different. The control area was sited on and below a ridge, however, including greater exposure, greater slope, and patches of more open vegetation compared to the treatment site. Other unmeasured habitat parameters could affect frog movement and abundance including food availability, rock pile depth, humidity (wind exposure, mist, and rainfall), water hazard (puddling), and temperature. Reduced competition for invertebrate prey by possums and rodents was expected following aerial 1080 application to the treatment area, but would be of short duration due to the rapid recovery of rodent populations within c. 3–4 months (Warburton 1989; Innes & Williams 1991) and immigration of both possums and rodents from the adjacent non-treatment area. Moreover any increase in prey abundance would not influence frog count data in this study unless it resulted in migration or changed the probability of detecting frogs. Bait dust may drift outside treatment boundaries (Wright et al. 2002), but was probably minimised in this operation by wet weather during and after the 1080 drop.

Local *L. archeyi* population decline was anecdotally dated from late 1996 as reasonable numbers were present earlier in the year (D.G. Newman, DOC pers. comm.), and has since been tentatively associated with chytrid fungus (Bell et al. 2004).

4.1.1 Symptoms of 1080 poisoning

One *L. archeyi* was found foaming at the mouth (Section 3.1). This was not identified in literature or captive trials (this study) as a symptom of 1080 poisoning in frogs and may relate instead to characteristics of prey items as it was observed previously in the Maud Island frog *L. pakeka* during feeding (B.D. Bell, pers. obs.). Foaming at the mouth has been reported from 1080-poisoned shingle-back lizards (*Tiliqua rugosa*), however, with symptoms otherwise largely akin to spotted grass frogs tested concurrently (McIlroy et al. 1985). 'Excessive salivation' has also been reported in the early stages of poisoning of various mammalian carnivores.[4]

Other possible symptoms of 1080 intoxication such as immobility, outstretched hind limbs, blinking eyes, attempts to regurgitate, shallow breathing and twitching legs or toes seen during frog captive trials (McIlroy et al. 1985; this study) were not seen during fieldwork but may have been difficult to detect. For example, cases of paralysis might not be recognised during brief observation, while periods of convulsions as described by Chenoweth & Gilman (1946) may not overlap with transect search times.

Eisler (1995) noted the latent period before onset of symptoms may complicate calculation of non-target impacts as poisoned animals may leave the area during this time. The scale of transects relative to frog movements, and the placement of monitoring areas within a large poison operation area implies this was not a factor with regard to *L. archeyi*. However the *L. hochstetteri* search areas H1–6 were not equivalent to their adjacent forest surroundings, and accordingly any tendency of poisoned animals to leave the creeks would affect monitoring results.

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[4] For example, dog (Egekeze & Oehme 1979), and also among feral cats, dingo *(Canis familiaris dingo),* and marsupial test subjects (McIlroy 1981b).

4.1.2 1080 bait

A number of factors affect the reliability of bait survey information, particularly the delay in surveying. A further complication was the need to hand-sow 1080 bait on some roadside creek transects; the effect of simulating aerial application was thought to be negligible. Hand sowing confirmed that bait surveys may have missed more 1080 baits adjacent to creek transects than in forest floor habitat, where they are more visible, but this may not account for the much smaller bait densities seen in creeks. A gap between flight paths is another possibility suggested by very low bait densities on creeks H3 and H4, which lay on the edge of the flight paths flown on 7 June, before poor weather delayed further application.

The contrasting habitats may explain some of the difference in bait densities and suggest *L. hochstetteri* may be less exposed to 1080 in typical steep hill creeks than either species on flatter forest floors, depending on habitat use (stream v. banks v. surrounding forest area) and bait breakdown. The sloping sides of the creeks increase the surface area covered by the baits applied. Local clumping may result from down slope movement, but baits reaching the watercourse are likely to detoxify, disintegrate, or be removed from the site more rapidly than baits in carcasses or on the ground. Water and soil research indicate that 1080 does not cause lingering contamination; even brief, very low levels of water contamination have rarely been recorded from pest control operations (Eason et al. 1992, 1993, 1994b; Hamilton & Eason 1994; Parfitt et al. 1994; Walker 1994; Meenken & Eason 1995). The flow-on pollution effect (if any) is negligible in the creek headwaters used in this study and favoured by *L. hochstetteri*.

Rainfall followed shortly after bait application in this pest control operation[5] and may affect comparability of this study to other operations in drier conditions which retard 1080 leaching and biodegradation (Wong et al. 1992; Eason et al. 1994a; Parfitt et al. 1994), although extended dry winter conditions are unlikely in the high rainfall and frequently misty areas with which *L. archeyi* are associated (Archey 1922; Turbott 1942; Stephenson & Stephenson 1957; Bell 1978; Bell et al. 1985; Thurley 1996).

The density of baits remaining on transects A1–3 following the operation suggests the sowing rate of 5 kg ha^{-1} (range 5.4–7.2 kg ha^{-1}) was higher than required. A subsequent operation in 1999 also used 5 kg ha^{-1} (Buchanan 2003). The most recent operation in 2002 used 2 kg ha^{-1} of 12 g bait (A. Styche, DOC pers. comm.), and probably represents a smaller risk to frogs as fewer baits are likely to remain after the initial possum and rat bait take. All operations used bait with c. 0.15% toxic loading (DOC 1995; Buchanan 2003; A. Styche, DOC pers. comm.).

4.1.3 Monitoring design

The highly variable frog counts in this study emphasise the need to control external sources of variation, including observer differences, recent weather, habitat, and site disturbance. An area-based index of counts made by consistent personnel is recommended over search effort indices or ad hoc personnel assignment (Perfect 1996).

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[5] Rainfall of 69.0 mm over 8 days at Coroglen, and 76.8 mm over 10 days at Whitianga.

Disturbance over multiple visits seemed to be successfully minimised by consistently omitting sites likely to be damaged or destroyed by examination (although potentially missing occasional frogs by doing so), placing feet carefully at all times on transects, walking in water or mud rather than on stones along stream transects, and avoiding the ground within forest floor transects after searching. Replicated control and treatment sites would be preferable to further reduce bias.

Searches covered the full range of available retreat types, including vegetation, logs and rocks, as either species may show a preference for specific retreat types (Thurley 1996; Perfect 1996) capable of affecting density estimates. Frog activity and counts are affected by recent climate, particularly moisture availability, as are relative frequencies of retreat types occupied (Bell 1978; Cree 1986, 1989; Slaven 1992; Thurley 1996; Perfect 1996), but ensuring that counts occur in comparable conditions is difficult. Searching all forest floor transects on one day, and all creek transects on another during each visit was a partial answer to differentiate climatic effects from control/treatment effects on frog counts (Perfect 1996).

Power analysis shows that a small, immediate impact on *L. archeyi* or *L. hochstetteri* is highly unlikely to be detected using this monitoring method, given the highly variable count data typical of these species, and indicates that more than one study is required to confidently accept the safety of native frog populations during 1080 operations. Test power is not substantially altered by mild seasonal effects or differences in numbers between treatment and control areas, so some inequalities (e.g. retreat site density) may be acceptable. However, differential seasonal effects may simulate or camouflage a population decline. The extended monitoring period helped to distinguish seasonal from disturbance or treatment effects.

Any comparable future study may require more extensive search effort to obtain sufficient frog counts, preferably using enough searchers that all treatment and control sites for the same species can be searched on the same day. Replication can be introduced and the potential for site disturbance or seasonal effects reduced by covering a larger total search area fewer times, than possible with only two searchers in this study. Intervals between counts must be sufficient to maintain statistical independence.

The transects in this study are too close to the possum control operation boundary (and hence re-invasion) to allow long-term monitoring of change in relation to pest control. More recently a decline in *L. archeyi* populations at Tapu and elsewhere indicate the species requires close attention. A long-term demographic study with mark-recapture conducted at Tapu by Bell (Bell et al. 2004) is better suited to this purpose than the anonymous frog count transects established for this study.

4.1.4 Summary

This short-term study found no evidence of a detrimental impact on the Tapu *L. archeyi* population from a single aerial application of 1080 cereal bait for possum control. Frog counts in both the treated and adjacent control area declined for a time, but a relative increase in the treated area compared to the control area toward the end of the study suggests this was a natural fluctuation.

L. hochstetteri data showed no evidence of a decrease associated with 1080, but no conclusion could be drawn as the variable, low monthly counts obtained for this species provided poor statistical power to detect a population decline.

Rapid poison breakdown was implied by high rainfall following bait application, and may affect the applicability of this study to other operations where low rainfall or a period of fine weather following bait application extends the period of toxicity. This study addressed only 1080 broadcast in cereal pellets and does not consider the effects of operations using other toxins, or other means or rates of poison application.

4.2 LABORATORY TRIALS

The results of the laboratory trials are discussed below including behaviour and mortality, and trial limitations. Fundamental constraints of small sample size, lack of replication, and unknown 1080 dosage rate arise from the trial design and limited availability of subjects. However, further difficulties were associated with maintaining frogs in a captive environment, interpreting frog behaviour, and with the existence (and delayed detection) of contamination in Trials 1 and 2 and in stock bait (below).

Rehydration in a toxic bait solution, extended proximity to poison bait, and feeding on poisoned prey resulted in 1080 tissue residues in both L. *archeyi* and *L. hochstetteri*. Some degree of oral uptake is possible in addition to cutaneous absorption of solution, but was not observed during Trial 1. Uptake through soil and litter contact, and (in at least some cases) via direct bait contact has been assumed in Trial 2 from the test results and previous work showing 1080 transmission in soil or litter (Corr & Martire 1971; Parfitt et al. 1994, 1995).

4.2.1 Mortality and symptoms

Individual assay results cannot be readily interpreted because the relationships between 1080 poison residues, dosage and metabolism are uncertain (Heyward & Norbury 1998; Gooneratne et al. 1995), the effects on frogs not commonly studied, and because contamination affected some trials. Limited examples in the literature suggest muscle residues can be variable and may be low, compared to the administered dose or to published LD_{50} figures, in animals killed by poisoning. For example, residues in ferrets (LD_{50} = 1.0–1.4 mg kg^{-1}) ranged from 0.68–1.8 mg kg^{-1}, and were 0.003 mg kg^{-1} in a ferret which died two days after consuming c. 1.7 mg kg^{-1} (Hugghins et al. 1988; Eisler 1995; Heyward & Norbury 1998). However, the 1080 tissue residues of up to 4.1 mg kg^{-1} found in these trials seem too low to suggest lethal poisoning compared to the 54.4–2000 mg kg^{-1} range of published lethal dose data for amphibians (Section 1.2).

Two control frogs, three toxic group animals, and one frog in the stock tank died during the laboratory trials, however, no deaths could be ascribed conclusively to 1080 poisoning. None of these frogs had residues > 1.4 mg kg^{-1}, although post-mortem detoxification may have affected two animals in Trial 2. Environmental stress was as likely an explanation in each case of death, and dehydration may have played a major role in the toxic group fatalities seen. In

Trial 1 the pilot *L. hochstetteri* lost substantial weight during post-immersion observation, whereas the later toxic group *L. hochstetteri* with similar 1080 tissue residues was given more moist conditions, and did not lose weight or share the symptoms expressed by the pilot frog.

Fatal dehydration was not expected in Trial 2 because each frog had access to water in a Petri dish, and moist conditions were indicated: by initial humidity readings of 95–100% RH in terraria, 96% RH ambient; rapid bait surface deterioration and mould growth; prolific condensation on covers; damp cardboard shelters; ad lib. spraying of distilled water; and the provision of litter to improve frog moisture retention.

Individual frogs were considered 'quite dry' on occasion despite the above factors, as were the terraria themselves at trial end, when surviving frogs seemed thin, but otherwise healthy. Observation checks were infrequent by this time, however, water dishes remained available, with the exception of an escapee whose death could have resulted from simple moisture loss. The two dead frogs were desiccated on recovery; it is unclear how long each had been dead when discovered, or how much desiccation occurred before or after death.

Frog condition in these trials may be explained by earlier laboratory and field experiments by Cree (1986) which documented dehydration on a dry substrate, slower rehydration rates in frogs unsettled by experimental treatment, and reduced activity in *L. archeyi* at relative humidity below 99%. The possibility that frog water balance was affected by a physiological or behavioural response to 1080 (e.g. Chenoweth 1949; this report Section 1.2) cannot be discounted, however, and may be worth investigating further in the literature or with less endangered frog species. The 'super-hydration' of poisoned frogs reported by Chenoweth (1949) was not observed; two *L. archeyi* were substantially (c. 15%) heavier than their initial weight by the end of Trial 1, but one was a control frog.

Recognised symptoms of poisoning were not seen, but some aspects of the behaviour and appearance of the pilot *L. hochstetteri* in Trial 1 were suspect, particularly the white watery faeces, and white specks on the limbs, sides and ventral surface. The timing of these symptoms corresponds with the latent period reported for 1080-poisoned spotted grass frogs (McIlroy et al. 1985), although death was more rapid in the pilot *L. hochstetteri* and the residues in this individual were not high (1.3 mg kg^{-1}). Similarly a sprawled posture, apparent spasms, and reduced rear limb control seen in this frog may equate to the convulsions and flaccid paralysis reported by Chenoweth & Gilman (1946) as products of fluoroacetate acting primarily on the nervous system in frogs,[6] however the same symptoms have been observed recently in chytrid infected *Leiopelma* (S. Carver, VUW, pers. comm.). The white flecked skin and faeces noted above have not been seen in New Zealand chytrid cases (S. Carver, VUW, pers. comm.; B. Waldman, University of Canterbury, pers. comm.; T. Thurley, DOC, pers. comm.).

General lethargy, weakness, dry skin, or a water preservation posture have commonly been observed when dehydrating *L. archeyi* and *L. hochstetteri* in

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[6] From examining 'very few frogs', species not noted.

the laboratory, while rapid buccal skin movement in Trial 1 pilot frogs and lack of a water preservation posture in several *L. archeyi* during the controlled dehydration phase may be symptomatic of stress in general (S. Carver, VUW pers. comm.; this study).

Two frog deaths were attributed to general stress. The first (*L. archeyi* #2) occurred apparently in response to weight loss in the dehydration phase of Trial 1, without experimental 1080 exposure. The second (*L. archeyi* #25) died in Trial 2. The latter was held in an urban garden until brought in as a control for Trial 1 (substituted for a frog which died before trials began), so was not well-habituated to laboratory conditions. It was considered lean one week later at the start of Trial 2. No frogs died in Trial 3, which was shorter and less manipulative than earlier trials.

4.2.2 Contamination

Contamination was discovered in Trial 1 and 2 control material, and stock control baits, when the first of two batches of material was assayed for 1080. The smallest amounts ($\leq$ 0.1 mg kg^{-1}) cannot be written off as an interferent agent as higher residues occurred in contemporary samples. The 0.2 mg kg^{-1} 1080 found in stock (unused) control bait, could be due to contaminated supply materials (G. Bentley, AgriQuality NZ Ltd pers. comm.), but was too small to account for the amounts found in control samples from the trials. Further assays of stock bait were not possible in the scope of this study.

The flaw in Trial 1 was attributed to handling error when removing frogs from bait solutions as smaller residues ($\leq$ 0.1 mg kg^{-1}) were found in the control solutions handled before processing the toxic groups, compared to those handled later (0.2 mg kg^{-1}). Surface contamination was indicated by high tissue residues in a control frog compared to the corresponding bait solution,[7] in contrast to the toxic group which all had tissue residues around two orders of magnitude lower than their bait solutions. Traces in frogs not exposed to bait but frozen on the same day (one found dead in stock, one died during dehydration) may be linked to handling around this time.

Trial 2 was conducted before assay results indicated tighter hygiene protocols were needed. The two control frogs and one toxic group frog assayed for 1080 in Trial 2 were also used as controls in Trial 1, six days before the second trial began.[8] While the degree of initial contamination and remaining tissue levels after two weeks is uncertain, the 0.7–1.4 mg kg^{-1} residues found seem to point to continued hygiene issues. Handling error is suspected.

No evidence of contamination was found in samples from Trial 3 or the remaining stock animals. Four frogs in this trial had previously been controls in Trials 1 or 2.[9] Other studies (Eason et al. 1993; Gooneratne et al. 1994; Eisler 1995 and references therein) suggest any slight 1080 residues left from that time would be detoxified or excreted during the long interval before the last trial; assay results for Trial 3 seem to confirm this.

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[7] *L. hochstetteri* #22, 0.4 mg kg^{-1} v. 0.1 mg kg^{-1} in solution.

[8] *L. archeyi* #9 and #25, *L. hochstetteri* #23.

[9] *Leiopelma hochstetteri* E and F in Trial 1, *L. hochstetteri* B and *L. archeyi* J in Trial 2.

4.2.3 Other aspects of trials

With the exception of the pilot *L. hochstetteri* in Trial 1 there was no obvious behavioural difference between toxic and control groups in any trial. Behavioural observations were problematic, as noted previously.

In Trial 1 the greater proportion of weight lost in dehydration in the *L. archeyi* compared to *L. hochstetteri* was attributed to the greater surface area:volume ratio of small frogs, but the greater rehydration seen relates to their physiology, and ecology (Cree 1985, 1986, 1988, 1989). *Leiopelma archeyi* have an accelerated water uptake response and reduced urination when dehydrated as an adaptation to their terrestrial lifestyle, accounting for the correlation in proportional weight change during de- and rehydration. The semi-aquatic *L. hochstetteri* does not share these traits and could not replace lost body weight during the 2 h immersion phase of Trial 1.

Rehydration rates in this trial were slow and variable in each species compared to studies which quantified and corrected for urination (Cree 1986).[10] Urination was not considered in this study but probably contributed to the case of slight weight loss in one *L. hochstetteri*, and some weight change in other animals. Stress may also affect results by reducing rehydration rates (Cree 1986, 1989). The solution avoidance behaviour observed in some individuals corresponds with previous findings that *L. archeyi* dehydrated to 85% of initial weight take up to an hour to regain lost weight and when hydrated may struggle to escape, or become comatose if left in water (Cree 1986). It may also result from unfamiliar conditions. The response seems unrelated to 1080 because it occurred in both toxic and control groups, well within the published minimum latent period of 13 h for amphibians (McIlroy et al. 1985), and was not seen in *L. hochstetteri*.

The strong cinnamon smell noticed in terraria during prolonged bait exposure in Trial 2 prompted a brief literature review which suggests cinnamon could affect frogs, but did not clarify whether the concentrations reached were sufficient to do so. Cinnamon has induced a variety of responses in tested species, including insecticidal, antimicrobial, antifungal and herbicidal properties described from essential oils (e.g. Chang & Cheng 2002; Tworkoski 2002), and fewer invertebrates are observed on baits lured with cinnamon compared to those without (Spurr & Drew 1999). The enclosed conditions of Trial 2 exaggerated the smell (and effect, if any) of cinnamon compared to field situations; frogs in their native habitat are highly unlikely to be at risk because neither air nor frog movement are restricted.

Houseflies were used in Trial 3 to investigate secondary poisoning as they were readily available, from a consistent environment, and formed the frogs' normal diet in captivity. The diverse range and behaviour of prey species, and normal frog behaviour and movement would affect the amount of secondary 1080 poisoning likely in their natural habitat, and may have affected results in this trial. Similar weight gains in the toxic group frogs of both species imply that the greater 1080 concentrations in *L. hochstetteri* than *L. archeyi* do not result from greater fly intake. In fact a comparison of the tissue residue and estimated 1080

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[10] 123 ± 19 mg g^{-1} h^{-1} in *L. archeyi* and 33 ± 8 mg g^{-1} h^{-1} in *L. hochstetteri*.

dose of each individual frog, calculated from weight gain and fly 1080 residues, found the estimated doses were between 9 and 14 times higher than residues detected in *L. archeyi*, and 0–7 times higher than residues in *L hochstetteri*.[11]

Prey choice combined with variable fly 1080 levels, as found in fly samples, seems a likely mechanism for these differences. Flies might be less active when intoxicated, as found in cockroaches which suffer a lack of co-ordination, lethargy, and other altered behaviour (McIntyre 1987). Theoretically this could make poisoned flies less attractive prey to frogs, which are visually stimulated predators. If so this suggests the fly samples taken at the end of the trial overestimate average 1080 concentrations, as more intoxicated flies would be easier to catch and less likely to have been eaten than those less affected.

The interspecific differences in residues of each frog species may relate to differences in normal prey and feeding behaviour. The greater tendency of *L. archeyi* when foraging (Cree 1986) to climb upward where less intoxicated flies probably predominate, could focus their feeding on these flies, for example. This would result in a bias toward more intoxicated flies remaining at trial end and contribute to the relatively high residues of the *L. archeyi* close contact regime fly sample.

The toxic group frogs of both species seemed slightly more moist than the controls at trial end, implicating hydration levels as a factor in the weight increase among the toxic group (Chenoweth 1949).

4.2.4 Experimental design and captive maintenance

The limited scope of this study constrained some aspects of this work. The relatively short length of Trials 1 and 3 compared to published latent periods and response times for other amphibian 1080 studies (e.g. Section 1.2) is not ideal and reflects the trade off with a small group of subjects between waiting to see if symptoms develop, and quicker termination to enable detection of tissue residues.

Laboratory maintenance of native frogs can be problematic, particularly when experimental stress is applied. Prolonged captivity prior to Trial 1 gave the frogs ample time to acclimatise but may have increased environmental stress when experiments commenced. Cree (1988) cites findings from experimental in vitro research on frog skin that increased time and wetter conditions in captivity both resulted in reduced water uptake through the skin, which might affect moisture balance in captive animals. The effect of unnaturally constant temperatures and humidity during their extended captivity on the frogs' ability to adjust to environmental changes during the trials is uncertain, and likewise the effect of their constant diet. Most individuals lost weight in captivity between capture and their first use in any trial: from –37% to +3% weight change in *L. archeyi*, and from –14% to +1% in *L. hochstetteri*.

Attempts to complement the study with behavioural observations were of variable success. Behavioural disruption was suspected due to artificial conditions, applied experimental stress and handling, while attempts to address

[11] Calculated from fly 1080 residues from (1) individual terraria samples; (2) average residues for each regime (close or casual); and (3) the mixed toxic fly sample.

hydration requirements (e.g. addition of litter, misting) resulted in poor visibility. Interpretation was also restricted because the subjects are relatively inactive animals for which there is little behavioural information.

The moist environment required to maintain the frogs may have affected bait toxicity, however, further bait assays were not possible within the scope of this study.

4.2.5 Summary

1080 residues were found in both native frog species exposed to the poison in simulations of worst-case scenarios which might be encountered in their normal habitat during pest control by aerially distributed cereal bait. Confounding factors affected the laboratory trials and suggest environmental stress may have caused deaths in frogs of both species in captivity. One *L. hochstetteri* showed symptoms which might indicate poisoning however no deaths in these trials could be definitively attributed to 1080.

4.3 POTENTIAL FOR 1080 IMPACT

Amphibians are up to several orders of magnitude more resistant to 1080 dosing in laboratory experiments than other animals (Eisler 1995). While this suggests New Zealand's native frogs are unlikely to be at risk, their resistance to 1080 is uncertain and their small size in relation to the targeted pests may affect their susceptibility. An average 5 g bait containing 7.85 mg 1080 constitutes a single lethal dose for most possums (DOC 1994a), but contains from 10 to 72 times the amount required to kill a 1.5 g frog (average weight for *L. archeyi*)[12] based on published lethal dose data for amphibians (Table 1, Section 1.2). Conservative figures should be assumed in the absence of lethal dose data for native frogs, particularly as some of the published data may be derived from species with previous exposure to fluoroacetate-bearing vegetation. Extrapolation of any laboratory findings to field situations also requires caution.

The risks to individuals or populations of a given species also depend on ecological and behavioural factors, poison application parameters, and environmental factors. Among these are ambient temperature, rainfall, and season; availability of alternative foods; and bait components, quality, dispersal, application rate and toxic loading (Batchelor 1978; Oliver & King 1983; McIlroy 1986; Morgan 1994). Population level effects are the main concern of frog conservation and management; occasional individual deaths, while regrettable, may be outweighed by benefits of reduced habitat destruction or represent short-term, recoverable costs.

4.3.1 Potential exposure of frogs to 1080

Possible sources of native frog intoxication after a typical aerial 1080 possum control operation include direct uptake through eating bait, contact with contaminated substrate, or contaminated water, and secondary poisoning via

[12] Derived from the c. 25 mm average SVL using Tapu weight-length data (B.D. Bell, unpubl. info.).

invertebrate prey. The risk of encountering toxic baits or leachates is influenced by bait dispersal, frog habitat use, habitat moisture levels (and thus frog emergence, activity, and degree of dehydration—Cree 1986, 1989), leaching, and the degree of toxin biodegradation by micro-organisms. Rainfall also accelerates 1080 detoxification by defluorination and leaching into the soil (Parfitt et al. 1994). Neither frog species showed an attraction to cereal pellets during field or laboratory work in this study; they are visually stimulated insectivores (Stephenson & Stephenson 1957; Meyer-Rochow & Pehlemann 1990) and seem unlikely to eat cereal baits. However laboratory trials indicate both frog species may acquire a dose of 1080 from contaminated substrate or prey, or rehydration in contaminated water.

Leiopelma archeyi were rarely discovered in close proximity to toxic baits under their rock retreats, and very few potential retreat sites held bait. Poison application around September to November would coincide with frog breeding and brooding, but very rarely result in prolonged close contact by eggs and offspring to baits and leachates. No baits were found in similar positions on creek transects, possibly due to the low bait numbers found generally and swift disintegration in wet conditions. The 1080 concentrations of 2.4–3.8 mg kg^{-1} in early (10 days) frog samples in laboratory Trial 2 probably represent a worst case scenario for these frogs, due to their close confinement in terraria and sampling based on apparent exposure to bait during the trial.

Encounter rates with bait or leachate are likely to increase during moist conditions, which encourage frog emergence, although such encounters may be short-lived. Contaminated water is available in the short-term from baits in moist depressions, or fine fragments on damp surfaces, or when rainfall washes fragments and leachate from the canopy (Meads 1994; Wright et al. 2002) onto the understorey and ground. In fact pellets were more often seen lying on coarse litter or moss on forest transects, or caught in angles between stones, rather than in depressions. Cree (1986, 1989) found 96% of emerging *L. archeyi* were above ground within an hour of darkness on wet nights, on vegetation. Frogs emerging on dry or moist nights stayed closer to the ground, but were fewer than on wet nights (2.6 *L. archeyi* per night, cf. 25.8) and generally stayed out for less than an hour.

Although there is no suggestion in the literature that age affects 1080 tolerance, young *L. archeyi* may be most at risk from contaminated vegetation because of their small size and behaviour. Small *L. archeyi* tended to occupy vegetative retreats at Whareorino (Thurley 1996) and were found in vegetation closer to the ground in nocturnal observation at Tapu (Cree 1989). Contact and systemic insecticidal properties were reported by David (1950) from experimental plants fed or surface treated with 1080 leachate. However a study of three New Zealand possum control operations using standard bait, sowing rates, and 1080 concentrations, found very little 1080 in tested plants in field situations (Wright et al. 2002), and does not suggest any threat to frogs.

Leiopelma hochstetteri may increase their exposure to 1080 by venturing away from creeks, apparently in association with moist ground conditions (Stephenson & Thomas 1945; Stephenson & Stephenson 1957; Bell 1978; Cree 1986; Perfect 1996; this report Section 4.1). Those found on forest floor transects were significantly larger than average for this study, and generally

found in moist sites during visits when the ground was most damp (Perfect 1996). Water monitoring from other 1080 operations strongly suggests there is no risk to *L. hochstetteri* or their aquatic prey from creek water (Hamilton & Eason 1994; Wright et al. 2002) and the risk posed by rare contamination of pools or puddles by pellets or carcasses was considered negligible. Contact with leachate or poisoned prey might occur when eating terrestrial invertebrates along streams (Turbott 1942) or venturing further afield.

Leiopelma archeyi and *L. hochstetteri* feed on invertebrates which may be susceptible to 1080 intake (e.g. beetles, flies, ants, and gastropods—Kane 1980; Notman 1989; Slaven 1992), but comparison of the mass of contaminated prey required relative to frog body weight (Table 9) strongly suggests that lethal secondary poisoning is unlikely. Part of each feed may comprise non-toxic prey, while field and laboratory time series studies of terrestrial invertebrates suggest that 1080 residues are rapidly reduced, limiting the period of risk to insectivores (Eason et al. 1993). In the absence of specific toxicity data for native frogs the most conservative published amphibian LD_{50} rate (54.4 mg kg^{-1} in bullfrogs; McIlroy et al. 1985) was compared to invertebrate residue data. On this basis the average 1.5 g *L. archeyi* would generally need to consume more than its own body weight of contaminated prey to acquire a lethal dose. Simulated worst-case scenarios of weta or flies in close contact with bait required c. 0.63–0.9 g of prey to convey the same dose; 0.3–0.5 g weight increases were recorded over 24 h in 1.4 g frogs in Trial 3 of this study.

Gatherings of invertebrates on baits have been reported previously (Batchelor 1978; Rammell & Fleming 1978; Notman 1989), and feed on pellets predominantly at night (Spurr & Drew 1999; Lloyd & McQueen 2000). Invertebrates were not attracted to bait by day at Tapu in this study; observations during future operations or trials would confirm invertebrate behaviour and indicate whether *L. archeyi* and *L. hochstetteri* are at risk. The frogs are believed to be ambush hunters (Bell 1985b; Cree 1989) and may therefore not be attracted to clusters of insects outside their ambush range.

TABLE 9. ESTIMATED WEIGHT OF 1080-CONTAMINATED INVERTEBRATES COMPRISING A 54.4 mg kg^{-1} DOSE OF 1080 FOR A 1.5 g FROG.*

INVERTEBRATE SAMPLE†	INVERTEBRATE 1080 RESIDUE (mg kg^{-1})	WEIGHT OF INVERTEBRATES (g)	NOTES
Mixed species[3]	minimum = 14.0	5.83	Simulated aerial operation, from bait
Mixed species[3]	mean = 56.8	1.44	Simulated aerial operation, from bait
Tree weta[3]	mean = 130.0	0.63	Simulated aerial operation, from bait (prob. over-estimate as n = 2)
Mixed species[3]	mean = 0.8	102.00	Aerial operation, random sample
Tree weta[2]	mean = 12.0	6.80	Aerial operation, random sample; 2 days post application
Tree weta[2]	maximum = 46.0	1.77	Aerial operation, random sample; 2 days post application
Mixed species[1]	≤ 0.00075	≥ 100 000	Outdoor pen trial; 14 days exposure to bait
House fly[4]	minimum = 26.0	3.14	Laboratory; 1 h close bait contact then 24 h casual access
House fly[4]	maximum = 91.0	0.90	Laboratory; 1 h close bait contact then 24 h casual access
House fly[4]	mean = 32.0	2.55	Laboratory; 24 h casual bait access (n = 2)

* Based on the lowest published amphibian LD_{50} data of 54.4 mg kg^{-1} in bullfrogs from McIlroy et al. (1985).

† Sources: [1] Pierce & Montgomery 1992; [2] Eason et al. 1993; [3] Lloyd & McQueen 2000; [4] this study (Section 3.2.4).

The effect of widespread cereal bait fragments and dust on invertebrates is uncertain, and may potentially be more attractive or accessible because of their size and wide dispersion. However, operation monitoring suggests contamination of litter, vegetative surfaces and soil from bait dust may be very low, and is short-lived (Wright et al. 2002), limiting the risk to invertebrates and insectivores.

Mouldy baits proved unattractive to weta in previous trials (Hutcheson 1989). Mould quickly developed during captive trials in this study, in humid terraria, but was not seen on pellets at Tapu; DOC quality specifications (DOC 1994a) require incorporation of a fungicide in cereal baits. Pellets gradually developed surface damage and disappeared over time, which might be attributable to rain impact rather than insect damage. The baits would have been detoxified by moisture and microbes well before disintegration (Department of Agriculture et al. 2002; Eason 2002). Field pitfall trap studies have found no effect of aerial 1080 operations on invertebrate populations (Spurr 1996).

Pigs can leave conspicuous trails of contaminated material because most (98%) vomit if they eat any 1080 bait (Rathore 1985; O'Brien 1988; O'Brien et al. 1988), however encounter rates with frogs are likely to be low. Native frog populations may be more affected by predation or habitat degradation if pigs are present (Perfect 1996) than by contamination from poisoned pigs. Frogs are not reported to be attracted to carcasses.

The risk posed by different potential sources of intoxication may be influenced by the speed at which the 1080 dose becomes available. A study on pigs found they were more susceptible to externally coated grain than pellet bait, which incorporates 1080 throughout, apparently because the poison could be more rapidly absorbed (O'Brien et al. 1988). This suggests a possibility that contaminated moisture uptake, rapidly distributing 1080 into the tissue, may be a greater threat than equivalent doses obtained through other routes. *Leiopelma archeyi* may be more vulnerable in this respect than *L. hochstetteri* because of their water-limited environment and ability to rapidly rehydrate, as suggested by the relative residues obtained during captive simulations in Trial 1.

4.3.2 Potential effects of 1080

Few studies on the effect of sodium fluoroacetate on frogs have been undertaken and most records relate little more than an LD_{50} estimate; collectively these suggest comparatively large doses by body weight are required to kill amphibians (Eisler 1995; this report Section 1.1). Investigations of sub-lethal effects on biochemistry, physiology, behaviour, and natural selection described from other species are rare for amphibia. The influence of repeated sub-lethal doses, as might be obtained via poisoned prey, is also yet to be explored. Consequences of sub-lethal doses are not always conspicuous and large doses may be necessary before changes occur in groups considered highly resistant to 1080. In this context results from lizard studies suggest possible effects of 1080 in frogs, as amphibia and reptiles are generally considered similar in their high tolerance of the poison and slow metabolism (Chenoweth 1949; Atzert 1971; McIlroy et al. 1985; McIlroy 1986; Twigg et al. 1986).

Defluorination of 1080 is dependent on glutathione (an antitoxin, antioxidant and enzyme cofactor) and results in rapid depletion of reduced glutathione in the liver; fluoroacetate is not effectively detoxified when glutathione levels are low and disruption of the Krebs cycle ensues. Liver glutathione levels are slow to recover in mammals and birds tested, and take up to two weeks to recover in the shingle-back skink (Twigg et al. 1986; Twigg 1994). Affected animals may be predisposed to liver damage and increased sensitivity to any further fluoroacetate intake during this period (Twigg et al. 1986; Twigg et al. 1988b).

Sub-lethal doses of fluoroacetate well below their respective LD_{50} affect fecundity in a variety of mammals (Spurr 1994). In laboratory rats this involved changes in spermatids and spermatocytes, reduced testicle weight and atrophied seminiferous tubules, although regeneration began when dosing stopped (Sullivan et al. 1979). The testes suffer dose-dependent damage when energy production is affected and may be the most vulnerable organ to fluoroacetate because of their high energy requirements combined with low glutathione levels (Sullivan et al. 1979; Twigg 1994). Twigg et al. (1988a) found single high 1080 doses of 100 or 200 mg kg^{-1} reduced plasma testosterone levels and caused degeneration of seminiferous tubules in the shingle-back skink but could not determine whether reproductive success was affected. The impact on lizard populations via this mechanism was deemed likely to be minimal since the above doses are much higher than those likely to be ingested by skinks in a pest-control campaign (Freeman et al. 1996), although Twigg (1994) suggests an influence on evolutionary timescales could explain the high tolerance of 1080 in some Australian fauna in habitats where fluoroacetate-bearing vegetation occurs.

The response in lizards suggests the possibility that 1080 could affect reproductive tissues in frogs, but also that damage may be temporary and requires a higher dose than is likely to be obtained by these species during typical aerial possum control operations.

Laboratory trials in this study did not clarify whether sub-lethal poisoning causes behavioural modification, as the frog poisoning symptoms described by Chenoweth & Gilman (1946) suggest, potentially affecting thermoregulation (McIlroy et al. 1985), water balance or predation risk in native frogs. Chenoweth (1949) noted that allowing cutaneous hydration caused poisoned frogs to die more quickly than those kept dry, with up to 50% weight gain. He also commented that symptoms in cattle were delayed by withholding water, and recovering cattle were very thirsty. This suggested water seeking and water conservation behaviours might be seen in poisoned frogs, but these were not obvious in our trials. Immobility and a tendency to seek shelter have moisture preservation benefits (Newman 1977; Newman et al. 1978; Bell 1985b; Cree 1986, 1989) and may help protect frogs from predators including rats, the introduced green and golden bell frog (*Litoria aurea*), tuatara (*Sphenodon punctatus*), and possibly pigs (Newman 1977; Thurley & Bell 1994; Thurley 1996; Perfect 1996).

Studies of pesticide impacts on the immune response of northern leopard frogs suggest these can be significant (Gilbertson et al. 2003), however a marked decline in the Tapu *Leiopelma archeyi* population by late 1996 (Bell 1999; Bell et al. 2004) has not been linked to 1080 use. Both the treatment and control

sites were affected (Perfect 1997a), and frogs were still comparatively plentiful at Tapu a year after the 1080 operation (D.G. Newman, DOC pers. comm.). Chytrid fungus was discovered in frogs throughout Coromandel over 2001–02, and is a possible agent of rapid *L. archeyi* population declines which seemed to occur from south to north over the peninsula (Bell et al. 2004). The declines are not limited to 1080-treated areas, nor has a decline been identified among populations at Whareorino where possum control using 1080 also occurs.

5. Conclusions and recommendations

The highly variable counts typical of native frogs result in poor statistical power and emphasise the need for replicated population monitoring of both species through further 1080 drops before concluding that 1080 operations have no effect on frog numbers.

Transect counts of *Leiopelma archeyi* at Tapu before and after the 1995 aerial 1080 possum control operation indicate there was no short-term detrimental impact on the population. No impact was found among *L. hochstetteri,* but power analyses show that a decrease was unlikely to be detected because of the low numbers of sightings. High rainfall following the poison application suggests that bait detoxification may have been atypically fast, and limits the applicability of these Tapu results to other 1080 operations over native frog populations.

The risk of potential contact with 1080 may be comparatively greater for *L. archeyi* than *L. hochstetteri* as a result of differences in habitat and behaviour. Higher relative bait densities and longevity were observed on forest floor transects compared to steep-sided creeks in this study (within noted bait monitoring limitations). Neither species was attracted to cereal bait in laboratory trials although they acquired 1080 through chance contact or leachates transmitted through soil, litter, and water during simulated 'worst-case scenarios'. Theoretically a greater proportion of moisture available to *L. archeyi* in their water-limited habitat may be contaminated to some degree in the short term, compared to *L. hochstetteri.* Some vegetative surfaces which *L. archeyi* tend to climb upon or hydrate from may also be contaminated. Frog behaviour suggests each species is most likely to encounter bait during moist conditions which enhance both frog emergence and activity, and hasten the detoxification of poison bait.

Captive studies showed both *Leiopelma* frog species may suffer secondary poisoning. Consideration of available prey residue and amphibian 1080 tolerance data suggests these frogs are unlikely to acquire a fatal dose via prey unless attracted to invertebrates on poison baits. Nocturnal forest observations of interactions between the frogs and their natural range of prey species are recommended to clarify the potential risk from secondary poisoning during pest control operations.

Most frogs remained alive during experimental treatment. Five individuals died in the trials, including three in 1080-treated groups, but environmental factors and captive maintenance issues which confounded some trials may have caused or contributed to their deaths.

Further literature review, followed by laboratory research if required (using more common frog species), is recommended. This would improve understanding of poisoning symptoms and the potential impact of 1080 on amphibian water balance, immune, and reproductive systems, which the literature hints may be affected. No further lethal studies of *Leiopelma* species are suggested at this time.

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Appendix 1

POWER ANALYSIS OF SIMULATED FROG DATA

The statistical power to detect 1080 impact (population decline) in simulated scenarios incorporating area and seasonal effects is presented below as power curves for 10% and 20% significance levels in Figs A1.1 and A1.2 respectively. Power curves for the 5% significance level are presented in Fig. 5 (see Section 3.1.3).

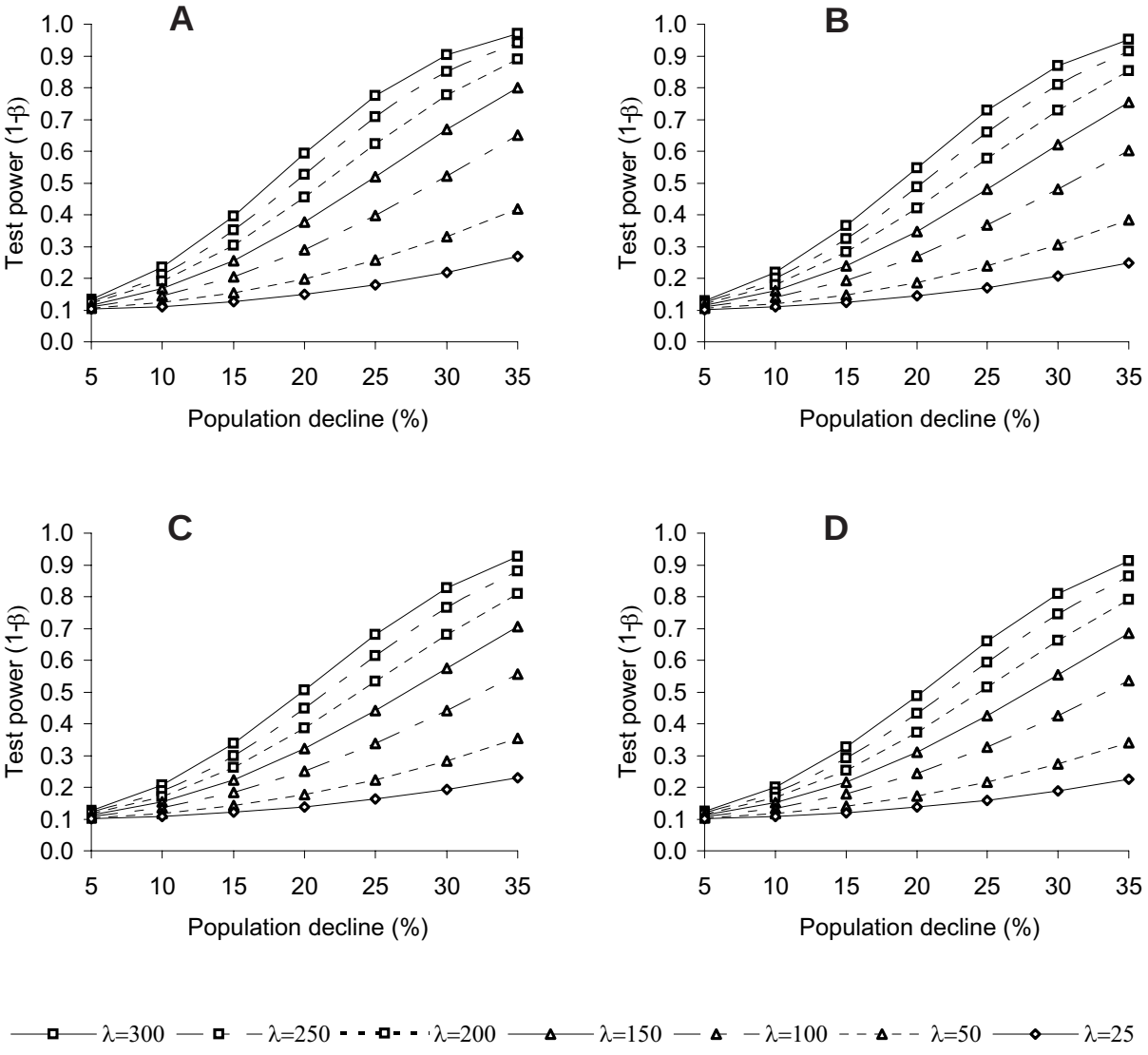

Figure A1.1. Power to detect 1080 impact (population decline) in simulated count data at 10% significance level. Varying degrees of seasonal and site-specific influence are modelled over a range of pre-1080 drop control counts (λ). Seasonal factor (s.f.) is the proportional difference between pre- and 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.

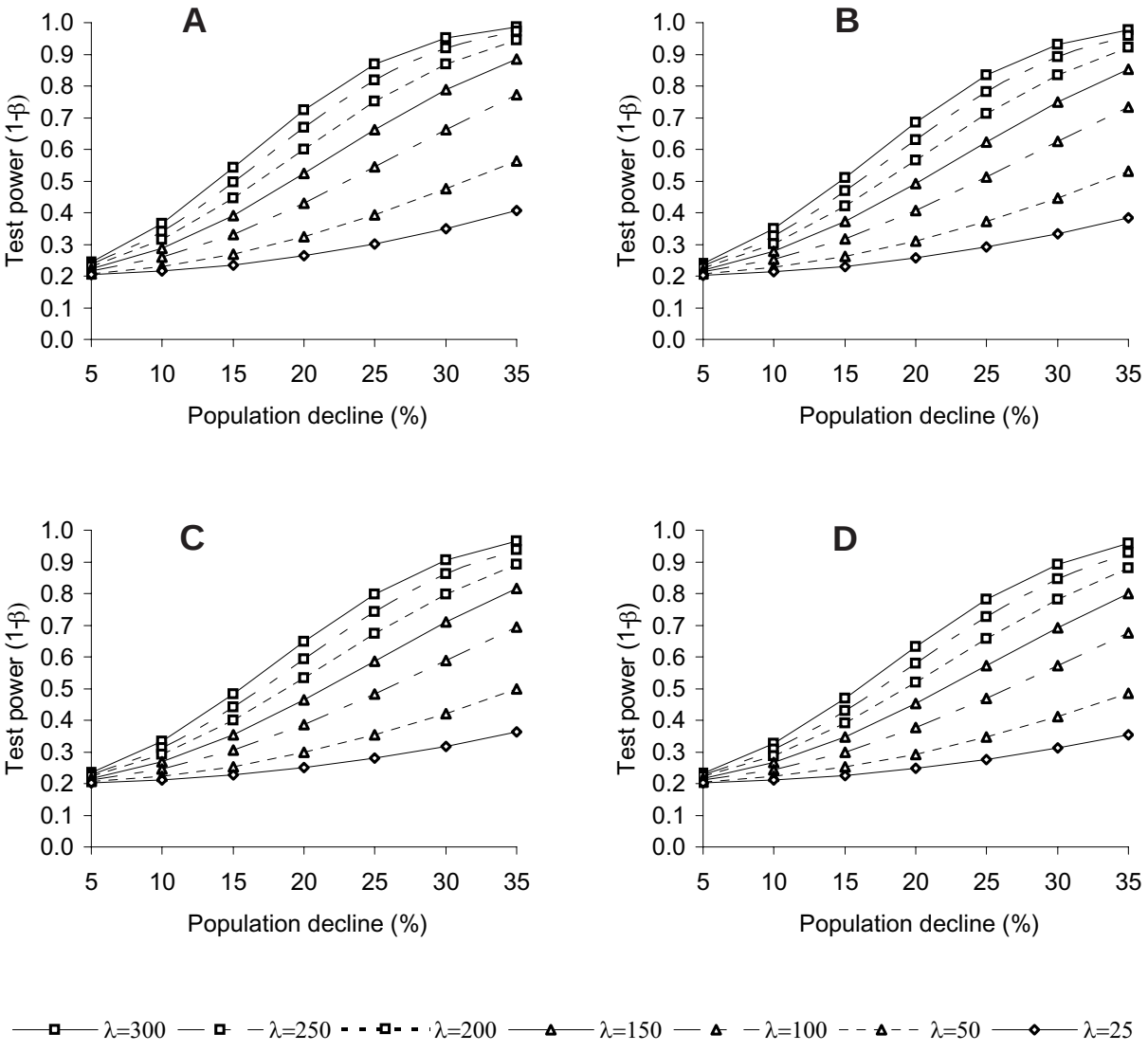

Figure A1.2. Power to detect 1080 impact (population decline) in simulated count data at 20% significance level. Varying degrees of seasonal and site-specific influence are modelled over a range of pre-1080 drop control counts (λ). Seasonal factor (s.f.) is the proportional difference between pre- and post-drop counts, and area factor (a.f.) the proportional difference between control and treated populations. **A, B, C,** and **D** as for Fig. 8.

Appendix 2

TWINSPAN ANALYSIS OF VEGETATION PLOTS

TWINSPAN results for forest transect vegetation plot data are presented in Fig. 10. This analysis provides a check on the validity of the control site, i.e. that vegetation did not differ substantially between control and treatment transects. Experimental (A1–3) and control (A4–6) quadrats were not differentiated in presence-absence analysis (Fig. A2.1A); in abundance analysis (vegetation cover, Fig. A2.1B) the two areas separate only at the third level. In both cases low eigenvalues[13] indicate that vegetation plots separated at each division was not particularly dissimilar from each other.

TWINSPAN analyses were not undertaken on vegetation data from H2, H3, H4, H5 and H6 because frog counts along creek transects were too small to allow meaningful comparison.

[13] The numbers at each partition, which portray the degree of variation explained by the split.

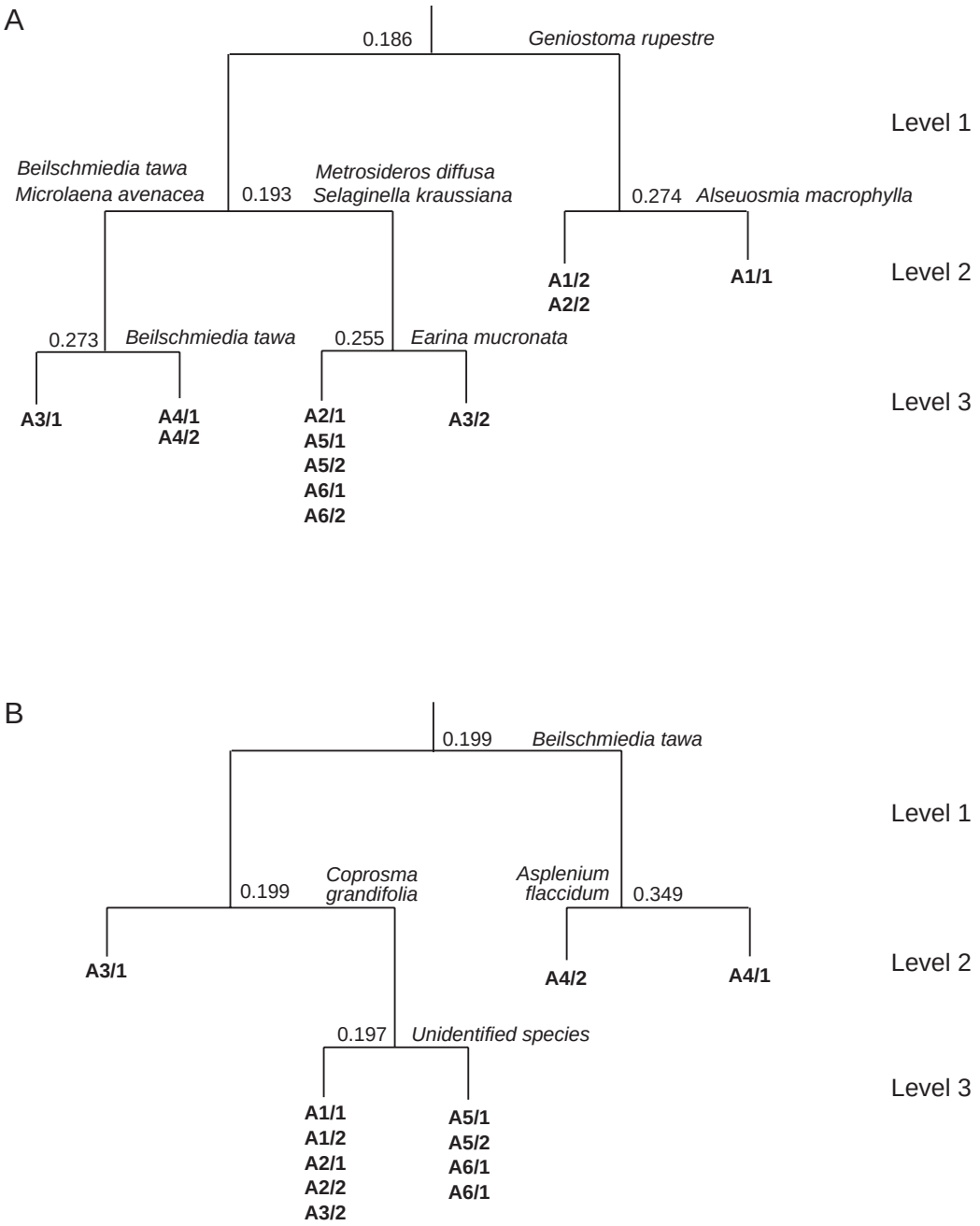

Figure A2.1. Results of TWINSPAN analysis of 12 vegetation plots from transects A1-6. Eigenvalues and indicator species (abbreviated) are shown at each level. **A.** Analysis of floristic data (presence–absence). **B.** Classification of percentage cover data.

Appendix 3

1080 ASSAY REPORT

Test reports for assays of sample materials from laboratory trials using the 1080Tox.v2 method by the Ministry of Agriculture and Fisheries at Wallaceville (following pages). Report Y3044 dated January 1997 comprises frogs and bait solutions from Trials 1 and 2, report Y3104 dated February 1997 comprises frogs and flies from Trial 3.

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

MAF

TEST REPORT

Contract No:
Lab No: Y3044

Client: DR BEN BELL
Address: SCHOOL OF BIOLOGICAL SCIENCES
VICTORIA UNIVERSITY
PO BOX 600
WELLINGTON

Fax Number:
Submitter:
Owner: Bell

Date Reported: 14/01/97
Date Received: 09/12/96
Date Analysed: 23/12-10/01
Number Received: 18+16

Condition on Receipt: Good
Storage: Freezer 3.10
Sample Type: Frogs, Baits

Your Reference:

Analysis Required: 1080(tox method)

Method: 1080Tox.v2

RESULTS

Frog	1080 mg/kg
1	3.9
3	2.0
5	4.1
14	0.3
19	1.1
21	1.3
24	1.2
4	< 0.1
9	2.4
10	0.6
25	1.4
18	3.8
22	0.4
23	0.7
2	< 0.1
6	< 0.1
12	< 0.1
16	0.8

Bait Soln	1080 mg/kg
1	250
3	210
4	0.1
5	170
9	0.1
10	290
14	230
15	0.2
19	200
20	0.2
21	230
22	0.1
23	0.2
24	160
25	<0.1

Stock Bait : 1080 = 0.17 %
Stock Blank Bait : 1080 = 0.2 mg/kg

CHEMICAL SERVICES WALLACEVILLE, NEW ZEALAND

Dr A.F. Erasmuson
Manager

14/1/97

Page 1of 1page(s)

The results obtained pertain only to the sample(s) as received.
This report shall not be reproduced except in full, without the written approval of the laboratory.

Notes: 1) There will be an additional charge of $5 for faxed results
2) Sample(s) will be discarded 8 weeks from test report date unless the client directs otherwise

National Chemical Residue Analytical Laboratory
Wallaceville Animal Research Centre, Ward Street, PO Box 40 063, Upper Hutt, New Zealand
Telephone (04) 528 6089, Facsimile (04) 528 0493

Form NCRL1 Iss 11/96

MINISTRY OF AGRICULTURE NEW ZEALAND
TE MANATU AHUWHENUA AOTEAROA

TEST REPORT

Contract No:
Lab No: Y3104

Client: DR BEN BELL
Address: SCHOOL OF BIOLOGICAL SCIENCES
VICTORIA UNIVERSITY
PO BOX 600
WELLINGTON

Fax Number:
Submitter: B. Bell
Owner:

Date Rep 97
Date Rec 97
Date Anal 02
Number Rec 3 + 9

Condition on Receipt: Good
Storage: Freezer 3.7
Sample Type: Frogs (9)/Flies(8)

Your Reference:

Analysis Required: 1080

Method: 1080Tox.v2

RESULTS

Fly Number	1080 (mg/kg)	Frog Letter	1080 (mg/kg)
1	70	A	N.D.
2	33	B	N.D.
3	31	C	1.3
4	91	E	2.6
5	26	F	N.D.
6	N.D.	G	N.D.
7	N.D.	H	0.41
8	N.D.	I	0.66
		J	N.D.

(N.D. = Not Detected.)

CHEMICAL SERVICES

V. SHANKS
Operations Manager

Dr A. Erasmuson
Manager

Page1 of 1 page(s)

The results obtained pertain only to the sample(s) as received.
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