Assessment of the impact of 1080 on the native frogs *Leiopelma archeyi* and *L. hochstetteri*

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ABSTRACT

New Zealand's native frogs are theoretically likely to possess the innate high tolerance of 1080 found in other ectothermic animals, however few studies have investigated the 1080 risk to *Leiopelma* or any frog species. Populations of *Leiopelma archeyi* and *L. hochstetteri* at Tapu, Coromandel Forest Park, New Zealand, were monitored through an aerial 1080 possum control operation in June 1995 using monthly counts of frogs along strip transects. Statistical power was analysed using collected and simulated data, to determine the minimum number of sightings required to successfully detect various levels of population decline. *Leiopelma archeyi* numbers did not decline in association with the poison bait drop, however too few *L. hochstetteri* were found to ascertain whether a decline occurred in this species. Heavy rainfall during and following poison application, and hence rapid breakdown of 1080, may have reduced the applicability of these results to other aerial operations. Laboratory trials simulating worst-case scenarios indicate that both these frog species can absorb 1080 from contaminated water, substrate, or prey. The chance of this occurring in the wild is ameliorated by a variety of factors, including frog ecology. Captive maintenance and contamination problems rendered parts of this study inconclusive. Further population monitoring is recommended to provide more conclusive evidence than provided by this single study.

Keywords: 1080, sodium monofluoroacetate, non-target impact, poison, *Leiopelma archeyi*, *Leiopelma hochstetteri*, Archey's frog, Hochstetter's frog, Coromandel, Tapu, conservation, possum control

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1. Introduction

The surviving endemic frog species of New Zealand are listed in the IUCN Red List of Threatened Species (Bell 1994, 1997a; IUCN 2000) and legally protected by the Wildlife Act 1954. Archey's frog *Leiopelma archeyi* is classified as Nationally Critical and Hochstetter's frog *L. hochstetteri* as Sparse under the New Zealand threat classification system (Hitchmough 2002). Both are found in the northern North Island (Cree 1986; Green & Tessier 1990; Bell 1993, 1994; Bell et al. 1995; Newman 1996), and *L. hochstetteri* on Great Barrier Island (Ogle 1981). They are generally restricted to podocarp–broadleaved forests which are also occupied by the introduced Australian brushtail possum *Trichosurus vulpecula*. The other extant native frog species—*L. hamiltoni* and *L. pakeka* (Bell et al. 1998)—occur on possum-free islands.

Possum control operations increased in the 1990s with greater recognition of the detrimental impacts of this pest on native habitats and fauna, and of possums harbouring bovine tuberculosis. The extensive use of the toxic compound sodium monofluoroacetate (1080) in possum control prompted interest in further research on the impact of 1080 use on non-target species, including native frogs (Atkinson et al. 1995). Amphibians are recognised as sensitive indicators of environmental change, including the impacts of agrochemicals (Blaustein et al. 2003), so the use of pesticides in New Zealand frog habitats warrants careful evaluation.

Leiopelma archeyi and *L. hochstetteri* are exposed to poison during pest control, but few studies have explored how they are affected by aerial 1080 application and no conclusive findings have been made (McNaughton & Greene 1994, DOC 1994b). This study was instigated by the Department of Conservation (DOC) to address the safety of *L. archeyi* and *L. hochstetteri* during pest control operations. Population monitoring was conducted by A.J. Perfect as part of her MSc programme, supervised by B.D. Bell and assisted by S. Pledger. Captive laboratory trials were conducted later by B.D.B. and A.J.P. Preliminary reports on this contract research were submitted to DOC in 1995 (Perfect & Bell 1995, 1996), and further related reports released later (Bell 1996; Perfect 1996, 1997a, b). This publication aims to record these preliminary findings in the scientific literature.

1.1 OBJECTIVES

The principal research objectives were:

- To document in the field the short-term impact, if any, of an aerial 1080 possum control operation on populations of *L. archeyi* and *L. hochstetteri* in Coromandel by determining the proportion of individuals which are at risk from poison baits or from secondary poisoning.
- To expose *L. archeyi* and *L. hochstetteri* to 1080 baits in controlled laboratory conditions, and observe their response (behaviour, survival rate) and the amount of 1080 absorbed into their body tissue.

1.2 PREVIOUS STUDIES

Tolerance of 1080 intoxication is roughly indicated by the amount of poison (mg kg^{-1} body weight) required to kill half an experimental group of animals, known as the LD_{50} (lethal dose, 50% of sample). Low tolerance is shown by low LD_{50} figures. High to extremely high LD_{50} doses ranging from 54.4 to > 1000 mg kg^{-1} have been calculated for all frogs investigated (Table 1), suggesting this is the most poison-resistant animal group.

Reptiles have a similarly high tolerance of 1080 poisoning (LD_{50} = 43.6–543.2 mg kg^{-1} in tested Australian species) (McIlroy et al. 1985), while birds, omnivorous mammals, marsupials, and carnivorous mammals (in ascending order) show greater susceptibility (Tomlinson & Gooding 1971; McIlroy 1981b, 1994; Eisler 1995). Carnivores are generally less tolerant of 1080 than herbivores, and animals with high metabolic rates are more susceptible than those with slower metabolisms (Chenoweth & Gilman 1946; Atzert 1971; McIlroy et al. 1985; McIlroy 1986; Twigg et al. 1986; Twigg & King 1991; Twigg 1994; Eisler 1995).

TABLE 1. LITERATURE RECORDS OF 1080 TOLERANCE IN FROGS.

SPECIES	LD_{50} (mg kg^{-1})	SOURCE
Bullfrog *Rana catesbeiana*	54.4	Eisler (1995) and references therein
Spotted grass frog *Limnodynastes tasmaniensis*	c. 60	McIlroy et al. (1985)
Unspecified species (average of four spp.)	93.69	McIlroy (1986)
Leopard frog *Rana pipiens*	150	Chenoweth (1949), Eisler (1995) and references therein
Unspecified species	300	Chenoweth (1949), Eisler (1995) and references therein
South African clawed frog *Xenopus laevis*	> 500	Chenoweth & Gilman (1946)
'various frogs'	1000–2000	Eisler (1995) and references therein

A 'latent period' of at least 30–120 minutes occurs between 1080 intake and the first signs of intoxication regardless of animal species or dose (Chenoweth & Gilman 1946). Amphibians and reptiles show the longest latent period seen in vertebrate captive trials (median times of 22 h and 56 h respectively), and the longest times between lethal dose and death (median times for amphibians 78 h and lizards 131 h). In both animal groups 'the most common signs of poisoning … are a lack of movement or convulsions' (McIlroy et al. 1985: 113). Chenoweth & Gilman (1946) commented that frogs (of unreported species) given an LD_{50} stopped convulsing by 24 h and entered flaccid paralysis, with surviving animals recovering over the next 24 h to seem normal.

Chenoweth & Gilman (1946) also noted that fluoroacetate acts primarily on the nervous system in frogs, with no noticeable cardiac effect. Other trials using isolated *Rana tigrina* frog hearts found a dose-dependent increase of up to 50% in the force, but not the rate of heart beat at doses of up to 320 µg 1080 (Burande et al. 1983). Whether this occurs in living frogs is uncertain, as the conclusion derives from experimental research on isolated frog material, not *in vivo* observation. For example, Chenoweth (1949) reported that sodium fluoroacetate and methyl fluoroacetate produce different effects on frog tissue, but an identical effect on whole animals.

Water balance appeared to affect amphibian tolerance to 1080 in at least one trial as 'frogs allowed to imbibe water through their skin after poisoning, which they do to the extent of a 50% weight gain, die more quickly than those kept dry' (Chenoweth 1949: 399–400).

It is unclear whether temperature affects amphibian response to 1080 poisoning. Chenoweth (1949) noted that sensitivity to fluoroacetate did not significantly increase in frogs kept in water at 32°C[1], and contrasted this to a finding by Boyarsky et al. (1949) that frogs are more sensitive in summer than winter. A seasonal difference might reflect differing metabolic demands associated with changing hormonal balance (McIlroy et al. 1985), but this remains questionable as the conclusion derives from experimental research on isolated frog nerve material. Other investigations have not examined the influence of temperature on frog 1080 response.

No influence on amphibian sensitivity to 1080 by sex, age, or other factors was found in the literature. Susceptibility may be influenced in other animal groups by temperature (mice and guinea pigs) (McIlroy 1981b, and references therein), extreme youth (mammals), and breeding condition (female waterfowl) (McIlroy 1981a).

1.3 FROG MONITORING THROUGH 1080 OPERATIONS

Non-target frog deaths have not been reported from New Zealand 1080 pest control operations. Numbers of *L. hochstetteri* were monitored at weekly intervals on 200 m long stream sections at three poisoned sites on the Mangatawhiri stream, Hunua Ranges, a total of three times before the aerial 1080 operation and four times afterwards. No evidence of 1080 affecting populations or individual frogs was found, but the study was inconclusive as a result of observer bias and lack of control sites (McNaughton & Greene 1994).

The Department of Conservation monitored *L. archeyi* and *L. hochstetteri* during an aerial 1080 operation around Waiau Falls, Coromandel, in June 1994 (DOC 1994b). Night searches were conducted on two 10 × 10 m quadrats for *L. archeyi*, and day searches along two 100 m stream transects for *L. hochstetteri*, in four searches over three weeks before the poison drop in June 1994, and four searches in one week afterward. Numbers of *L. archeyi* found were too low for meaningful comparison. Increased numbers of *L. hochstetteri* found after the drop were attributed to weather conditions; the increase was statistically significant outside the 1080 area, where larger numbers were found initially.

Previous investigations of the effect of aerial 1080 operations on native frogs have been problematic, however, the apparently innate tolerance of amphibians implies the risk to Leiopelma species may be low. Spurr (1993, 1994: 129) suggested native frogs are unlikely to be at risk from direct or secondary poisoning during 1080 pest control as lizards and frogs in general are more tolerant than other animal groups and require large quantities of poisoned

[1] Presumably in warmer than ambient conditions, although this is not stated explicitly.

food (bait or insects) to receive a lethal dose. Lethal dose (LD_{50}) tests are unsuitable for *Letopelma* species because of their conservation status.

1.4 COROMANDEL 1080 OPERATION AND THE TAPU SURVEY AREA

Waikato Conservancy of the Department of Conservation (DOC) instigated possum control over 12,239 ha of Coromandel Forest Park to address extensive possum browse damage on native vegetation reported by the vegetation monitoring programme (DOC 1995). Both *L. archeyi* and *L. hochstetteri* may be present throughout much of this area. Ground-based possum control using cyanide and traps was conducted on 960 ha around Castle Rock and the Manaia catchment. The main block of 11,279 ha was aerially treated with 61 tonnes of 1080 pollard (cereal pellet) bait by helicopter over 7, 8, 23 and 24 June 1995. The Tapu–Coroglen Road formed the southern boundary of the aerially treated area (DOC 1995).

The toxic content of baits was estimated at 0.157% 1080 by weight (DOC 1995). Bait weight data were not available, but the predominantly 4–6 g pellet range specified by the DOC National Possum Control Plan equates to 6.28–9.42 mg 1080 per bait and would provide a single lethal dose for most possums (DOC 1994a). The toxic loading and tonnage figures represent a total of 95.77 kg of sodium monofluoroacetate used in the operation, averaging 8.5 $g.ha^{-1}$.

Frog monitoring was carried out in the Tapu River catchment, adjacent to the Tapu–Coroglen Road (see Fig. 1) between c. 250–580 m a.s.l. The study area lies in the Thames Ecological District between the Papakai and Waiomu Ecological Areas, falling mainly within the Tapu RAP (Recommended Area for Protection) of Humphries & Tyler (1990). The vegetation is warm–temperate mixed podocarp–broadleaf forest on Komata clay-loam (Cree 1986). Historical modification included selective kauri (*Agathis australis*) logging in the 1880s and 1950s, development of tracks toward Papakai and Crosbies Clearing (B. Haynes, Tapu–Coroglen Road pers. comm.; F. Buchanan, DOC pers. comm.), and road construction. The Tapu–Coroglen Road continues to result in noise, dust, litter, and road maintenance activities. The local habitat has been modified by the continuing presence of introduced mammal species including goats (*Capra hircus*—subject to DOC pest control), pigs (*Sus scrofa*), possums, rats (*Rattus* spp.), mice (*Mus musculus*), cats (*Felis catus*), and mustelids (*Mustela* spp.).

Warm humid summers and mild winters are usual, with both frequent droughts and torrential rain and flooding. Mean annual rainfall at Thames township is 1278 mm and most falls in June. Cree (1986) measured rainfall of 2729 mm over October 1983–September 1984 on the Tapu ridge, and cites mean annual rainfall of between 1775–2550 mm for the area. Rainfall increases with altitude, particularly on the west side of the ranges (Humphreys & Tyler 1990), and mists are common around the hilltops.

A long-term mark-recapture study of *L. archeyi* was initiated at Tapu in 1982, following earlier surveys in the area (Bell 1978, 1994, 1996).

1.5 LABORATORY TRIALS

A series of laboratory trials explored whether *Leiopelma archeyi* and *L. hochstetteri* are susceptible to 1080 uptake in situations where they might encounter 1080 pollard bait during aerial operations to control possums. The threats simulated were: rehydration in a contaminated water puddle (Trial 1); extended close proximity to bait on the ground (Trial 2); and secondary poisoning via prey items exposed to bait (Trial 3).

2. Methods

2.1 FROG POPULATION MONITORING, TAPU AREA

2.1.1 Reconnaissance and establishing transects

Three experienced searchers undertook ad lib. reconnaissance in December 1994 near the Tapu–Coroglen Road (Fig. 1), on the western side of the ranges. Apparently suitable rocks and debris were examined in accessible typical frog habitats (forest floors for *L. archeyi*, small forested creeks on headwater tributaries of the Tapu River for *L. hochstetteri*). Trial transects were established in January 1995, if initial searches produced two or more animals within c. 5 minutes.

Six 50 × 2 m terrestrial strip transects (hereafter referred to as 'forest floor' or 'forest' transects) were established in two locations populated by *L. archeyi*. A1, A2 and A3 lay within the planned 1080 treatment zone (c. 450 m a.s.l.) and the non-treatment lines A4, A5 and A6 were outside the zone boundary (c. 580 m a.s.l.). A1, A2 and A4 run along tracks no longer maintained by DOC. Other forest transects lie adjacent to the same tracks and were marked with pink flagging tape to aid relocation.

A similar experimental structure (three treatment v. three control lines) was planned for *L. hochstetteri* creek transects, each covering a 50 m length of stream. This proved impractical because of low frog counts. Transects on one creek (H1a,b) were discontinued after March when insufficient numbers of frogs were found. Further transects were added in February and March to total six treated (H2a,b; H3a,b; H4a,b) and three control lines (H5; H6a,b) on five streams (H2–6) on most monitoring visits. Altitudes at the lowest point of the creek transects were approx. 220 m (H4a,b), 250 m (H1a,b), 320 m (H2a,b) and 370 m (H3a,b) on treatment lines, and 250 m (H5) and 400 m a.s.l. (H6a,b) on control lines. Transects were marked with metal pegs and flagging tape at completion of the study; location maps are held by Waikato Conservancy, DOC, and the authors.

2.1.2 Transect monitoring technique

Frog counts were conducted monthly five times before and fives times after the poison drop. All forest transects were searched on one day, and all creeks on

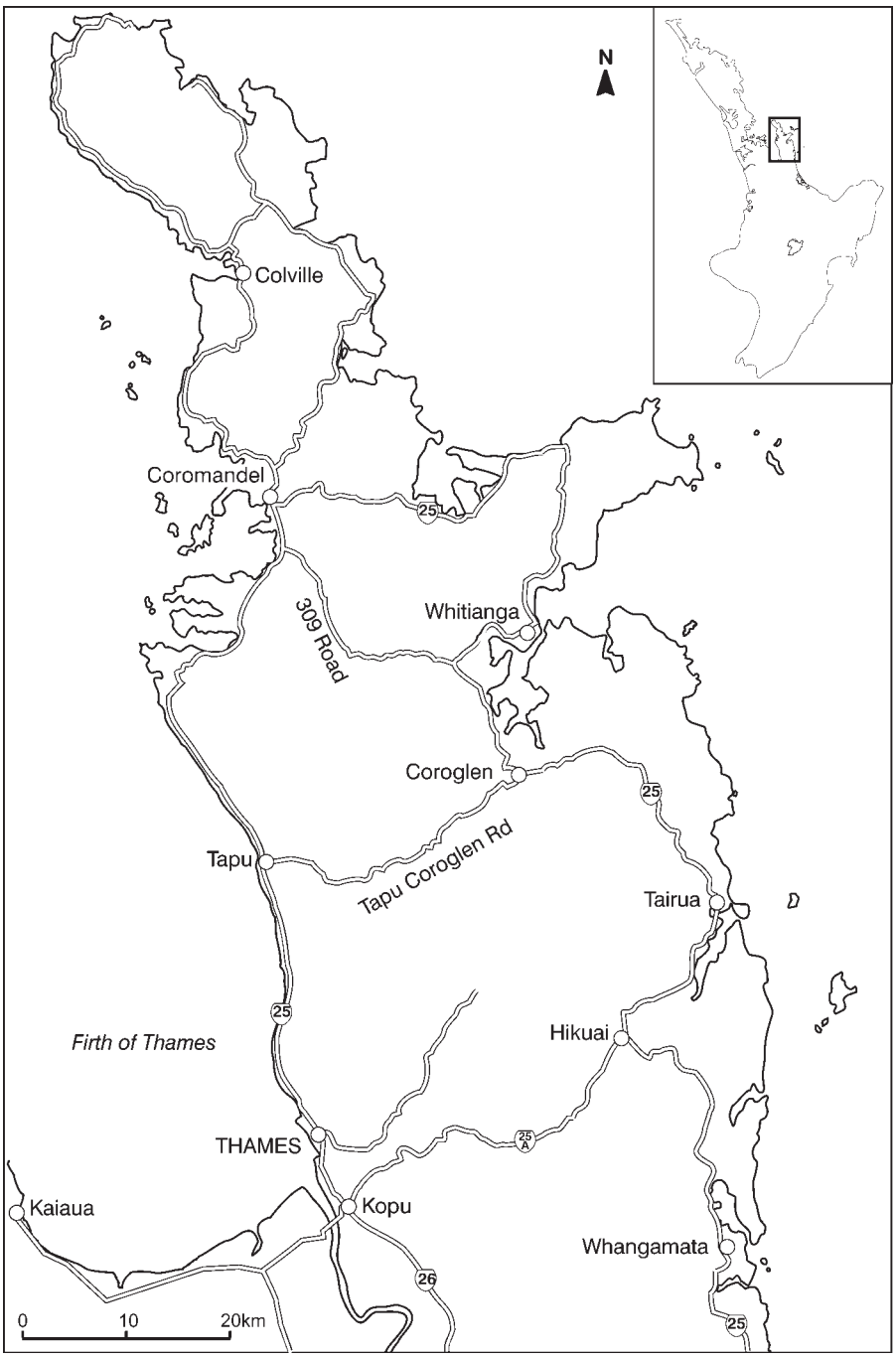

Figure 1. Coromandel Peninsula, showing the location of the Tapu–Coroglen Road.

another to reduce extraneous variation between transects. The first forest transect search in January 1995 was an exception, carried out over two consecutive days, as extra time was needed to reconnoitre areas, establish transects, and refine search methodology.

Two people with similar experience of searching for native frogs worked the transects on each occasion, each person covering half of every forest and stream transect. On stream transects Observer 1 consistently searched from 0 to 15 m and from 40 to 50 m, while Observer 2 covered from 15 to 40 m. The forest transects A1, A4, and from 0 to 25 m on A2 and A6 were covered by Observer 1, and A3, A5, and from 25 to 50 m on A2 and A6 were surveyed by Observer 2. All searches were conducted during daylight. Potential retreats were examined carefully to avoid damaging them.

Forest floor lines were searched by lifting all rocks of clenched fist size or larger, looking in and beneath fallen logs and branches, and examining vegetation (e.g. rice grass *Microlaena avenacea*; hook grass *Uncinia uncinata*; ferns *Blechnum discolor* and immature *Dicksonia* and *Cyathea* spp.; juvenile nikau *Rhopalostylis sapida*). Trees were briefly checked for resting frogs to c. 2 m above the ground, a height limited by reliable visibility. Each c. 100 m^2 search area was defined by a 50 m tape along the ground and the area up to 1 m distant on either side of it. On track transects (A1, A2, A4) the area up to 1 m from the track was used, except where rocks, logs or vegetation could be searched on the track itself. The search area would otherwise be comprised mainly of beaten earth or mud, and thus not comparable to other forest transects.

Searches along creeks were similar but covered the creek bed and adjacent bank. The total area searched varied from metre to metre along the transect because of the variation in bank slope or overhang, substrate, and the extent of raised creek-bed. The area also varied slightly from month to month, and the number of retreat sites to a greater degree, as water levels changed according to rainfall and runoff. As a result, although the search area was delineated by relocatable streamside strata and a set length of 50 m, the extent of the search area may not be strictly 100 m^2.

Search effort and morphometric and distribution data on frogs were recorded during monitoring; these are analysed and discussed in Perfect (1996). Handling was avoided where possible to reduce disturbance and only occurred if required to safely replace the frog's cover or prevent escape when estimating frog size. Otherwise the animal's details were recorded and the shelter carefully replaced over the frog. Covers too heavy to lift with sufficient control, or likely to be damaged by inspection, were left alone on every occasion. Transects were searched in the same order each month.

2.1.3 Bait density

Changes to the possum control operation boundaries to include a buffer zone along the Tapu–Coroglen Road left the creek transects H2a, b outside the flight area. 1080 bait was hand-sown by DOC staff on these lines to maintain numbers of treatment transects.

Forest floor strip transects were searched intensively for 1080 baits following application, and bait position and condition recorded. Bait surveys were scheduled for the day of application, but were conducted 1–2 days post-application because of a miscommunication from DOC (M. Frank, DOC pers. comm.), except on H2a,b where baits were hand sown and the survey occurred immediately after application. 1080 pellets are often cleared from public tracks, but were left along track transects in this study by request.

2.1.4 Generalised Linear Models of frog count data

Variation in transect counts (frogs per transect per month) of each species was investigated (by Shirley Pledger, Victoria University of Wellington, VUW) in the software package S+ v. 3.2, by analysis of deviance using Generalised Linear Models (Dobson 1983). Models were constructed to explain count variation in

terms of potentially influential factors, including exposure to 1080 poison, transect-specific and month-specific factors. 'Transect effect' is a generalised parameter encompassing differences in counts between transects (e.g. because of differences in retreat site quality and quantity, or local frog abundance). Similarly 'month' acts as a conglomerate local condition parameter, incorporating a number of anonymous influences which probably include rainfall, temperature, wind and sunshine, ground moisture, seasonal differences, and any lag effects of conditions from previous months. The independence problem associated with a repeated measures design is allowed for by including the 'transect' parameter, and was reduced by monthly search intervals and the use of counts. Pairs of alternative models were compared by log likelihood ratio (LR) and χ^2 goodness of fit (GOF) tests, and groups of models by Akaike's Information Criterion (AIC) (Burnham & Anderson 1992), separately for *L. archeyi* on forest transects and *L. hochstetteri* on stream transects.

A lattice diagram was produced to document the statistics and AIC scores of different models, and the results of pairwise LR tests. All combinations of parameters were examined to provide a comprehensive trial of available models.

2.1.5 Ability to detect decline

The power of a dataset (1–β) expresses the chance of recognising a significant difference if it is present, i.e. the probability a real decline in frog population will be recognised through a statistically significant difference in frog count data before and after 1080 application. Statistical power was determined using the non-central χ^2 distribution (Agresti 1990) in S+ from both real and simulated count data for α = 0.05, 0.1 and 0.2 in a BACI design.

1995 Tapu count data

The probability of detecting a 1080 impact from the varying numbers of frogs found at Tapu in 1995 was investigated using forest transect *L. archeyi* data, and February–October counts from transects H2, H3, H5 and H6 for *L. hochstetteri* to obtain a balanced dataset (i.e. to exclude transect-months with no count data). Expected post-treatment numbers were calculated to simulate a post-drop decline of from 5% to 35% in the 1080 treated area.

Simulated population means and environmental influences

Hypothetical scenarios based on control site populations of 25–300 frogs, subject to some degree of natural seasonal and site specific (area) influence on numbers, were also investigated to determine statistical power to detect declines of from 5% to 35% in a treated-area frog population following poison application.

Five situations were simulated for each combination of initial population and post-treatment decline: no seasonal or area effect; mild seasonal and no area effect; no seasonal and mild area effect; mild seasonal and area effects; and larger seasonal and no area effect.

2.1.6 Vegetation

Vegetation plots were measured on all transects in use in October 1995 to verify the comparability of treatment and control areas. On 10 × 10 m plots vascular plant species, diameter (1.4 m above ground) and estimated height of trees with at least one stem of >10 cm diameter were noted, as well as aspect and slope, and drainage on an arbitrary five-point scale. Species cover and ground cover of rocks and earth were estimated in nested 5 × 10 m plots.

One plot was located a random distance along each 25 m half-transect length. On streams the plot was sited parallel to the watercourse on a randomly designated bank, above the bed and any immediate overhang, with the nested plot beside the creek. Plots on forest-floor transects were offset a random distance of from 2 m to 8 m to the left of the transect tape, and divided at 90° to the transect before randomly selecting one half as the nested plot.

Two-way indicator species analysis (TWINSPAN: Hill 1979) was conducted on vegetation data entered in a DECODA database (Minchin 1990).

2.2 LABORATORY TRIALS

The trials were exploratory and did not quantify 1080 dosage, rates of detoxification, or provide definitive risk data for primary, secondary, or repeated poisoning. Experiments were run on both frog species simultaneously and included toxic and control (non-toxic) treatments (frogs subject to each treatment are hereafter referred to collectively or individually as toxic and control groups or frogs). Toxic and control groups of each species were matched as closely as possible in size and sex for each trial. Funding and limited sample sizes (because of the frogs' threatened status) constrained some aspects of this work. Some control frogs were used again in a later trial because of the limited number of animals available.

Safety and ethical regimes approved by the University Safety Officer and the University Animal Ethics Committee (BB95R1, 26 November 1996) were followed, and access to the experimental lab was restricted to authorised personnel only. The former Institute of Statistical and Operational Research (VUW) provided statistical advice on experimental design.

2.2.1 Frogs and bait

Twelve frogs of each species were collected at Tokatea Ridge, northern Coromandel, over 4–5 November 1995 (Bell 1996)[2]. Adult frogs were selected where possible. On 5 November the frogs (in chilled containers) were taken by car to temporary terraria in Wellington, and housed from 11 November in glass covered stock tanks (46 × 25 × 25 cm glass aquaria) in a constant temperature room at the School of Biological Sciences, VUW. An equal dark/light photoperiod started with darkness from 15:50 h. The initial temperature of

[2] A further frog was taken inadvertently at the same time, and later replaced a frog which died in captivity before trials commenced. Two frogs from existing captive stock were also used in Trial 3.

from 12 to 13°C was increased to 15°C from mid March 1996 to allow multiple use of the room for a different project, and varied for individual trials as noted in each section below. Other projects and items in the room were vetted to avoid the risk of toxins or disease.

Frogs were referenced individually with a number (Trials 1 and 2) or letter (Trial 3, as some frog identities were uncertain by this date) and sexed if possible. Flat layered branches and bark on moist leaf-litter and soil from Tokatea were provided in the *L. archeyi* tank to imitate natural retreat sites, sprayed regularly with distilled water, while the *L. hochstetteri* tank also had 5 cm of Tokatea creek water. Laboratory-reared house flies (*Musca domestica*) dusted in 'Bonegro' calcium powder were supplied and occasionally supplemented with captured moths.

Wanganui Conservancy, DOC, supplied 'Wanganui number 7' 1080 pollard bait (dyed green, cinnamon lured) and non-toxic pollard bait (not dyed or lured) from the Wanganui Poison Factory in November 1995. Bait was held in plastic bags inside plastic containers, in a locked metal cupboard in a separate room, and kept at ambient room temperature until used in trials in May 1996 and January 1997.

1080 assays were conducted by the Ministry of Agriculture and Fisheries (MAF, Wallaceville) using the 1080Tox.v2 method which has a lower detection limit 0.05 mg kg^{-1}, and lower quantification limit 0.1 mg kg^{-1} (G. Bentley, AgriQuality NZ Ltd pers. comm.). Assay sample preparation was discussed with C. Eason (Landcare Research) and MAF staff (G. Bentley, K. Glenn, and V. Shanks) prior to the trials. Frogs were killed humanely by freezing. Samples of frogs, groups of flies, and bait/water solutions were frozen separately in plastic bags or containers. MAF staff removed baits from water before thawing to avoid further leaching from baits prior to analysis, and used a mixed tissue sample for each frog assay.

Toxic and non-toxic baits were selected and assayed with Trial 1 and 2 materials in January 1997 (1st batch). Three animals from the frog stock tanks were also assayed (one found dead in May 1996, in the 1st batch; one remaining frog of each species left in stock following Trial 3 in January 1997—2nd batch of assays, along with Trial 3 material).

2.2.2 Trial 1: Exposure to 1080-contaminated water

This trial simulated active rehydration in a small water puddle contaminated by 1080 pollard bait. Frogs were dehydrated under controlled conditions (after Cree 1985, 1986) to a state of negative water-balance to encourage active rehydration in the trial solution. The trial was conducted over 5–8 May 1996 at c. 12°C.

The frogs were weighed after gentle forced urination (W_0) and placed individually in labelled 55 mm diameter × 20 mm deep plastic dishes, covered with fine (<1 mm) plastic mesh attached by a rubber band. Controlled gradual desiccation was conducted in the dark at 40–48% relative humidity (RH) using silica gel crystals with a blue gel moisture indicator for up to 19 h, with periodic checks on frog condition and weight. Individual frogs were removed on reaching their target of c. 85% of initial body weight (0.85 W_0) and held

separately in labelled jars at 100% RH until ready to continue. The dehydrated frogs were then reweighed; no further action was taken to address frogs not at 0.85 W_0 at that time.

Toxic and control solutions were prepared using baits of similar weight for each regime (6.2–6.3 g toxic or control), soaked for an hour in 40 ml distilled water in 400 ml plastic jars. Each dehydrated frog was placed in a solution jar with the cap lightly screwed on and left in the dark for a two hours. Frogs found climbing the jar during regular checks were replaced in their solutions. Urination was not monitored.

After the two-hour immersion period each individual was removed, dried with filter paper, and reweighed. Samples were frozen for assay (two toxic and one control frog of each species, and all jars of bait and water solutions). The other frogs were held individually in terraria for 24 h and checked intermittently, with one toxic and control frog of each species under a video camera lit by LEDs. Following the observation period the remaining toxic group frogs were frozen for assay and controls returned to the stock tanks.

A staggered start was used, beginning with one frog of each species on 5 May 1996 (hereafter referred to as a pilot), and continuing with seven further animals ('main run') of each species from 6 May 1996. Distilled water was supplied and periodically sprayed over main run frogs in the post-immersion observation phase, prompted by observations of dehydration in the pilot frogs, but glass covers were not used. A hygrometer reading from the pilot *L. archeyi* terrarium c. 9 h post-immersion was at least 92% RH. Post-immersion terraria readings for the main run frogs were 98–100% RH.

Toxic and control jars were placed in separate parts of the room to reduce risk of cross contamination and care was taken to avoid contamination of controls by frogs exposed to 1080 during handling.

2.2.3 Trial 2: Exposure to 1080 pollard bait in terraria

This trial simulated extended close proximity to 1080 pollard bait on the forest floor. It was conducted from 13 May to 24 May 1996, with follow up observations extending from 24 May to 21 June 1996, at c. 12°C.

Individual chambers for two toxic and two control frogs of each species were prepared by dividing four 46 × 25 × 25 cm terraria in half with 2 mm mesh. The frogs were introduced on 13 May and each provided with moistened peat (standard garden product) topped with a moist paper towel, petri dish with distilled water (25 ml for each *L. archeyi*, 50 ml for each *L. hochstetteri*), cardboard corner shelter, glass cover, and a hygrometer to measure humidity (c. 98% RH during the trial: ambient humidity recorded outside terraria ranged from 95%–100% RH, down to 77–86% RH in periods when frequent movement in and out of the room affected airflow). One toxic (5.3 g) or control (3.5 g) bait was added to each chamber on 14 May (day 0), keeping these treatments in separate terraria to avoid contamination.

One treatment and one control frog of each species (those most active or apparently exposed to bait) were to be frozen for 1080 assay after 10 days. The remaining frogs were checked periodically for a further 28 days to observe possible effects of longer term exposure. The informal notes taken later

allowed a crude assessment of behaviour for possible aversion or attraction to bait. Data were derived from checks at intervals of ≥ c. 45 min. from just after initial bait addition until day 38.

Periodic observations were made on the frog chambers using night-vision equipment or a torch, avoiding disturbance where possible. Occasionally fogged glass covers were taken off briefly and condensation removed to allow observation. One toxic and control chamber for each species were under video surveillance for the first 10 days of bait exposure.

Distilled water was sprayed ad lib throughout the trial. The paper covering the potting mix substrate was replaced with a 3–4 cm layer of native plant leaf litter from Tokatea on day 4, despite greatly reduced visibility, because of concerns about dehydration in the exposed conditions. Baits were repositioned on top of the litter. Some litter and mouldy baits were replaced on day 14.

Live house flies were provided intermittently during the trial, and left at least overnight to allow the frogs to feed. Glass containers were placed over baits at these times to avoid secondary poisoning of frogs.

2.2.4 Trial 3: Exposure to 1080-contaminated prey

This trial investigated secondary poisoning via housefly prey exposed to pollard bait. It was conducted over 22–23 January 1997.

Two regimes of close or casual contact by prey with bait were used. Seven 46 × 25 × 25 cm, glass topped terraria were divided in half with 5 mm mesh to restrict frogs, but allow free ranging by houseflies. In each terrarium one half was equipped as a frog chamber with moist potting mix substrate (drier for *L. archeyi* than *L. hochstetteri*), corner shelter, and a hygrometer to measure humidity (range 60–100% RH over experiment). The other half held one moist and one dry bait on a petri dish for the casual contact regime, and an additional dry bait in the close contact regime.

Flies (c. 75 per terrarium) for the casual contact regime were introduced directly to the terraria. Groups of flies for the close contact regime were each confined in a small plastic jar with a bait, near a light source to encourage fly activity, for 1 h prior to release. Frogs were not fed for 10 days prior to the trial start. The room temperature was increased to between 13.8 and 19°C to promote fly activity and encourage feeding, and lights positioned to attract flies into the frog chamber of each terrarium during the daylight cycle.

The frogs and flies were held in the experimental terraria for 24 h. Samples were then frozen for 1080 assay including all frogs, separate samples of 20 flies per terrarium, and a further mixed 20 fly sample from toxic terraria only.

The trial comprised one frog per treatment (close/casual, toxic/control). The casual contact control *L. archeyi* was omitted because inadequate numbers of healthy frogs remained.

3. Results

3.1 FROG POPULATION MONITORING, TAPU AREA

Monthly counts of each frog species are presented in Tables 2 and 3. A total of 874 *L. archeyi* sightings were made on terrestrial transects A1–6, with a fall in numbers in both the control and treatment areas over the course of the study (Table 2). *L. hochstetteri* sightings along streams totalled 393, more or less evenly divided between pre- and post-drop periods (Table 3). Seventy *L. hochstetteri* sightings made on forest floor transects and three *L. archeyi* sightings on stream transects were excluded from analyses.

TABLE 2. *Leiopelma archeyi* COUNTS FROM TRANSECT SEARCHES, 1995.

	1080 TRANSECTS				CONTROL TRANSECTS			TOTAL	
	A1	A2	A3	SUM 1080	A4	A5	A6	SUM CONTROL	SUM A1–6
Jan	5	10	16	31	3	9	21	33	64
Feb	24	18	28	70	3	18	30	51	121
Mar	18	22	25	65	4	19	35	58	123
Apr	20	11	25	56	4	15	30	49	105
May	17	19	19	55	13	14	27	54	109
1080 application									
Jun	16	16	13	45	4	12	26	42	87
Jul	11	15	14	40	4	7	19	30	70
Aug	10	8	22	40	1	5	16	22	62
Sep	9	8	13	30	3	3	6	12	42
Oct	14	13	26	53	2	16	20	38	91
TOTAL	144	140	201	485	41	118	230	389	874

TABLE 3. *Leiopelma hochstetteri* COUNTS FROM TRANSECT SEARCHES, 1995.

	1080 TRANSECTS									CONTROL TRANSECTS			TOTAL	
	H1		H2		H3		H4		SUM	H5	H6a		SUM	SUM
	a	b	a	b	a	b	a	b	1080		a	b	CONTROL	H1–6
Jan	2	1	0	9	–	–	–	–	12	6	15	5	26	38
Feb	0	0	8	7	3	3	–	–	21	10	6	13	29	50
Mar	0	0	2	3	2	4	1	3	15	9	7	11	27	42
Apr	–	–	1	8	1	1	1	2	14	4	1	11	16	30
May	–	–	3	5	1	2	0	4	15	4	7	7	18	33
1080 application														
June	–	–	3	6	1	3	3	3	19	2	3	6	11	30
July	–	–	2	5	2	2	2	1	14	3	4	10	17	31
Aug	–	–	3	6	2	0	1	7	19	6	4	5	15	34
Sep	–	–	3	13	3	3	0	4	26	7	7	15	29	55
Oct	–	–	3	10	3	1	2	0	19	12	10	9	31	50
TOTAL	2	1	28	72	18	19	10	24	174	63	64	92	219	393

Extensive raw field data has been omitted from this report. Electronic copies are held by Science Technology and Information Services (STIS) and Waikato Conservancy of DOC, the authors at VUW, and can be found attached to Perfect (1996).

No dead frogs were found. Possible poisoning symptoms were seen only once during searches. On 15 June a frog (*L. archeyi*) was described by a field assistant as foaming at the mouth, but appeared normal c. 10–15 minutes later when checked again and was not relocated subsequently.[3] No other behaviour possibly symptomatic of poisoning was found during searches.

3.1.1 Bait location

Bait numbers and distribution on frog transects (Table 4) are indicative only and may underestimate the initial poison densities on most lines because of consumption by possums and rats in the 1–2 day period between application and survey.

TABLE 4. 1080 BAIT RECORDED FROM TREATED TRANSECTS.

TRANSECT	NUMBER OF BAITS RECORDED		
	WITHIN TRANSECT	VISIBLE WITHIN c. 1–2 m OF TRANSECT	MAXIMUM PER 10 m^2
A1	22	19	9
A2	15	9	8
A3	20	6	11
H2a*	2	8	5
H2b*	3	6	6
H3a	2	1	1
H3b	0	0	0
H4a	2	0	1
H4b	4	5	2

* Surveyed immediately after 1080 application. All other transects at an uncertain interval (up to 1–2 days) after application.

Very few baits were recorded from the treated stream transects, or seen on the surrounding slopes. Only a small proportion of bait hand sown on H2a,b was recorded immediately after application, suggesting that densities from other creek transects may also be underestimated because of the steep terrain and vegetative debris. Clumps of bait were found on H2a,b, but were otherwise rare on creek transects.

Higher numbers of bait found on forest floor transects A1, A2, and A3 indicate that a minimum of 173, 118, and 157 mg 1080 respectively were initially present, based on average bait weights prescribed by DOC national quality standards and reported toxic loading (DOC 1994a, 1995). The greatest densities of bait seen per 10 m^2 on each transect equated to c. 70.65 mg 1080 before

[3] DOC permit for population monitoring did not allow removal of live animals.

leaching or breakdown on A1, c. 62.8 mg on A2, and c. 86.35 mg on A3 calculated as above.

Several pellets showed surface damage; it was not determined whether this related to invertebrate or rodent feeding, or mechanical damage during application or later rainfall.

3.1.2 Generalised Linear Models of frog count data

Generalised Linear Models of count variation in *L. archeyi* forest transect data, and *L. hochstetteri* stream transect data, are compared in Figs 2 and 3 respectively. The arrows between models and their associated p-values in the lattice diagrams indicate the outcome of pairwise likelihood ratio (LR) tests. Except where goodness of fit (GOF) is specified, p-values given in the text refer to LR tests.

Leiopelma archeyi

No significant 1080 impact was detected. On the contrary, slightly lower (although not statistically significant) post-operation frog counts in the control area compared to the treatment area ('count ~ transect + month + 1080', AIC = 76, p = 0.0784) is reflected in the 1080 model having slightly better GOF and AIC values than the additive 'transect + month' model, despite pairwise testing favouring the latter. Variation in *L. archeyi* count data was best explained by local and temporal effects ('count ~ transect + month', GOF p = 0.3700, AIC = 78). Neither transect nor month effects alone explain *L. archeyi* count data (GOF p = 0.0000 for both), although transect effects seem more influential than month.

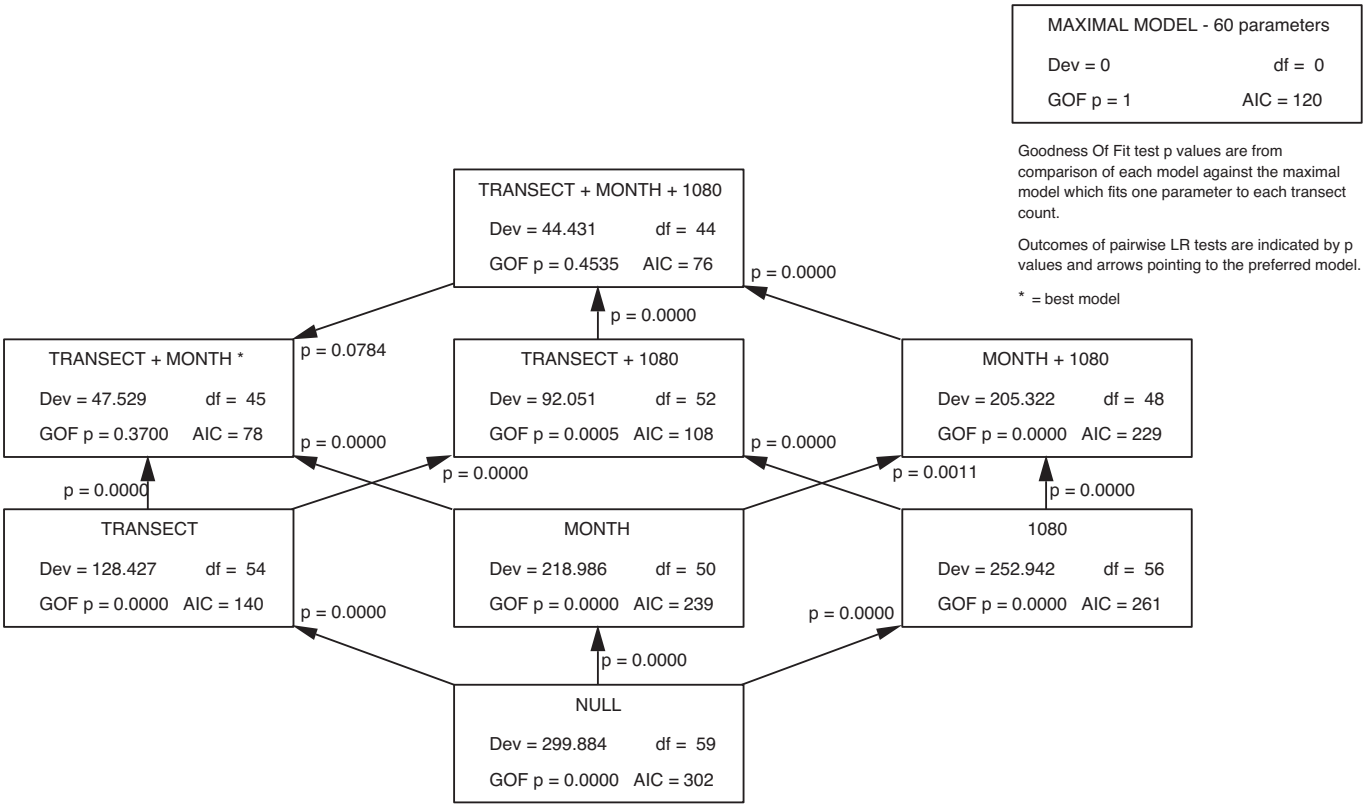

Figure 2. Generalised linear models of *Leiopelma archeyi* count data.

Leiopelma hochstetteri

Leiopelma hochstetteri results were similar to those of *L. archeyi* and did not show any impact by 1080. Location appears to strongly influence frog counts; models including the 'transect' factor had similar, low AIC scores over a tight range of 128–136. Some refinement was provided by including month effects ('count ~ transect + month', p = 0.0072), but neither 'transect' only, or 'transect and month' models were improved by allowing for 1080 impact ('count ~ transect + 1080', AIC = 136, $p = 0.4928$; 'count ~ transect + month + 1080', AIC = 129, $p = 0.3204$). The low AIC scores of these two models when a 1080 effect is included reflect the good fit of the other parameters, rather than any influence by 1080, as indicated by the arrows showing LR test outcomes in Fig. 3.

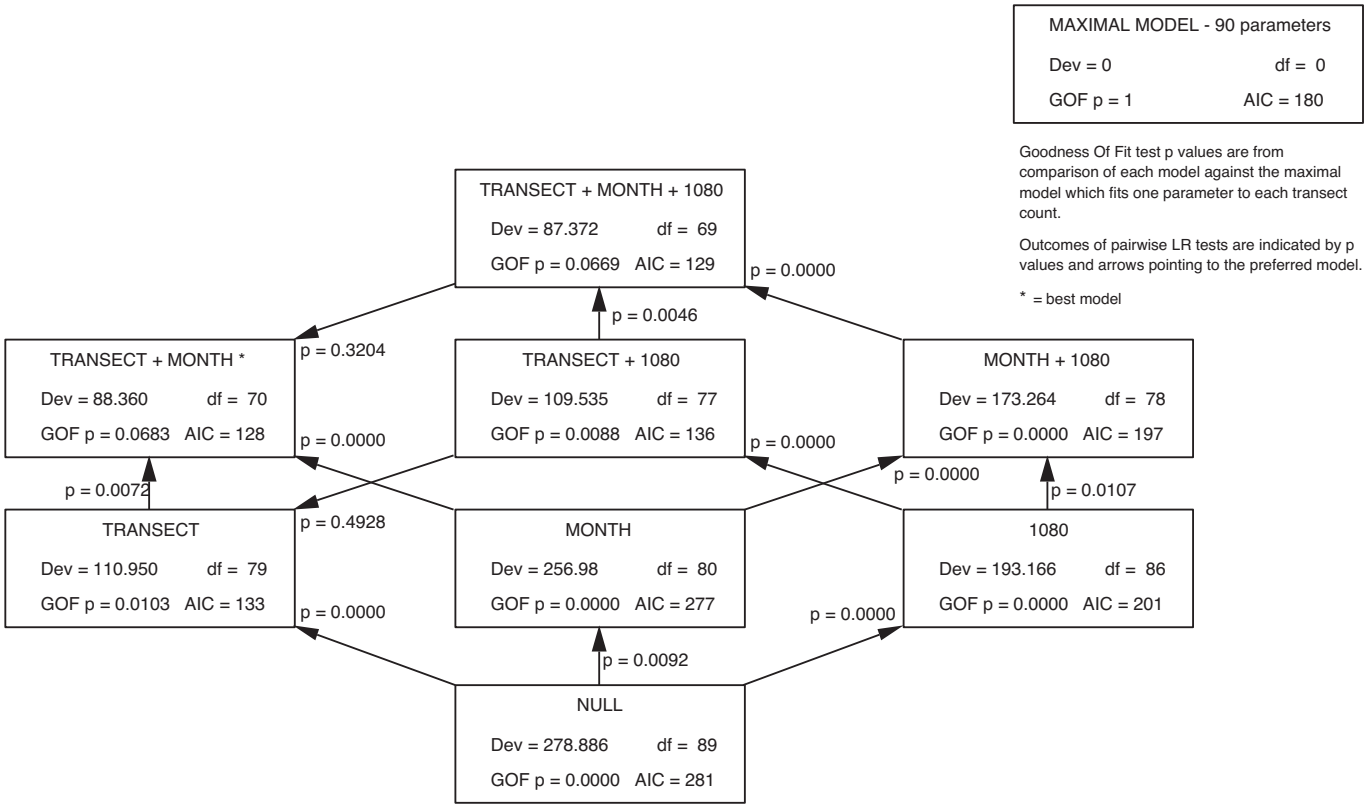

Figure 3. Generalised linear models of *Leiopelma hochstetteri* count data.

3.1.3 Ability to detect decline

For this report test power (1–β) of 80% was considered reasonably good; other circumstances or objectives may require stricter, or more relaxed, probabilities of correctly detecting a real decline.

Tapu count data, 1995

Power curves derived from 1995 transect counts of *L. archeyi* and *L. hochstetteri* (Fig. 4) are similar to those from simulated scenarios (Fig. 5). Figure 4A indicates a reasonably high likelihood ($1-\beta \geq 0.8$) of detecting large declines of 35% at $\alpha = 0.05$, ≥ 32% decline at $\alpha = 0.10$, and ≥ 28% of the population at $\alpha = 0.20$. Smaller reductions are less likely to be detected unless the probability of falsely concluding an impact occurred (α) is relaxed.

Figure 4B indicates *L. hochstetteri* counts were too low to allow robust tests for post-treatment decline. Even decreases of 30–35% might not be detected given the numbers and variation in counts before and after the 1080 operation within and outside the poisoned area.

Simulated population means and environmental influences

Power analysis results from simulated scenarios are presented as power curves for the 5% significance level in Fig. 5; curves for 10% and 20% significance levels are given in Figs A1.1 and A1.2 (see Appendix 1). Test power improved if larger initial numbers of animals were obtained or significance levels were relaxed, particularly for large population declines. However, the probability of detecting a small decline was very low, regardless of the size of pre- and post-treatment counts used.

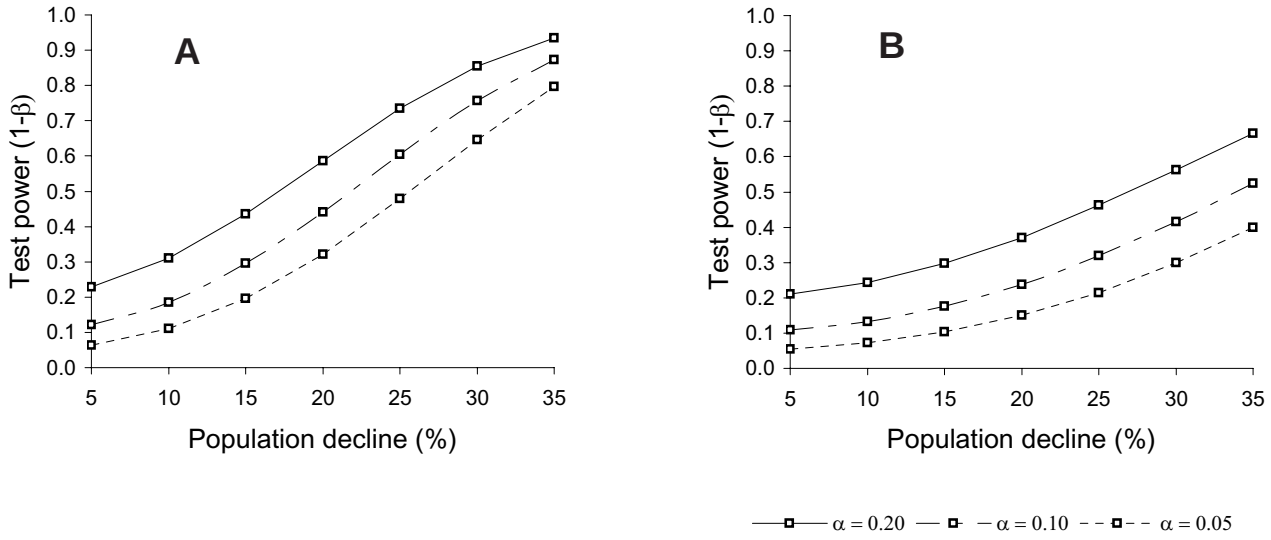

Figure 4. Probability of detecting a frog population decline associated with 1080 application at Tapu, 1995. **A**. *Leiopelma archeyi*. Jan-Oct 1995 counts on forest floor transects. **B**. *Leiopelma hochstetteri*. Feb-Oct 1995 counts on stream transects.

Figure 5A shows that a reasonably high likelihood ($1-\beta \geq 0.8$) of detecting a 30% decline in the treated area is only obtained with initial control area counts of at least 250 frogs, or a 35% reduction coupled with an initial control count of 200 frogs. The introduction of seasonal or area effects (i.e. s.f. or a.f. $\neq 1.0$) reduced test power slightly further. Mild seasonal or area effects as applied in this model were largely interchangeable; the power of detection was identical for the two situations s.f. = 0.8, a.f. = 1.0 and s.f. = 1.0, a.f. = 0.8 (Fig. 5B, and see Figs A1.1B and A1.2B in Appendix 1) which represent 20% difference in counts because of mild seasonal or mild area effects. Power was reduced slightly further by combining both mild seasonal and area effects (s.f. = 0.8, a.f. = 0.8; Fig. 5C, and see Figs A1.1C and A1.2C in Appendix 1), and similar powers resulted when a stronger seasonal influence in the absence of area differences was simulated (s.f. = 0.6, a.f. = 1.0; Fig. 5D, and see Figs A1.1D and A1.2D in Appendix 1).

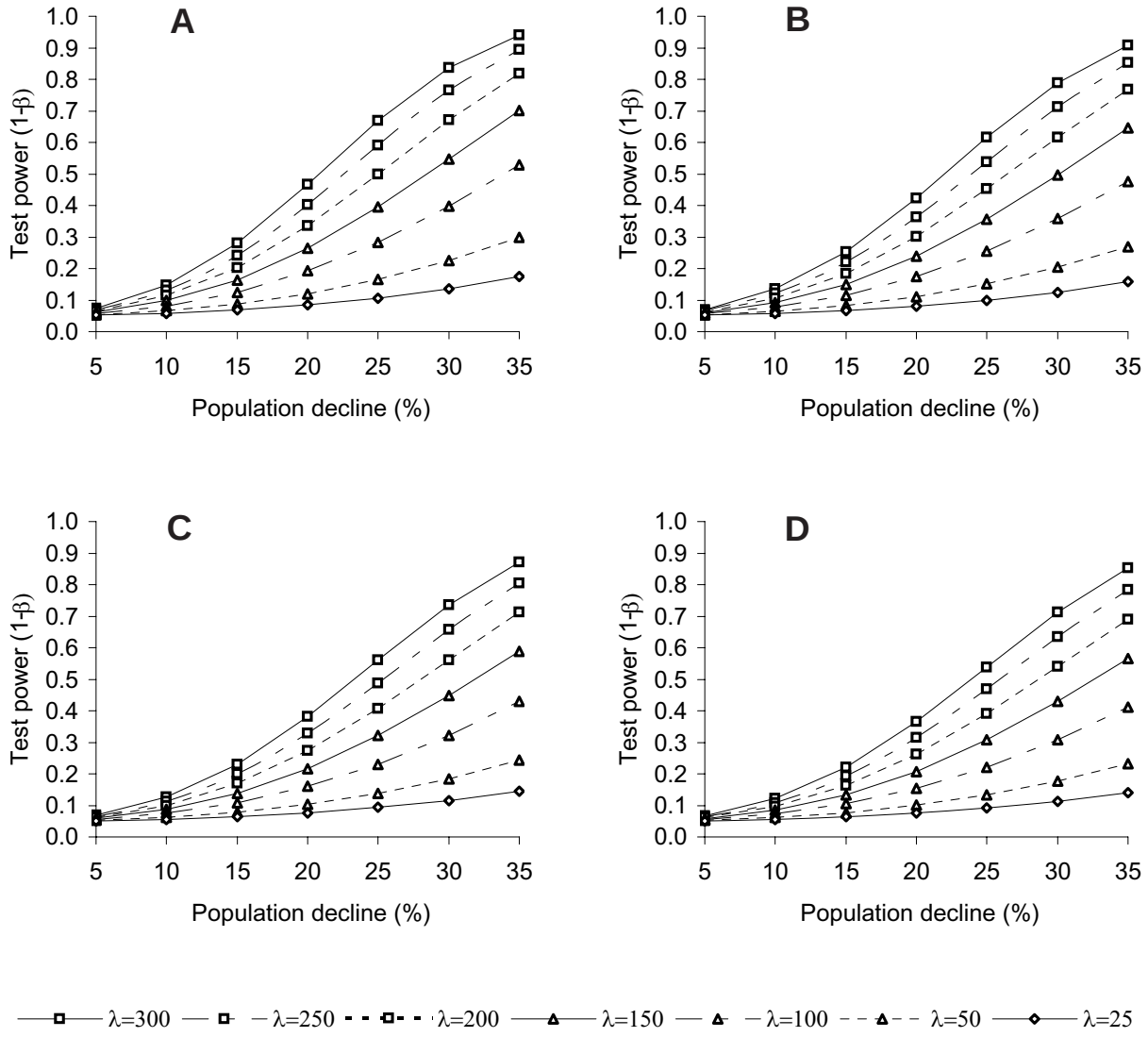

Figure 5. Power to detect 1080 impact (population decline) in simulated count data at 5% significance level. Varying degrees of seasonal and site-specific influence are modelled over a range of pre-1080 drop control counts (λ). Seasonal factor (s.f.) is the proportional difference between pre- and post-drop counts, and area factor (a.f.) the proportional difference between control and treated populations. **A** s.f.=1.0, a.f.=1.0; **B** s.f.=0.8, a.f.=1.0; or s.f.=1.0, a.f.=0.8; **C** s.f.=0.8, a.f.=0.8; **D** s.f.=0.6, a.f.=1.0.

3.1.4 Vegetation

TWINSPAN analysis confirmed there were no substantial differences in vegetation plots at control and treatment *L. archeyi* monitoring sites in terms of vascular plant species present or their relative plant cover (see Figs A2.1A and B in Appendix 2). Vegetation data collected from stream transects were not analysed because insufficient numbers of frogs were found for *L. hochstetteri* population monitoring.

3.2 LABORATORY TRIALS

Laboratory 1080 assay reports are presented in Appendix 3 and summarised here.

3.2.1 Frog and bait stock assay

Tests found 1080 concentrations of 0.17% (1700 mg kg^{-1}) in stock 1080 bait, and 0.2 mg kg^{-1} in stock control bait. The lean *L. archeyi* found dead in the stock tank on 7 May 1996 had residues of < 0.1 mg kg^{-1}. No 1080 was detected in the remaining frog of each species left in the stock tanks at trial end in January 1997.

TABLE 5. *Leiopelma archeyi* RESULTS FROM TRIAL 1—EXPOSURE TO 1080-CONTAMINATED WATER.

	1080 GROUP				CONTROL GROUP			
Frog reference	#3	# 5	# 1[P]	# 10	# 2[†]	# 4	# 9	# 25
SVL (mm)	31.2	31.7	31.1	34.3	33.3	31.6	29.8	29.8
Apparent sex	F	F	F	F	F	F	F	?
Start date	6 May	6 May	5 May [P]	6 May	6 May	6 May	6 May	6 May
Hours observation*	2	2	2+24	2+24	0[†]	2	2+24	2+24
Final 1080 (mg kg^{-1})								
Frog	2	4.1	3.9	0.6	<0.1	<0.1	*n.s.*	*n.s.*
Bait and solution	210	170	250	290	n/a[†]	0.1	0.1	<0.1
Weight (g)								
Corrected initial (W_0)	2.04	2.35	2.92	3.59	2.78	2.97	2.61	2.17
During dehydration[§]								
9 h from start			2.61	[¬]				
13½ h	1.65	1.95	2.47	3.16	2.41	2.66	2.20	1.78
14½ h			2.44					
15½ h	1.58	1.92	2.44	3.11	2.37	2.62	2.18	1.76
16½ h			2.44					
17½ h			2.44					
18 h	1.59	1.92		3.05	2.31[Θ]	2.57	2.18	1.76
19 h	1.54[¬]	1.92	2.44[¬]	2.64[¬]	2.43[†]	2.54	2.18	1.75
Immersion 19–21 h	toxic	toxic	toxic	toxic	control	control	control	control
Post treatment (h from start)								
21¼–½ h	**2.38**	**2.27**	2.83[¬]	2.77		**2.88**	2.41	2.07
39¼ h			2.40[Θ]					
41 h			2.52					
45¾ h			**weight not avail.**	**3.35**			2.69	2.48
% change dehydration (W_0–19 h)	–24.4%	–18.4%	–16.5%	–26.6%	–12.8%	–14.5%	–16.6%	–19.1%
% change immersion (19–21 h)	54.3%	18.6%	16.3%	5.2%	–	13.1%	10.6%	18.1%
Rehydration rate (19–21 h, mg g^{-1} h^{-1})	206	76	68	19	–	56	44	73
Final % difference from W_0	16.7%	–3.3%	–13.7%	–6.9%	–	–3.3%	3.0%	14.5%

[P] = pilot run; [†] = frog died; ? = uncertain sex; * = 2 h during immersion +/– 24 h subsequent observation; *n.s* .= not sampled; [§] = times approximate; [¬] = frog had defecated; [Θ] = provided with water; **Bold** = frog killed.

3.2.2 Trial 1: Exposure to 1080-contaminated water

Individual frog and treatment data are given in Tables 5 and 6. Toxic group *L. archeyi* averaged 32.1 mm snout-vent length (SVL) and 2.9 g initial weight (control 31.3 mm SVL, 2.7 g). Toxic group *L. hochstetteri* averaged 35.7 mm SVL and 4.7 g initial weight (control 35.7 mm SVL, 4.5 g).

The toxic group *L. archeyi* had 1080 residues of 0.6–4.1 mg kg^{-1} (mean 2.65 mg kg^{-1}) with a lower range of 0.3–1.3 mg kg^{-1} in toxic group *L. hochstetteri* (mean 0.98 mg kg^{-1}). Toxin residues in the poison bait solutions, frozen immediately post-immersion, were substantially higher than found in any frog samples (mean 230 mg kg^{-1} and 205 mg kg^{-1} respectively for *L. archeyi* and *L. hochstetteri* solutions). Toxic residues were also measured in the two control frogs assayed (<0.1–0.4 mg kg^{-1}), and in control bait solutions for both species (<0.1–0.2 mg kg^{-1}) (Tables 5 and 6).

TABLE 6. *Leiopelma hochstetteri* RESULTS FROM TRIAL 1—EXPOSURE TO 1080-CONTAMINATED WATER.

	1080 GROUP				CONTROL GROUP			
Frog reference	# 14	# 19	# 21[P†]	# 24	# 22	# 15	# 20	# 23
SVL (mm)	34.5	36.8	37.2	34.3	38.3	38.2	32.9	33.3
Apparent sex	F	F	F	M	F	F	?	M
Start date	6 May	6 May	5 May[P]	6 May	6 May	6 May	6 May	6 May
Hours observation*	2	2	2+24	2+24	2	2+24	2+24	2+24
Final 1080 (mg kg^{-1})								
Frog	0.3	1.1	1.3	1.2	0.4	*n.s.*	*n.s.*	*n.s.*
Bait and solution	230	200	230	160	0.1	0.2	0.2	0.2
Weight (g)								
Corrected initial (W_0)	4.07	5.42	5.04	4.11	5.17	5.46	3.54	3.23
During dehydration[§]								
9 h from start			4.62					
13½ h	3.74	5.06	4.45	3.73	4.80	5.02	3.09	2.82
14½ h			4.41					
15½ h	3.70	5.01	4.36	3.69	4.76	4.97	3.04	2.78
16½ h			4.32					
17½ h	3.66	4.96	4.28	3.63	4.71	4.90	2.99	2.73
19 h	3.63	4.92	4.28	3.26[¬]	4.68	4.86	2.96	2.73
Immersion 19–21 h	toxic	toxic	toxic	toxic	control	control	control	control
Post immersion (h from start)								
21 h	**3.65**	**4.83**	4.56	3.27	**4.81**	5.24	3.19	2.81
39¼ h			3.61[¬Θ]					
41 h			4.47[†]					
45 h				**3.77**		5.48	3.37	3.02
% change dehydration (W_0–19 h)	–10.8%	–9.2%	–15.2%	–20.8%	–9.6%	–11.0%	–16.5%	–15.3%
% change immersion (19–21 h)	0.6%	–1.8%	6.6%	0.4%	2.9%	7.7%	7.9%	2.8%
Rehydration rate (19–21 h, mg g^{-1} h^{-1})	3	–8	28	2	13	32	33	12
Final % difference from W_0	–10.3%	–10.8%	–11.3%	–8.2%	–7.0%	0.4%	–4.8%	–6.4%

[P] = pilot run; [†] = frog died; ? = uncertain sex; * = 2 h during immersion +/– 24 h subsequent observation; *n.s* .= not sampled; [§] = times approximate; [¬] = frog had defecated; [Θ] = provided with water; **Bold** = frog killed.

A crude index of individual 1080 dose was obtained by calculating the amount of 1080 present in a portion of toxic bait solution equivalent to the final weight change measured in each frog, and compared to the amount of 1080 present in each toxic group frog (Fig. 6). There was a significant correlation if *L. archeyi* #3, with an excessive weight gain of 50.8%, was excluded as an outlier (r = 0.673, p = 0.049) and a less significant correlation (partial correlation coefficient = 0.7072, p = 0.058) between factors when the variable interval between immersion and sampling was accounted for.

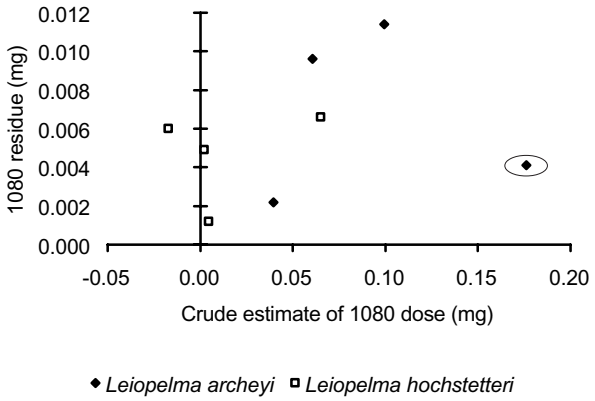

Figure 6. Estimated 1080 dose via water gain during immersion compared to the amount of 1080 found in toxic-treated frogs in Trial 1. An outlier is circled.

Dehydration and rehydration

Mean weight loss at 19 h compared to corrected initial weight W_0 was 21.5% in the toxic group *L. archeyi* (15.8% control) and 14.0% in the toxic group *L. hochstetteri* (13.1% control). All frogs were substantially dehydrated before immersion although *L. hochstetteri* had not quite attained target weight.

Leiopelma hochstetteri exhibited less weight change during both dehydration and immersion than *L. archeyi* (Mann-Whitney *U*, $p = 0.021$ in each test), and a significantly smaller weight change during rehydration than dehydration (Wilcoxin signed ranks test, $p = 0.12$). The extent of dehydration seems to relate directly to volume:surface area (crudely indexed by weight/snout-vent length), however, rather than differences in species, as small or lean frogs generally lost a greater proportion of their weight (Fig. 7; r = –0.576, $p = 0.01$).

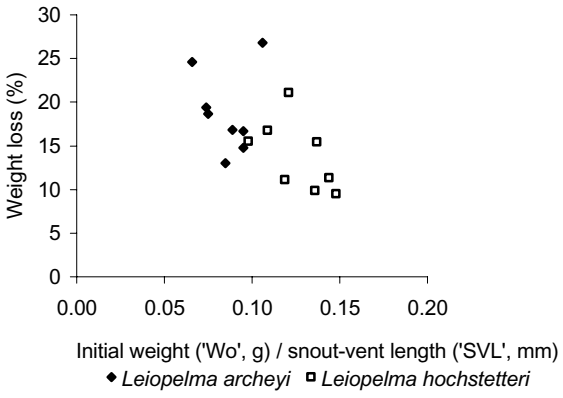

Figure 7. Comparison of crude volume:surface area ratio (weight/length) with extent of controlled dehydration in Trial 1 frogs.

Exaggerated weight loss of > 20% during dehydration was recorded in three frogs which defecated immediately prior to weighing at 19 h; a stronger correlation between small size and weight loss was found when these data points were omitted (r = –0.689, p = 0.007).

Average rehydration rates were 77 ± 44 (95% confidence interval) mg g^{-1} h^{-1} in *L. archeyi* and 14 ± 11 mg g^{-1} h^{-1} in *L. hochstetteri* (17 ± 10 mg g^{-1} h^{-1} for *L. hochstetteri* with positive weight gain, i.e. excluding #19; negative weight change may result from urination).

Proportional weight change during immersion was more variable in toxic groups than controls. In *L. archeyi* this ranged from 5.2 to 54.3% of W_0 in toxic solutions (mean 23.6%) and 10.6–18.1% in controls (mean 13.9%; Table 5). *Leiopelma hochstetteri* weight change was –1.8% to 6.6% in toxic solutions (mean 1.5%) and 2.8 to 7.9% in controls (mean 5.3%; Table 6).

No significant difference was found between conspecific toxic and control groups in their extent of dehydration or rehydration, or in rehydration rate (mg g^{-1} h^{-1}) (Mann-Whitney U test, p >0.05).

Behaviour and survival

The pilot *L. hochstetteri* (#21) died in Trial 1. Apparent distress symptoms were seen c. 8 h after immersion, i.e. rapid buccal skin movement, non-water conserving posture with forelimbs held out, sprawled out rear limbs, apparent spasms, and reduced rear limb control. At c. 16 h after immersion the same frog showed curled digits, a dry dorsal surface, white watery faeces, and white specks on the limbs, sides, and ventral surface. The frog died 4 h later. The pilot *L. archeyi* (#1) also showed rapid buccal skin movement at c. 23 h post-immersion.

Dehydration in the pilot frogs was indicated by continued weight loss, reaching 28.4% (*L. hochstetteri* #21) and 17.5% (*L. archeyi* #1) less than W_0 by 18 h after the immersion phase, c. 39 h into the trial. At that time the *L. hochstetteri* was placed in a petri dish of distilled water, and the *L. archeyi* offered moistened paper; both subsequently gained weight. A record of at least 92% RH was taken from the *L. archeyi* tank 9 h after immersion.

In the main run, one *L. archeyi* (#2) from the control group died prior to immersion; it showed no obvious symptoms of poor health, and lost 16.7% body weight during dehydration. The single *L. archeyi* and *L. hochstetteri* retained for post-immersion observation in the main run showed none of the distress symptoms seen in pilot frogs.

Three of the six remaining *L. archeyi* in the main run were found partially or completely out of the bait solutions during the immersion phase, two of these twice (control #9 and toxic group frog #5). Frog #10 was found with only cloaca and hind limbs in a toxic solution and gained little weight in the immersion phase (–23% body weight at 21 h compared to W_0), but partially rehydrated from moisture available during observation. The *L. archeyi* were generally immobile during post-immersion observation, with occasional climbing attempts. The *L. hochstetteri* were quite settled during immersion, but more active during post-immersion observation, changing position between checks more often than *L. archeyi* and one individual escaping twice.

Observations showed all *L. hochstetteri*, but fewer *L. archeyi* were touching bait for an undetermined length of time during immersion.

3.2.3 Trial 2: Exposure to 1080 pollard bait in terraria

Individual frog data and treatment regimes are given in Table 7. Toxic group *L. archeyi* averaged 28.2 mm SVL and 2.1 g initial weight (control 29.6 mm SVL, 2.2 g). Toxic group *L. hochstetteri* averaged 29.4 mm SVL and 2.1 g initial weight (control 31.9 mm SVL, 2.9 g).

1080 residues of ≥ 2.4 mg kg^{-1} were found in the single frog of each species sampled after 10 days exposure to toxic bait. Remaining toxic group frogs were found dead 28 days after baits were added (see below) with lower 1080 residues of ≤ 0.8 mg kg^{-1}, after an unknown degree of post-mortem detoxification; they were last seen 7–14 days after baits were added.

Toxic residues were also measured in the two control frogs assayed, one of which (*L. archeyi* #25) died early in the trial following an extended period of abnormal behaviour. It was exposed and immobile most of the time, sometimes raised against the glass, and continuously lost weight. Providing a continuous food supply from day 4 was unsuccessful; the frog was found on its back with hind legs outstretched and died some hours later on day 8.

Behavioural data should also be interpreted cautiously as frog visibility was highly variable, and generally very poor because of condensation and concealment by leaf litter. No attempt has been made to account for behaviour and mortality in relation to relative 1080 residues because of the small sample size and evidence of contamination from assay results. Video footage was not analysed for the above reasons. Extended periods of immobility were observed in one frog (#25, described above), but no behaviour believed to be symptomatic of 1080 poisoning.

TABLE 7. TRIAL 2 RESULTS—EXPOSURE TO 1080 PELLETS IN TERRARIUM.

	Leiopelma archeyi				*Leiopelma hochstetteri*			
	1080 GROUP		CONTROL GROUP		1080 GROUP		CONTROL GROUP	
Frog reference	#9	#6†	#25†	#11	#18	#16†	#23	#13
SVL (mm)	29.8	26.6	29.8	29.3	29.8	29	33.3	30.5
Apparent sex	F	?	?	F	?	F	M	F
Weight (g) 13 May 96	2.45	1.71	2.09	2.23	2.01	2.20	3.12	2.75
No. days observed	10	38	8	38	10	38	10	38
No. times seen*	20	3	67	1	64	4	26	1
No. of sightings (and %) within bait quarter*	5 (25%)	0 (0%)	1 (1.5%)	0 (0%)	18 (28%)	1 (25%)	5 (19%)	0 (0%)
Dates								
Start exposure	14 May	14 May	14 May	14 May	14 May	14 May	14 May	14 May
End exposure	24 May	24 May	22 May	24 May	24 May	24 May	24 May	24 May
Frog sampled	**24 May**	21 Jun†	22 May†	*n.s.*	**24 May**	21 Jun†	**24 May**	*n.s.*
Final 1080 (mg kg^{-1}) frog	2.4	<0.1†	1.4†	*n.s.*	3.8	0.8†	0.7	*n.s.*

* = from trial notes at intervals of c. 45 minutes or more; † = frog died (#6 and #16 bodies found, date of death uncertain); ? = uncertain sex; **Bold** = frog killed; *n.s.* = not sampled.

Data were drawn from observation checks at intervals of no less than 45 minutes (Table 7); records of frogs under their cardboard shelter were ignored as in some instances these notes were mistaken due to poor visibility. Three individuals were seen in the toxic bait quarter of their terraria in frequencies equating to random proximity (25–28% of sightings), indicating no attraction to or avoidance of 1080 baits. The most contact was seen in *L. hochstetteri* #18 which rested near the bait for several hours at times, and on occasion transported or rested on bait fragments. In contrast *L. archeyi* #6 was seen only three times outside the shelter (and only briefly under the shelter itself) and never near the bait. No conclusions about bait attraction are made for the control group because of insufficient sightings of most animals and the abnormal behaviour of frog #25.

The low frequency of frog sightings combined with a non-disturbance regime delayed the discovery of poor condition, and mortality, in concealed frogs. Two *L. hochstetteri* escapes into adjacent chambers probably occurred when their glass covers were removed to spray distilled water, or clean off condensation to improve visibility. One was found within 3½ h in good health (frog #18). The other escapee (frog #16) was dead when revealed at the trial conclusion on day 38; it was quite mobile when last seen on day 8, appearing to retreat under the shelter in response to light or movement associated with observer entry. The body of *L. archeyi* #6 was also found on day 38, last seen on day 15 partially hidden by a leaf fragment. Each dead frog was hidden in leaf litter, and showed no signs of being unwell previously during low numbers of sightings. Dates and cause of death could not be determined, but partial desiccation, and mould on the *L. archeyi*, suggest death occurred several days earlier.

A strong smell of cinnamon was noted in the enclosed terraria, derived from 1080 baits which incorporate the compound as a lure for possums.

3.2.4 Trial 3: Exposure to 1080-contaminated prey

Individual frog data and treatment regimes are given in Table 8. Frogs are lettered, cf. numbered, because of uncertainty about frog identity by the start of Trial 3. Only F and E were reliably identified as frogs #15 and #20, respectively.

TABLE 8. TRIAL 3 RESULTS—EXPOSURE TO 1080-CONTAMINATED PREY.

	Leiopelma archeyi			*Leiopelma hochstetteri*			
BAIT TYPE FLY/BAIT CONTACT	1080 CASUAL	1080 CLOSE	CONTROL CLOSE	1080 CASUAL	1080 CLOSE	CONTROL CASUAL	CONTROL CLOSE
Frog reference	H	I	J	C	E	B	F
SVL (mm)	23.5	29.7	29.3	30.4	32.9	30.5	38.2
Apparent sex	?	F	F	?	F	?	F
Weight (g)							
22 Jan 97	1.4	2.7	2.6	2.8	3.8	2.9	6.1
Change to 23 Jan 97	+0.3	+0.3	0.0	+0.4	+0.5	–0.1	–0.2
Final 1080 (mg kg^{-1})							
Frog	0.41	0.66	*n.d.*	1.3	2.6	*n.d.*	*n.d.*
Fly sample	33	91	*n.d.*	31	26	*n.d.*	*n.d.*

? = uncertain sex; *n.d.* = not detected.

1080 was found in all four frogs given access to flies exposed to toxic bait, in concentrations ranging from 0.41 to 2.6 mg kg^{-1}, and was not detected in control frogs or flies. Close contact toxic group fly samples had a greater mean 1080 concentration (58.5 mg kg^{-1}, SEM = 32.5) than casual contact samples (32 mg kg^{-1}, SEM = 1), but were also more variable. Within each species, the frog enclosed with 'close contact' flies had a higher 1080 concentration than that with 'casual contact' flies. Residues of toxic group *L. archeyi* were lower than *L. hochstetteri* in the same regime.

Weight change over the trial period was positive in toxic group frogs (11–21% gain), but zero or slightly negative in controls (0–3% weight loss). Control frogs appeared slightly drier than the toxic group at final weighing. Toxic group *L. archeyi* averaged 26.6 mm SVL and 2.1 g initial weight (single control, see Table 8). Toxic group *L. hochstetteri* averaged 31.7 mm SVL and 3.3 g initial weight (control 34.4 mm SVL, 4.5 g).

4. Discussion

4.1 FROG POPULATION MONITORING, TAPU AREA

Leiopelma hochstetteri

Monitoring detected no change in *L. hochstetteri* counts associated with the 1080 drop, however, power analysis indicated only a very small probability of detecting a real impact given the frog numbers found at Tapu (Section 3.1.3). No clear conclusion can be drawn from the frog data collected.

Leiopelma archeyi

Count data suggest the *L. archeyi* population was not affected by the 1080 pest control operation. Declines in both treatment and control areas over June to September implied a cumulative habitat disturbance factor or seasonal factor affected frog counts. Higher final counts in October 1995 favour the latter explanation, illustrated by periodic flooding over parts of transect A5. The literature (Turbott 1942; Bell 1978; Bell et al. 1985; Cree 1986, 1989; Thurley 1996) reporting increased *L. archeyi* emergence—even in daytime hours—in wet conditions, may also favour this explanation. Variation in counts is attributed to frog movement in general, including emergence or retreat into immovable rock piles as well as lateral movement out of transects. Frog size and monitoring dates assure that differences in search results were not due to breeding, while immigration was unlikely as the species seems quite territorial (Bell 1985a, 1997b, c).

Higher monthly counts on treatment transects than control transects on all but one occasion suggest the former may encompass slightly better frog habitat, contributing to the relatively smaller decline in frog counts found there through winter and early spring. TWINSPAN analysis indicated the vegetation and proportion of rocks and bare ground in the two areas were not substantially

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