genetic structure. Auckland, New Zealand, Manaaki Whenua Landcare Research.

Goldberg CS, Kaplan ME, Schwalbe CR 2003. From the frog's mouth: buccal swabs for collection of DNA from amphibians. Herpetological Review 34(3): 220.

Horn S 2012. Target enrichment via DNA hybridization capture. In: Shapiro B, Hofreiter M eds. Ancient DNA: methods and protocols. New York, Humana Press. Pp. 177–188.

Irisarri I, San Mauro D, Green DM, Zardoya R 2010. The complete mitochondrial genome of the relict frog *Leiopelma archeyi*: insights into the root of the frog tree of life. Mitochondrial DNA 21(5): 173–182.

Johnson CE, Herbert SM, Gilbert J, Armstrong DP 2024. A comparison of methods for estimating abundance of unmarked Hochstetter's frogs. New Zealand Journal of Ecology 48(1): 3572.

Luedtke JA, Chanson J, Neam K, Hobin L, Maciel AO, Catenazzi A, Borzée A, Hamidy A, Aowphol A, Jean A, Sosa-Bartuano Á, Fong GA, de Silva A, Fouquet A, Angulo A, Kidov AA, Muñoz Saravia A, Diesmos AC, Tominaga A, Shrestha B, Gratwicke B, Tjaturadi B, Martínez Rivera CC, Vásquez Almazán CR, Señaris C, Chandramouli SR, Strüssmann C, Cortez Fernández CF, Azat C, Hoskin CJ, Hilton-Taylor C, Whyte DL, Gower DJ, Olson DH, Cisneros-Heredia DF, Santana DJ, Nagombi E, Najafi-Majd E, Quah ESH, Bolaños F, Xie F, Brusquetti F, Álvarez FS, Andreone F, Glaw F, Castañeda FE, Kraus F, Parra-Olea G, Chaves G, Medina-Rangel GF, González-Durán G, Ortega-Andrade HM, Machado IF, Das I, Dias IR, Urbina-Cardona JN, Crnobrnja-Isailović J, Yang J-H, Jianping J, Wangyal JT, Rowley JJL, Measey J, Vasudevan K, Chan KO, Gururaja KV, Ovaska K, Warr LC, Canseco-Márquez L, Toledo LF, Díaz LM, Khan MMH, Meegaskumbura M, Acevedo ME, Napoli MF, Ponce MA, Vaira M, Lampo M, Yánez-Muñoz MH, Scherz MD, Rödel M-O, Matsui M, Fildor M, Kusrini MD, Ahmed MF, Rais M, Kouamé NGG, García N, Gonwouo NL, Burrowes PA, Imbun PY, Wagner P, Kok PJR, Joglar RL, Auguste RJ, Brandão RA, Ibáñez R, von May R, Hedges SB, Biju SD, Ganesh SR, Wren S, Das S, Flechas SV, Ashpole SL, Robleto-Hernández SJ, Loader SP, Incháustegui SJ, Garg S, Phimmachak S, Richards SJ, Slimani T, Osborne-Naikatini T, Abreu-Jardim TPF, Condez TH, De Carvalho TR, Cutajar TP, Pierson TW, Nguyen TQ, Kaya U, Yuan Z, Long B, Langhammer P, Stuart SN 2023. Ongoing declines for the world's amphibians in the face of emerging threats. Nature 622(7982): 308–314.

Martin M 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. Journal 17(1): 10–12.

Martin ME, Delheimer MS, Moriarty KM, Early DA, Hamm KA, Pauli JN, McDonald TL, Manley PN 2022. Conservation of rare and cryptic species: challenges of uncertainty and opportunities for progress. Conservation Science and Practice 4(11): e12809.

Martin R, Mullin K, White N, Grimason N, Jehle R, Wilkinson J, Orozco-terWengel P, Cunningham A, Maddock S 2024. Optimising recovery of DNA from minimally invasive sampling methods: efficacy of buccal swabs, preservation strategy and DNA extraction approaches for amphibian

studies. Ecology and Evolution 14(9): e70294.
Melzer S, Clerens S, Bishop PJ 2011. Differential polymorphism in cutaneous glands of archaic *Leiopelma* species. Journal of Morphology 272(9): 1116–1130.
Mudke MM 2020. First report on the fossorial tadpole of *Micrixalus kottigeharensis* (Rao, 1937). Herpetology Notes 13: 645–648.
Nájera-Hillman E, King P, Baird AC, Breen BB 2009. Effect of pest-management operations on the abundance and size-frequency distribution of the New Zealand endemic frog *Leiopelma hochstetteri*. New Zealand Journal of Zoology 36(4): 389–400.
Nakayama Y, Yamaguchi H, Einaga N, Esumi M 2016. Pitfalls of DNA quantification using DNA-binding fluorescent dyes and suggested solutions. PLoS One 11(3): e0150528.
Narayan EJ, Molinia FC, Kindermann C, Cockrem JF, Hero J-M 2011. Urinary corticosterone responses to capture and toe-clipping in the cane toad (*Rhinella marina*) indicate that toe-clipping is a stressor for amphibians. General and Comparative Endocrinology 174(2): 238–245.
Nielsen SV, Bauer AM, Jackman TR, Hitchmough RA, Daugherty CH 2011. New Zealand geckos (Diplodactylidae): cryptic diversity in a post-Gondwanan lineage with trans-Tasman affinities. Molecular Phylogenetics and Evolution 59(1): S1055790310004872.
Paris JR, Stevens JR, Catchen JM 2017. Lost in parameter space: a road map for stacks. Methods in Ecology and Evolution 8(10): 1360–1373.
Peig J, Green AJ 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118(12): 1883–1891.
Peig J, Green AJ 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Functional Ecology 24(6): 1323–1332.
Pichlmüller F, Straub C, Helfer V 2013. Skin swabbing of amphibian larvae yields sufficient DNA for efficient sequencing and reliable microsatellite genotyping. Amphibia-Reptilia 34(4): 517–523.
Pidancier N, Miquel C, Miaud C 2003. Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. Herpetological Journal 13(4): 175–178.
Poschadel JR, Möller D 2004. A versatile field method for tissue sampling on small reptiles and amphibians, applied to pond turtles, newts, frogs and toads. Conservation Genetics 5: 865–867.
Prunier J, Kaufmann B, Grolet O, Picard D, Pompanon F, Joly P 2012. Skin swabbing as a new efficient DNA sampling technique in amphibians, and 14 new microsatellite markers in the alpine newt (*Ichthyosaura alpestris*). Molecular Ecology Resources 12(3): 524–531.
R Core Team 2025. R: A language and environment for statistical computing. Version 4.5.0. Vienna, Austria, R Foundation for Statistical Computing. http://www.R-project.org/.
Rainey TA, Tryc EE, Nicholson KE 2024. Comparing skin swabs, buccal swabs, and toe clips for amphibian genetic sampling, a case study with a small anuran (*Acris blanchardi*). Biology Methods and Protocols 9(1): bpae030.
Reilly S, Essner Jr R, Wren S, Easton L, Bishop PJ 2015. Movement patterns in leiopelmatid frogs: insights into the locomotor repertoire of basal anurans. Behavioural Processes 121: 43–53.
Ringler E 2018. Testing skin swabbing for DNA sampling in dendrobatid frogs. Amphibia-Reptilia 39(2): 245–251.
Rochette NC, Rivera-Colón AG, Catchen JM 2019. Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology 28(21): 4737–4754.
Shafer AB, Wolf JB, Alves PC, Bergström L, Bruford MW, Brännström I, Colling G, Dalén L, De Meester L, Ekblom R 2015. Genomics and the challenging translation into conservation practice. Trends in Ecology & Evolution 30(2): 78–87.
Stock SE, Klop-Toker K, Wallace S, Kelly O, Callen A, Seeto R, Mahony SV, Hayward MW, Mahony MJ 2023. Uncovering inbreeding, small populations, and strong genetic isolation in an Australian threatened frog, *Litoria littlejohni*. Conservation Genetics 24(5): 575–588.
Taylor HR, Dussex N, van Heezik Y 2017. Bridging the conservation genetics gap by identifying barriers to implementation for conservation practitioners. Global Ecology and Conservation 10: 231–242.
Theissinger K, Fernandes C, Formenti G, Bista I, Berg PR, Bleidorn C, Bombarely A, Crottini A, Gallo GR, Godoy JA 2023. How genomics can help biodiversity conservation. Trends in Genetics 39(7): 545–559.
Waddle JH, Rice KG, Mazzotti FJ, Percival HF 2008. Modelling the effect of toe clipping on treefrog survival: beyond the return rate. Journal of Herpetology 42(3): 467–473.
Wickham H 2016. ggplot2: elegant graphics for data analysis. R package version 3.5.2 https://ggplot2.tidyverse.org.
Zamora-Camacho FJ, Comas M, Pascual G, Moreno-Rueda G 2023. The effect of toe-clipping on locomotor performance and return rates in a frog. South American Journal of Herpetology 28(1): 38–46.
Zeileis A, Hothorn T 2002. Diagnostic checking in regression relationships. R News 2(3): 7–10.
Zemanova MA 2019. Poor implementation of non-invasive sampling in wildlife genetics studies. Rethinking Ecology 4: 119–132.
Zemanova MA 2020. Towards more compassionate wildlife research through the 3Rs principles: moving from invasive to non-invasive methods. Wildlife Biology 2020(1): 10.2981/wlb.00607.

Received: 3 September 2025; accepted: 10 March 2026
Editorial board member: Natalie Forsdick

Supplementary material

Additional supporting information may be found in the supplementary material file for this article:

Appendix S1. Practical buccal swabbing protocol.

Appendix S2. Scatterplot showing DNA yield (ng) against snout-vent-length (mm).

The New Zealand Journal of Ecology provides online supporting information supplied by the authors where this may assist readers. Such materials are peer-reviewed but not copy-edited. Any issues relating to this information (other than missing files) should be addressed to the authors.