Mortality of New Zealand native frogs in captivity

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ABSTRACT

Three species of New Zealand native frogs (*Leiopelma archeyi*, *L. hochstetteri* and *L. pakeka*) have been held in captivity in various institutions since 2000 as part of a programme to maintain and breed these threatened species. Following the death of a large number of these captive frogs, the Department of Conservation Native Frog Recovery Group decided that an investigation was needed to determine the cause. In this mortality study, we obtained data from captive populations to analyse mortality rates and causes of death for 252 wild-caught *Leiopelma* spp. These were held in captivity at the University of Canterbury and later transferred to other institutions between 2000 and 2006. *Leiopelma archeyi* and *L. hochstetteri* had similar overall average mortality but different yearly mortality patterns, whereas *L. pakeka* had much lower overall mortality. The major cause of death for *L. archeyi* and *L. hochstetteri* was bacterial infection, which was thought to be induced by a combination of husbandry factors, but mainly from oversterilising the substrate. Consequently, Auckland Zoo instigated a change in management, whereby soil was only heated to a temperature and for a length of time that was just sufficient to kill amphibian chytrid. New disease syndromes (skin blisters and muscle deterioration (rhabdomyolysis)) were also detected. Knowledge of disease is an important component of captive husbandry, so that healthy breeding populations can be maintained and we can gain an insight into diseases that may be affecting free-living populations. It is recommended that the staff at each institution undertake a review of all captive mortalities and report back to the Native Frog Recovery Group on an annual basis, so that any husbandry or disease issues that have arisen can be identified quickly.

Keywords: *Leiopelma*, amphibian disease, captive management, New Zealand, frogs, amphibian chytrid, rhabdomyolysis, septicaemia, blisters

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1. Introduction

The New Zealand frog fauna currently comprises four species of the genus *Leiopelma*: *L. archeyi* (Archey's frog), *L. hamiltoni* (Stephens Island frog), *L. hochstetteri* (Hochstetter's frog) and *L. pakeka* (Maud Island frog) (Bishop & Germano 2006). All four species are considered threatened (Hitchmough et al. 2007) and permits are required from the Animal Ethics Committee, Department of Conservation (DOC) to manipulate these animals for research purposes. As part of DOC's native frog recovery programme, captive populations of all species except *L. hamiltoni* have been established in a number of localities, either to aid research or for breeding.

During 1996–2001, a major population decline of *L. archeyi* occurred on the Coromandel Peninsula, which was possibly associated with *Batrachochytrium dendrobatidis* (*Bd*)—the amphibian chytrid (Bell et al. 2004). The only other population of *L. archeyi* was located in Whareorino, where no decline or amphibian chytrid had been detected. Therefore, since *Bd* was thought to be the cause of the decline, a new captive population of *L. archeyi* from Whareorino was also established at the University of Canterbury (CU) in response to this perceived threat of disease.

Up until 2004, the majority of native frog captive populations were held at CU. However, in late September 2004, a decision was made to move all species to separate institutions; this was achieved over the following 2 years. All living *L. archeyi* from CU were moved to Auckland Zoo (AZ); these consisted of progenies from both Coromandel Peninsula and Whareorino populations. All living *L. hochstetteri* were moved to Hamilton Zoo (HZ). Twelve living *L. pakeka* were moved to the University of Otago (OU) for further study and 30 were transferred to Karori Wildlife Sanctuary, Wellington, for release.

It was known that many frogs had died at CU, but no one had yet looked at the data to identify causes. In addition, a large number of *L. archeyi* died in the first year of arrival at AZ. Following these deaths in captivity, the DOC Native Frog Recovery Group decided that an investigation was needed to determine their cause. In this mortality study, we obtained data from each institution holding native frogs in captivity to examine the relationship between mortality rate and species, husbandry technique, duration in captivity, collection site and sex. We also investigated cause of death.

2. Methods

This study used information from records between 26 November 2000 and 27 November 2006. Therefore, it excludes data regarding the Whareorino population of *L. archeyi* that was caught from the wild in late 2006, individuals from which are now held at both Auckland Zoo and the University of Otago.

2.1 ACQUISITIONS, TRANSFERS AND DEATHS

All raw data on acquisitions, transfers and deaths of individual frogs were compiled and verified by DOC staff, based on collection labels, field notes and correspondence. Any data entries that were uncertain or unverifiable (i.e. not labelled at all, collection date not clear, date of death unclear, identification uncertain) were excluded.

2.2 HUSBANDRY

Although the DOC Native Frog Recovery Group has produced a husbandry manual for keeping native frogs in captivity (Webster 2002), the exact method of husbandry varies between captive institutions and with species. Therefore, each of the institutions that held frogs was asked to complete the same questionnaire (Appendix 1). One institution chose not to participate. Any follow-up clarifications that were required were obtained by email or phone. The University of Canterbury was not given a questionnaire to be filled out initially, as the principal investigator was no longer available; however, each question was later asked by email to the primary caretaker of the frogs, as it was decided that the comparison data would be useful. Based on the responses, key parameters of each institution's frog management methods were categorised and summarised.

2.3 MORTALITY RATE

To identify any patterns in frog mortality, the raw data were analysed by species, year, number of days in captivity, collection group (frogs from generally the same time and place) and transfer cohort. The two populations of *L. archeyi* were analysed separately. Age was not included as it was unknown at the time of capture. Snout-vent length was also not used, as few data were available for that parameter.

2.3.1 Mortality rate by year

Mortality rate measures the rapidity with which new deaths occur over time. However, since often the exact time of death was unknown, an estimation was made that used a denominator that represented the average number of frogs at risk. This was calculated for each year as:

$$M = \frac{D}{(n_1 + n_2)/2}$$

where M = mortality rate, D = total number of deaths for each year, n_1 = number of frogs at the start of each year, and n_2 = number of frogs at the end of each year.

This calculation was made for each species and was expressed as a percentage. This was used to indicate whether mortality events were associated with a particular period of time (Thrushfield 2007).

2.3.2 Mortality rate by days in captivity

To investigate the relationship between the length of time an individual had been in captivity and mortality rate, the number of days from collection to death was counted for each individual of a species and categorised. The categories were up to 90 days in captivity, 180 days in captivity, and then every 180 days through to 1980 days in captivity. The number of dead individuals divided by the number of live individuals at the beginning of that time period gave a cumulative mortality rate for each category.

2.3.3 Mortality rate by collection group (CG)

To determine whether the capture circumstances influenced mortality, each significant collection (five or more individuals) of frogs from approximately the same time and place was identified by a collection group (CG). The mortality rate for each CG was calculated by dividing the number of individuals that died for each CG over the total number of frogs in that CG. Nearly all collection groups of *L. archeyi* and *L. hochstetteri* were from Coromandel, and the majority were from a single area (Tapu); the only exception was a collection from Whareorino in 2002. Although *L. pakeka* had two significant cohorts, these were not analysed, as it had already been determined that their mortality was very low so further analyses would not be worthwhile.

2.3.4 Mortality rate by transfer cohort

Leiopelma archeyi were transferred from CU to AZ in four cohorts, with each transfer differing to some degree in regards to substrate and handling. Three of these comprised only individuals from one population while the last cohort was a mix of individuals from Coromandel and Whareorino. The quarantine substrate (3 months) was either paper towels or soil, while the post-quarantine substrate was soil in all cases. Some CU frogs in the initial transfer cohort were found to have skin blisters of unknown aetiology prior to transfer. Therefore, as a precaution, all blistered frogs were housed separately from non-blistered frogs, and all following cohort transfers contained either only frogs with blisters or only those without.

To examine whether transfer technique affected mortality, the following equation was used (Thrushfield 2007):

$$M = \frac{D}{(n \times t)/12}$$

where M = mortality rate, D = total number that died from each cohort, n = the total number in that cohort, and t = the number of months that cohort was present.

Since *L. pakeka* and *L. hochstetteri* were transferred in a single cohort, they were not analysed in this way.

2.3.5 Mortality rate by sex

The sex of individual frogs was determined either by post-mortem at Massey University (MU) (for all deaths that occurred at AZ), or by CU staff, who used a combination of methods, including observing eggs in females, ultrasound and/or post-mortem for frogs that died there. Since different protocols were used, MU and CU data were analysed separately as well as combined (to increase the sample size). The mortality rate for each sex and species was calculated as the number of animals of known sex that died divided by the total number of known sex individuals.

2.4 PATHOLOGY

Pathology reports for the period covered by this study had been prepared by Dr Richard Norman at Massey University. A summary of all reports from native frogs that died in captivity was prepared. We attempted to group causes of death into general categories, e.g. a 'bacterial' category, which consisted of bacterial infections of the skin, gastrointestinal tract and coelom. All the dead *Leiopelma* from CU not yet necropsied are stored in preservative at the DOC Waikato Conservancy Office awaiting post-mortems.

3. Results

3.1 ACQUISITIONS, TRANSFERS AND DEATHS

In total, 252 individual frogs (106 *L. archeyi*, 100 *L. hochstetteri* and 46 *L. pakeka*) were brought into captivity at CU between 2000 and 2004 (Appendix 2). These were mainly obtained from the wild; the exception was four *L. archeyi* and seven *L. hochstetteri*, which first went to VU and were then transferred to CU.

In 2005 and 2006, 154 frogs (67 L. *archeyi,* 45 *L. hochstetteri* and 42 *L. pakeka*) were transferred live from CU to another institution.

In total, 113 frogs (54 *L. archeyi*, 55 *L. hochstetteri* and 4 *L. pakeka*) died while in captivity.

3.2 HUSBANDRY

Key husbandry parameters varied between institutions, particularly with respect to group housing (Table 1, Appendix 3). All of the institutions kept individual animals in separate, small plastic containers on paper towels. However, the group housing varied from indoor on paper towels to outdoor on natural substrate. Two institutions (UC and HZ) sourced all the natural substrate components locally rather than from the original habitat of the species, whereas AZ sourced the soil and leaf litter from Coromandel. The method used to sterilise the leaf litter and soil also varied.

3.3 MORTALITY RATE

The frogs included in this study had been captured and brought into captivity mainly for research and captive propagation. For some, the specific research purpose was known, e.g. *L. archeyi* (Coromandel) were intended for amphibian chytrid studies. However, according to databooks and notes at CU, no invasive research ever took place that could be considered to have affected their mortality. The only known exception to this was four *L. archeyi* (Coromandel) and seven *L. hochstetteri* that were collected in 2002 and initially went to VU for manipulative chytridiomycosis research. These were part of a larger collection of frogs (ten of each of the two species), the rest of which died at VU. Three of the four *L. archeyi* and four of the seven *L. hochstetteri* that were transferred from VU subsequently died at CU, and it is possible that they arrived at CU in a weakened state. Nine of the 46 *L. pakeka* and two *L. archeyi* collected were brought into captivity over some health concerns (dermatitis, eye problems, blisters, bleeding or head injury). All of these individuals except the one frog with a head injury survived for the duration of this study.

TABLE 1. HUSBANDRY BASICS AT EACH CAPTIVE FACILITY. FOR ADDITIONAL INFORMATION, SEE APPENDIX 3.

PARAMETER	INSTITUTION			
	UNIVERSITY OF CANTERBURY*	AUCKLAND ZOO	HAMILTON ZOO	UNIVERSITY OF OTAGO†
Temperature				
Group	Controlled; 11–15°C	Controlled; 12–16°C	Ambient	Controlled; 12–16°C
Individual	Controlled; 11–15°C	Controlled; 12–16°C	Air conditioning; 12–15°C	Controlled; 12–16°C
Humidity				
Group	Unknown	Controlled; 100%	Ambient	Controlled; >85%
Individual	Unknown	Controlled; 100%	Ambient	Controlled; >85%
Watering				
Group	Automatic—ceramic filtered	Manual—reverse osmosis	Automatic—carbon filtered	Automatic—filtered 2 µm
Individual	Manual moistening—ceramic filtered	Manual—reverse osmosis	Unknown	Automatic—filtered 2 µm
Lighting				
Group	Fluorescent light; 12 h cycle	Incandescent bulb; 12 h cycle	Ambient	Fluorescent; 11 h ramped on
Individual	Fluorescent light; 12 h cycle	2 bulbs during day, 1 filtered at night	Ambient	Fluorescent; 11 h ramped on
Handling				
Group	Weekly	2–6 times/month	Twice a month	Monthly
Individual	Weekly	Weekly	Weekly	Monthly
Substrate				
Group				
Source	Local origin	Tapu, Coromandel	Local origin	Maud Island
Preparation	Autoclaved; dried at 140°C for 72 h	Baked at 200°C, then sun-dried for 90 days	Dried at >20°C for 14 days	150°C for 3 h, acclimatised for 30 days
Individual	Paper towels	Paper towels	Paper towels	Paper towels
Feeding				
Group	Weekly	Twice a week	Three times a week	Weekly
Individual	Weekly	Twice a week	Three times a week	Weekly

* University of Canterbury had seven frog areas and conditions could vary; information is based on main holding areas.

† University of Otago had group tanks prepared but had not used them at the time of this paper.

3.3.1 Mortality rate by year

Mortality rates by year and species are shown in Fig. 1.

Leiopelma archeyi (Whareorino) had an average mortality rate of 14.5% across all years. Mortality rate was lowest in the first (2002 = 12.2%) and third (2004 = 7.8%) years of significant holdings (i.e. year in which there was more than one frog in the captive population). Mortality rate was higher in the second and fourth years (2003 = 20.5%; 2005 = 24.2%), and was highest of all in the last year (2006 = 36.7%), when frogs were transferred to AK.

Leiopelma archeyi (Coromandel) had an average mortality rate of 10.3% across all years, which was about half that of *L. archeyi* (Whareorino). Mortality rate was highest in the third and fourth years of holdings (2002 = 17.9%; 2003 = 27.8%), which accounted for most of the overall deaths. Since the fifth year (2004), the

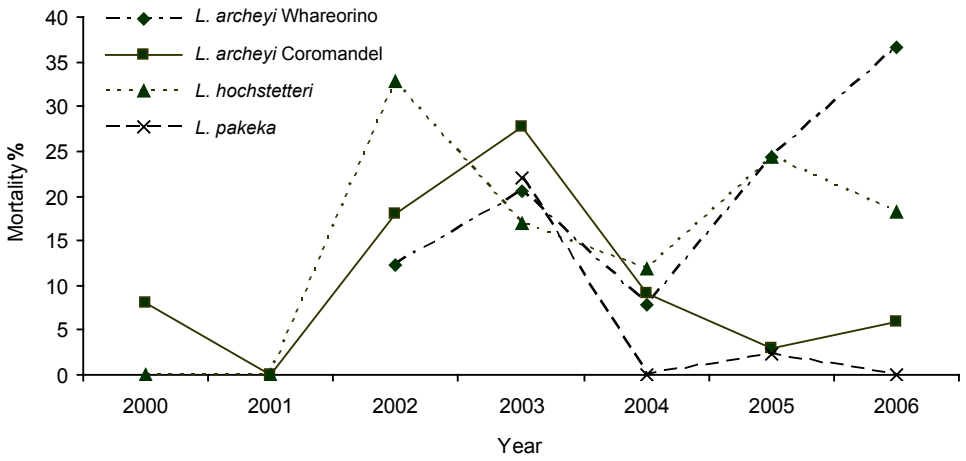

Figure 1. Mortality rate of *Leiopelma* spp. by year.

mortality rate has been at or below the average rate; this includes during and following the transfer to AK.

Leoiopelma hochstetteri had an average mortality rate of 14.9% across all years. No deaths occurred in *L. hochstetteri* in the first 2 years of holdings (2000 and 2001); since then, the mortality rate has fluctuated between 11.8% and 24.4%. No deaths occurred at HZ during the initial 6 months following transfer.

Leiopelma pakeka had an average mortality rate of 3.5% across all years, which was the lowest of all the species. The mortality rate was highest in the first year of significant holdings (2003); since then, the mortality rate has been very low (2.4 % in the third year (2005) and 0.0% in other years), including during and following the transfer to OU.

3.3.2 Mortality rate by days in captivity

Mortality rate by the total number of days in captivity for each species is shown in Fig. 2. Since cumulative data are presented, increases in the slope of the lines indicate where deaths mainly occurred.

For *L. pakeka*, all deaths occurred within the first 1.5 years (540 days) of captivity (8.7%). Rates of mortality for the remaining three taxa were higher than *L. pakeka* but were broadly similar to each other for the initial 900 days in captivity; following this, each species had different mortality rates.

Leiopelma archeyi (Whareorino) had a low mortality rate (1.9%) in the first 90 days of captivity. Following this, there were fairly uniform increases in overall mortality from day 90 to day 1620 (between 5.7% and 11.6%), except for the lower increases (1.9%) between 540 and 900 days in captivity. The mortality rate was very low for those frogs that had been in captivity longest, i.e. between 1620 and 1980 days, with between 0.0% and 1.2% increases in overall mortality rate.

Leiopelma archeyi (Coromandel) had low increases in mortality rate (2.1% and 2.2%) up to 180 days in captivity. From 180 to 900 days, increases were between 6.3% and 12.7%, except for the low increases (2.2%) between 360 and 540 days. After 900 days, increases in mortality rate were consistently low (between 0.0% and 2.2%).

Leiopelma hochstetteri had continual increases in overall mortality rate through time, varying from 2.1% to 7.6%.

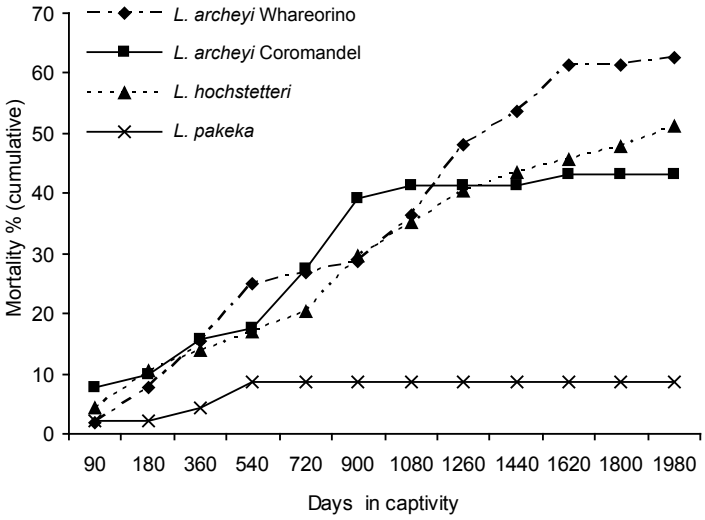

Figure 2. Mortality rate of *Leiopelma* spp. by total number of days in captivity.

3.3.3 Mortality rate by collection group (CG)

Mortality rates by collection groups are shown in Table 2.

All three *L. archeyi* (Coromandel) collection groups had similar mortality rates (between 35.7% and 40.0%). However, their average mortality rate (38.6%) was about half that of the Whareorino collection group of the same species (65.3%).

The range of mortality rates amongst the *L. hochstetteri* collection groups was similar to that of both populations of *L. archeyi* combined (ranging from 28.6% to 65.2%), and the overall average (47.2%) was about halfway between the two populations of *L. archeyi*. Mortality rate was highest for the oldest two collections and lowest for the second-youngest collection.

TABLE 2. MORTALITY RATE OF *Leiopelma archeyi* AND *L. hochstetteri* BY COLLECTION GROUP (CG).

CG	NO. INDIVIDUALS IN CG	YEAR OF DEATH							TOTAL DEATHS	% MORTALITY
		2000	2001	2002	2003	2004	2005	2006		
L. archeyi										
Coromandel 2000	30	1	0	5	5	1	0	0	12	40.0%
Coromandel 2002	14	0	0	0	3	1	0	1	5	35.7%
Coromandel 2004	5	0	0	0	0	0	1	1	2	40.0%
Total Coromandel	49	1	0	5	8	2	1	2	19	38.6% (average)
Whareorino 2002	49	0	0	3	9	3	8	9	32	65.3% (average)
L. hochstetteri										
Coromandel 2000	23	0	0	4	5	1	2	3	15	65.2%
Coromandel 2002	28	0	0	4	2	3	7	1	17	60.7%
Coromandel 2003 A	12	0	0	0	3	0	1	1	5	41.7%
Coromandel 2003 C	14	0	0	0	0	0	1	3	4	28.6%
Coromandel 2004	10	0	0	0	0	3	0	1	4	40.0%
Total Coromandel	87	0	0	8	10	7	11	9	45	47.2% (average)

3.3.4 Mortality rate by transfer cohort

Mortality of the third cohort of *L. archeyi* from Whareorino that was transferred was about twice as high as the two other cohorts from the same population (64.0% v. 54.5% v. 150.0%, for cohorts 1–3 respectively). The later cohort from Coromandel also had a higher mortality rate (Table 3). All three Whareorino cohorts had higher mortality rates than the Coromandel cohort.

Individuals from both the Whareorino and Coromandel cohorts that had blisters had higher mortality rates than those with no blisters. The one Whareorino cohort that had a mixture of blistered and non-blistered individuals had a similar mortality rate as the Whareorino cohort with no blisters. The quarantine substrate did not appear to have influenced mortality.

TABLE 3. MORTALITY RATE BY TRANSFER COHORT OF *Leiopelma archeyi* DURING TRANSFER FROM CANTERBURY UNIVERSITY TO AUCKLAND ZOO.

W= Whareorino; C= Coromandel; P= papertowels; S = natural substrate; N= no; Y= yes.

	TRANSFER COHORT				
	1	2	3	4a	4b
Population	W	C	W	W	C
Date of arrival	15 Mar 05	10 June 05	4 July 05	9 Nov 05	9 Nov 05
Quarantine substrate	P	P	S	P	8P/8S
Post-quarantine substrate	S	S	S	S	S
Blistered	Some	N	N	Y	Y
Total in transfer cohort	15	17	11	8	16
Total that died	8	1	3	2	1
% mortality rate	64.0%	10.1%	54.5%	150.0%	37.5%

3.3.5 Mortality rate by sex

Females consistently outnumbered males in the number of deaths for both *L. archeyi* and *L. hochstetteri* (Table 4).

For *L. archeyi*, 80% of individuals that died were female, regardless of whether sex was determined by a single method/institution or a combination of methods/ institutions.

For *L. hochstetteri*, 84% of individuals that died were female.

TABLE 4. MORTALITY RATE BY SEX OF *Leiopelma archeyi* AND *L. hochstetteri*.
AZ = Auckland Zoo; CU = University of Canterbury; MU = Massey University. Data exclude froglets.

	FEMALE	MALE	UNKNOWN	TOTAL OF KNOWN SEX	TOTAL NO. INDIVIDUALS
***L. archeyi* from AZ (sexed by MU)**					
Number dead	11	3	2	14	16
Dead/dead of known sex	78.6%	21.4%			
***L. archeyi* from CU (sexed by MU and/or CU)**					
Number dead	8	2	31	10	41
Dead/dead of known sex	80.0%	20.0%			
***L. hochstetteri* from CU (sexed by MU and/or CU)**					
Number dead	21	4	27	25	52
Dead/dead of known sex	84.0%	16.0%			

3.4 PATHOLOGY

Since pathology reports were only available from early 2005 and only for a limited number of frogs that died at CU, it is probably more useful to examine trends in deaths in post-Canterbury holdings. About a third of all deaths (34.8%, $n = 16$) were attributed to bacterial causes (Table 5). The remainder of deaths were fairly evenly attributed to the other categories of causes, with 1–4 frogs (2.2%–8.7%) in each category. About a fifth (21.0%, $n = 10$) of all deaths were of unknown cause.

TABLE 5. PATHOLOGY SUMMARY FOR *Leiopelma* spp.
CU= University of Canterbury; AZ= Auckland Zoo; HZ= Hamilton Zoo; Coro = Coromandel; Whare = Whareorino.

CAUSE OF DEATH	*L .archeyi*		*L. hochstetteri*	*L. archeyi*		*L. hochstetteri*	TOTAL	%
	CORO CU	WHARE CU	CU	CORO AZ	WHARE AZ	HZ		
Bacterial (skin/gastrointestinal/coelom)	1	2	7		6		16	34.0%
Mycobacterial					1		1	2.1%
Fungal skin		1	3				4	8.5%
Kidney			1		1		2	4.3%
Trauma		1	1	1			3	6.4%
Foreign body			1	1			2	4.3%
Reproductive			1				1	2.1%
Rhabdomyolysis					3	1	4	8.5%
Eustachian tube impaction			1				1	2.1%
Ophthalmic						1	1	2.1%
Poor nutrition/weight loss			2				2	4.3%
Unknown		1	6		3		10	21.3%
Total dead with pathology reports	1	5	23	2	14	2	47	

4. Discussion

Leiopelma pakeka had the lowest overall mortality of the three species, both in terms of mortality rate by year and days in captivity. None of the data examined could explain why this species had a greater ability to withstand captures, transfers and captivity than the other *Leiopelma* species investigated. However, an earlier collection of 11 *L. pakeka* in 2000 that were exclusively housed at OU (and therefore were not included in this report) showed a much higher mortality than in this study (9 out of 11 frogs died). These deaths occurred over a brief period and were attributed to problems in husbandry (enclosure humidity, substrate and food amounts) by university staff. These factors were corrected and the remaining two frogs were still alive at the end of the period covered by this study. This indicates that husbandry conditions will influence mortality in *L. pakeka*, although perhaps not to the same degree (or for the same specific conditions) as for the other species.

The annual mortality rate of *L. hochstetteri* at CU was relatively low but steady each year. Since the population was aging each year and their start age was unknown, this decline could have been simply an aging pattern, as would be expected if all individuals in the population were of different ages. However, results indicate that husbandry conditions also played some role here. At CU, *L. hochstetteri* were kept in controlled indoor conditions. In contrast, at HZ, where they were kept in outdoor enclosures in quite uncontrolled conditions, the mortality declined in the initial 6 months to lower levels than at CU. Therefore, it seems likely that the steady decline at CU was due to husbandry factors.

The overall average mortality rate for *L. archeyi* was similar to that of *L. hochstetteri*, but annual patterns of increases and decreases differed between the species. The difference in mortality between the two populations of *L. archeyi* is also interesting, with the mortality rate of the Whareorino population being higher than that of the Coromandel population. This difference arose because although the Coromandel population consistently had higher mortality while at CU, following transfer to AZ their mortality decreased. In the Whareorino population, the reverse occurred. There are several possible reasons why *L. archeyi* Whareorino population had higher mortality than its Coromandel counterpart.

First, it is possible that the resilience of frogs differed between the two populations. For example, the single collection of Whareorino individuals may have been from a weaker population so they did not cope as well as the Coromandel individuals with the stress of being transferred and/or having a change in husbandry. Alternatively, the Coromandel individuals that were transferred may have already survived greater mortality events than the Whareorino population, both in the wild and at CU, so that the stronger individuals remained, which were better able to cope with the stress of transfer. However, the evidence does not support this, as during the first year in captivity at CU the Whareorino population had a lower mortality rate than the Coromandel population.

The second possibility is that the origin of natural substrate used in group housing had an effect on mortality. Institutions differed in which portions of the

natural substrate came from the frogs' original habitat. At AZ, the soil and leaf litter portion of the natural substrate was from Coromandel. At CU, the leaf litter portion was local, favouring neither population of *L. archeyi*. It is possible that the use of a substrate from an origin other than their original habitat could have caused an imbalance of minerals, bacteria or another unknown factor contributing to mortality. However, at times, L. *archeyi* Coromandel had higher mortality rates than Whareorino individuals at CU when neither was on native substrate. In addition, if the origin of leaf litter/soil was a major factor, *L. hochstetteri* would have been expected to have fared poorly at Hamilton Zoo, which they did not. Therefore, although there seems to be some merit in this argument of soil origin, it is likely to be a minor contributing factor rather than a primary one.

A third possibility is that susceptibility to disease had an effect on mortality. Bacterial causes (dermatitis, septicaemia and infections in the coelom) were the main single confirmed cause of death in *L. archeyi*. Primary bacterial disease is unusual in amphibians and outbreaks are often associated with a variety of situations that could result in immunosuppression, alteration of non-specific host defences, or exposure to overwhelming bacterial numbers (Pessier 2002). Even when the known primary pathogenic bacterium *Aeromonas hydrophila* is isolated, it may not be diagnostic because this and other bacteria are frequent inhabitants of frogs' environments (Pessier 2002). Bacterial septicaemia may arise as a result of the complex interaction of multiple taxa of bacteria, or the overwhelming presence of a single species (Taylor et al. 2001). In a few cases, foreign bodies of plant material had caused trauma to the skin and started an infection that eventually overwhelmed the system. Although the epidermis provides some protection from abrasive substances, it is easily damaged if the frog is handled inappropriately or is in contact with rough substances. The resulting damage from even an apparently minor injury can have serious consequences, as there is no longer an effective barrier against opportunistic micro-organisms (Helmer & Whiteside 2005). Environmental stressors may also change amphibians' bacterial skin flora (Harris et al. 2006).

The captive environment may also have had high numbers and/or new bacteria to which frogs were naïve. This exposure combined with the stress of captivity may have resulted in bacterial infections. At the different institutions, there was great variation in the temperature to which soil and leaf litter were heated in an attempt to kill any *Bd* that may have been present. Over-heating soil kills off healthy invertebrates, bacteria and fungi, leaving the soil sterile and vulnerable to colonisation of bacteria that are opportunistic invaders. Both AZ and CU used very high temperatures to sterilise the soil and subsequently kept the soil in indoor enclosures where there was little to no possibility of insects being introduced. In contrast, HZ used lower temperatures, which may have been too low to kill amphibian chytrid (Johnson et al. 2003), and the outdoor enclosure favoured easy re-colonisation by insects, etc. A comparison of bacteria present on *L. archeyi* in the wild and on the Coromandel and Whareorino populations in captivity revealed that both the captive Whareorino and Coromandel frogs had a bacterial flora on their skin that was substantially different from the flora found on free-living Coromandel and Whareorino frogs, and bacteria that have been previously implicated in contributing to disease were only found in the captive populations (Potter & Norman 2006). Therefore, it is possible that some frogs on substrate at AZ were exposed to types and numbers of bacteria that caused

disease when combined with stress or some other unknown factor. In response to these deaths, a change was made to the husbandry of the Whareorino frogs, whereby they were all placed on paper towels, in a hope that this would reduce the number of bacteria to which they were exposed. Since then, there have been no more deaths of *L. archeyi* to date.

This would lead us to conclude that the major contributing factor to the death of *L. archeyi* Whareorino was that of husbandry. Hygiene and handling protocols have always been in place to minimise the frogs' exposure to new bacteria. However, based on these findings, it is recommended that any substrate component that is sterilised for the purposes of killing chytrid is only heated for 4 hours at 37°C to preserve healthy soil that has a balanced bacterial flora.

Another disease finding in *L. archeyi* was rhabdomyolysis. This is where an acute stress or an inherited enzyme deficiency causes muscle necrosis, the degradation products of which lead to renal tubular damage and death. The preceding clinical signs of this were noted in the history of extensor spasms. As far as we are aware, this is the first report of this syndrome in an amphibian and thus it requires further investigation.

Finally, the blistering syndrome was present in many *L. archeyi* when they arrived at AZ. The third Whareorino transfer cohort and the second Coromandel transfer cohort of *L. archeyi* to AZ, which were all blistered frogs, had higher mortality rates than pure non-blistered cohorts. This was only a total of three blistered frogs, however. Blisters have been seen in both wild and captive *Leiopelma* (A. Smale, DOC, pers. comm. 2007). Blisters are a syndrome of unknown etiology that is currently under investigation. So far, this investigation has shown that they are not infectious but are believed to be caused by an immune disorder (R. Speare, James Cook University, pers. comm. 2007).

According to both the MU pathology reports and the CU notes, there was a definite female sex bias in mortality rates for *L. archeyi* and *L. hochstetteri*, notwithstanding a large number of animals (mainly those from CU) where the sex was undetermined. Similar sex-biased mortality was found in the California red-legged frog (*Rana rana*) during a decline due to chytridiomycosis (Muths et al. 2003). The hypothesis of sex-biased mortality could easily be tested for *Leiopelma* if the surviving frogs or even source populations could be reliably sexed to confirm whether there was already a sex bias in the source or collected population. Currently, snout-vent length is used to determine sex in the field (Bell 1994; Tocher et al. 2006). However, this is not very accurate, so researchers are now trying to develop a method to assign gender of individual *L. archeyi*.

5. Conclusions

Most deaths of captive frogs appear to have been caused by husbandry factors. It appears that requirements differ between species and even populations of the same species. Although the specific causes of death in each species have not been clearly identified, several possible explanations have been proposed. At the time of writing, all three species had stable mortality rates, indicating either that populations have undergone their main mortality events or that causes of death have been removed through changes in husbandry. The apparent higher mortality in female frogs in both *L. archeyi* and *L. hochstetteri* requires further scrutiny. The recent disease findings of blistering and rhabdomyolysis in *L. archeyi* also need further investigation.

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Appendix 1

Leiopelma HUSBANDRY QUESTIONNAIRE

1) Enclosure temperature day/night/seasonal and how it's monitored
2) Type of light and timing
3) Humidity and how monitored
4) Number of animals/enclosure
5) Define what the substrates you use are exactly and where you got them
6) Did the substrates get any sort of pre treatment?
7) How often are these substrates changed?
8) Describe what enclosure is made of that they have contact with, i.e. plastic container/dirt/wire mesh, etc.
9) What are your hygiene protocols? Describe brand gloves, brand disinfectants/ strength, etc.
10) Feeding regime
11) Type of water used and how delivered
12) Anything else you think pertinent?

Appendix 2

ACQUISITIONS AND TRANSFERS OF *Leiopelma* spp.

CU = University of Canterbury; AZ = Auckland Zoo; OU = University of Otago; HZ = Hamilton Zoo; VU= Victoria University of Wellington.

YEAR	INSTITUTION	SPECIES	NO. FROGS ALIVE (AT 1 JAN)	NO. FROGS ACQUIRED	SOURCE OF ACQUIRED FROGS	NO. FROGS THAT DIED (1 JAN – 31 DEC)	NO. FROGS TRANSFERRED OUT	SITE TRANSFERRED TO	DATE TRANSFERRED OUT	NO. FROGS AT END OF YEAR
2000	CU	*L .archeyi* (Whareorino)	0	1	Wild	0	0	–	–	1
		L. archeyi (Coromandel)	0	26	Wild	1	0	–	–	25
		L. hochstetteri	0	23	Wild	0	0	–	–	23
		L. pakeka	0	0	–	–	–	–	–	0
2001	CU	*L .archeyi* (Whareorino)	1	0	–	0	0	–	–	1
		L. archeyi (Coromandel)	25	2	Wild	0	0	–	–	27
		L. hochstetteri	23	5	Wild	0	0	–	–	28
		L. pakeka	0	1	Wild	0	0	–	–	1
2002	CU	*L. archeyi* (Whareorino)	1	50	Wild	3	0	–	–	48
		L. archeyi (Coromandel)	27	15+4	Wild/VU	6	0	–	–	40
		L. hochstetteri	28	29+7	Wild/VU	13	0	–	–	51
		L. pakeka	1	0	–	1	0	–	–	0
2003	CU	*L .archeyi* (Whareorino)	48	1	Wild	9	0	–	–	40
		L. archeyi (Coromandel)	40	2	Wild	10	0	–	–	32
		L. hochstetteri	51	26	Wild	10	0	–	–	67
		L. pakeka	0	20	Wild	2	0	–	–	18
2004	CU	*L. archeyi* (Whareorino)	40	0	–	3	0	–	–	37
		L. archeyi (Coromandel)	32	5	Wild	3	0	–	–	34
		L. hochstetteri	67	10	Wild	8	0	–	–	69
		L. pakeka	18	25	Wild	0	0	–	–	43
2005	CU	*L. archeyi* (Whareorino)	37	0	–	4	15	AZ	15 Mar 2005	0
							10	AZ	4 Jul 2005	0
							8	AZ	9 Nov 2005	0
		L. archeyi (Coromandel)	34	0	–	1	17	AZ	10 June 2005	0
							16	AZ	9 Nov 2005	0
		L. hochstetteri	69	0	–	15	0			54
		L. pakeka	43	0	–	1	42	OU	28 Oct 2005	0
	AZ	*L. archeyi* (Whareorino)	0	33	CU	4	0			29
		L. archeyi (Coromandel)	0	33	CU	0	0	–		33
	OU	*L. pakeka*	0	42	CU	0	0	–	–	42
2006	CU	*L. hochstetteri*	54	0	–	9	45	HZ	24 May 2006	0
	AZ	*L. archeyi* (Whareorino)	29	0	–	9	0	–	–	20
		L. archeyi (Coromandel)	33	0	–	2	0	–	–	31
	HZ	*L. hochstetteri*	0	45	CU	2	0	–	–	43
	OU	*L. pakeka*	42	0	–	0	0	–	–	42

Appendix 3

HUSBANDRY DETAILS BY INSTITUTION

Although the Department of Conservation (DOC) Native Frog Recovery Group oversees all native frog holdings, the exact methods of husbandry used for each species varies by institution. The husbandry techniques of each institution for the period of this analysis are summarised below.

A3.1 University of Canterbury

Species

This was the original holding institution for all three native frog species (*Leiopelma archeyi*, *L. hochstetteri* and *L. pakeka*). The frogs were kept in many different rooms. The main areas for group and individual housing are described below.

Group housing

Breeding groups were housed in large glass tanks with perplex and mesh lids. The frogs were not in contact with the perplex or mesh as the containers were very high. The substrate in the tanks was mainly peat and leaf litter. The peat was a commercially dried product that came in a block and was rehydrated in water, then autoclaved. The leaf litter was collected on the University of Canterbury campus (Christchurch) and was then autoclaved and dried at 140°C for 72 h. The substrate was changed as required, which was usually every 6 months.

Individual housing

All *L. hochstetteri* and *L. pakeka*, and some *L. archeyi* were kept in individual housing. Individuals that were found together in the wild were kept together in an individual tank. Each tank was a plastic container that contained two interfolded paper towels, one moist and one dry. *Leiopelma hochstetteri* also had a bowl of water in the container. The containers were cleaned and the towels were changed weekly.

Temperature

In summer, the day temperature was 15°C and the night temperature was 11°C; this 4°C drop happened over 2 h. In winter, both day and night temperatures were 1–2°C lower.

Humidity/watering

Artesian spring water that had been twice filtered through ceramic micron-sized filters was used. The large breeding tanks had an automatic misting system while the plastic boxes were manually moistened. Moisture levels were monitored by carers

Lighting

Low-intensity (15 watt) fluorescent light was angled towards the ceiling to simulate moonlight. Low-heat white or fluorescent light was used to simulate daylight. Lighting was maintained on a photoperiod that roughly reflected the seasons.

Hygiene protocols

LabServ Nitril powder-free gloves were used at all times when handling frogs and equipment. The containers were cleaned out using only water, and no disinfectants were allowed on any equipment that would contact the frogs. The full hygiene protocols adhered strictly to the instructions of the DOC Native Frog Husbandry manual (Webster 2002).

Observations/handling

Each container was picked up daily to view the frog through the container; thus, every animal was sighted every day. No animals were touched except for a monthly weight check; this included during cleaning of the container each week. Snout-vent length was measured once every 6 months. If a frog was sick, observations increased to twice a day and treatment was given to the frogs if necessary.

Feeding

Individuals were fed weekly with a variety of invertebrates—crickets, fruit flies, houseflies and moth larvae.

A3.2 University of Otago

Contact

Dr Phil Bishop, academic staff member, Department of Zoology (email: phil.bishop@stonebow.otago.ac.nz).

Species

Leiopelma pakeka ex CU, wild-caught *L. archeyi*, and *Litoria* spp. are each kept in separate rooms, and all the *Leiopelma* are housed individually. Some chytrid-positive specimens are present. Only *L. pakeka* are referred to in this study.

Group housing

Both the group tanks and individual housing are in a designated frog room in the animal suite. The frog room is wired to an alarm that will sound if any of the temperature, lighting, watering or humidity systems fail. At the end of 2008, they will start to house a maximum of six *L. pakeka* in group tanks. Each group tank is essentially a glass tank with a hole in the bottom and the top and half of the front made from stainless steel mesh. The floor is made of plastic floor tiles that have large holes in them, which are supported by PVC plumbing pipes. These tiles are covered with a layer of fibreglass mesh (1 mm × 1 mm) and a layer of pebble (3 cm deep). Covering this is another layer of fibreglass mesh, followed by a layer of sand (3 cm deep), another layer of fibreglass mesh, and a layer of topsoil

(3 cm deep). Several large pieces of schist and leaf litter are on the topsoil. The substrates were obtained locally from a garden supplier and autoclaved at 150°C for 3 h, following which they were allowed to air dry. The tank was then set up with the sprinkler system and allowed to acclimatise for 30 days. Leaf litter and dead wood were then introduced from Maud Island (thought to be free from amphibian chytrid) to seed the microfaunal component. After a week or two, fungi and many small soil invertebrates and dipterans were present.

Individual housing

At the time of this study, all frogs are being held individually in the frog room in clear, plastic, airtight lunch boxes (30 cm × 20 cm × 10 cm) with opaque coloured lids. There are no holes drilled into these and the collection of frog boxes is completely covered with a blackout curtain. The frogs are on two pieces of damp, unbleached paper towel that have been scrunched up to give frogs some topography. The frogs remain in these containers unopened for a week, as they are only physically checked or weighed once a week. When they are checked, any soiled paper towels and all faeces and uneaten food are removed.

Temperature

The frog room is accurately temperature controlled. The temperature varies from 12°C min. and 14°C max. in winter, to 14°C min. and 16°C max. in summer.

Humidity/watering

Water is filtered through 2 µm and allowed to stand for more than 48 h. It is then attached to a misting system supplied by Ecologic (Rainmaker Misting System Kit™). It rains for 1.5 min every 12 h in the group frog tanks. The relative humidity (RH) of the room is around 60% and is expected to exceed 85% in the actual tanks. The watering in the individual tanks is manually monitored.

Lighting

Fluorescent tubes are used to light the frog room. During the summer, these ramp up for an hour from 0% at night to a maximum of 10% to simulate dawn. They then remain at 10% for 11 h before ramping down for an hour to 0% to simulate dusk. This regime is adjusted by decreasing the total amount of daylight time to roughly simulate seasonal variance. There is no natural light source.

Hygiene

Everything that goes into the tanks (except live insects) is sterilised at 150°C for 3 h. Rubber gloves (LabTex Plus Powder-free Multi-Purpose Laboratory) are used for handling everything, including the frogs. The lab scales and measuring equipment are permanently kept in the frog room.

Observations/handling

Leiopelma pakeka are observed once a week, and *L. archeyi* 2–3 times a week. Frogs are examined visually *in situ* and their boxes are cleaned around them. They are weighed once a month and snount-vent length is measured twice a year. Individuals are handled when being moved from one box to another during experiments or treatments.

Feeding

Leiopelma pakeka are fed once a week, usually with five very small crickets (< 6 mm long). This diet is supplemented with wax moth larvae, houseflies, locusts or fruit flies once a month, during which time the number of crickets per frog is reduced to three

A3.3 Auckland Zoo

Contact

Andrew Nelson, Team Leader, NZ Fauna (email: Andrew.Nelson@aucklandcity.govt.nz).

Species

Leiopelma archeyi from both the Coromandel and Whareorino populations, which are not mixed. All individuals included in this study were ex University of Canterbury; however, they have recently acquired some *L. archeyi* that were wild-caught form the Whareorino population.

Group housing

Up to eight frogs are housed in each enclosure, but this maximum may be increased to ten as necessary. Enclosures are kept in a purpose-built frog house, within which are two separate rooms that have the same watering/humidity and temperature regimes (as outlined below). The enclosures have three sides of glass and a fourth side that has glass on the bottom half and sliding doors on the top half for access. These doors are made from untreated pine that has been well covered by black enamel paint and aluminium mesh; silicon-based glue was used in their construction. All individuals included in this study were kept on natural substrate. However, in early 2007 all the Whareorino population group housing substrate was changed to dampened commercial Hygenex paper towels that are changed weekly. All Whareorino frogs are kept in one room (room 2), while the Coromandel frogs are kept in a different room (room 1) on natural substrate, which consists of commercially sourced sand and gravel that have been boiled for 1 h; commercially bought palm peat made up with boiling water; and soil substrate with leaf litter from Tapu (Coromandel), which has been sun dried for 3 months (some soil was baked at 200°C for 1 h before being dried in the sun for 3 months). The substrate is never changed but is spot cleaned. Substrate tanks have enclosure 'furniture' sourced from either Tapu or Auckland Zoo, which includes punga logs, broken terracotta pots and drip tray shelters.

Individual housing

Individual housing is currently used for quarantined or sick animals. Frogs are held in plastic Pet-pals containers that contain dampened commercial Hygenex paper towels, which are changed weekly. The tops are covered with Glad wrap to ensure the correct humidity is maintained and containers are kept within the same type of glass terrarium as is used for group housing but without lids. These animals are held in the same room as the Whareorino frogs (room 2).

Temperature

The frog house temperature is kept between 11°C and 15°C. Once a week, the temperature is reduced over 3 consecutive days to 8°C to help reduce bacterial load. There is a monitored temperature alarm system, which activates an alarm if the temperature goes above 15°C.

Lighting

In room 1, a standard fluorescent light is used during the day in addition to natural west-facing daylight filtered by glass. In room 2, a 15-watt shaded bulb is used during the night and an additional 15-watt unfiltered incandescent bulb is used during the day. Both rooms are on 12-h day/night cycles. In addition, both rooms are exposed to reptile/amphibian Acadia™ compact light 5 minutes a week. In early 2007, the fluorescent light in room 1 was discontinued.

Watering/humidity

The enclosures are watered with reverse osmosis water for 5 min four times a week, using hand sprayers that put 3–10 mL in each terrarium, the exact amount depending on how dry the soil is. Since early 2007, this system has been replaced by a manual turn-on irrigation system that uses Nylex irrigation spouts and a hose in each terrarium. There is also a small pot plant drip tray in each enclosure, which is filled with water so the frogs can soak themselves. Both rooms aim for 100% humidity, but this can vary down to 80%. The monitored humidity alarm is set for 60%. The water was changed to filtered instead of reverse osmosis water in early 2007.

Hygiene

Medishield (chlorhexidine) hand disinfectant is used to wash hands, and rubber gloves (Med X synthetic) are used when handling all items and are changed between enclosures. LabServ Nitril powder-free gloves have been used for handling frogs since mid-2006, as the Med X synthetic gloves seem to lather up when wet. Ammonia bleach is used to disinfect items that have been used in the enclosure and reverse osmosis water is used to rinse these items to ensure there is no residue.

Observations/handling

The individually housed frogs are weighed once per week. Colony Whareorino frogs are handled and weighed a maximum of every 6–8 weeks. Colony Coromandel frogs have also been on that regime, but are now being handled once every 6 months to minimise disturbance for breeding. The tanks are observed three times a week and any frogs seen are noted.

Feeding

The frogs were originally fed once a week. However, the colony frogs were changed to twice a week 1 year ago and the individual frogs were changed to this regime 3 months ago. Under this new regime frogs are given the same amount of food—just divided into two feedings. Each group-housed frog is fed six wax moth larvae (dusted with Miner-All Outdoor supplement at every feed

and Herptavite on the first feeding of each month), two or more house flies and 20 or more fruit flies; crickets are fed out to colony enclosures on Fridays. Each individually housed frog is fed six wax moth larvae, four house flies, and 20–30 fruit flies, all of which are dusted with Miner-All Outdoor supplement or Herptavite; four crickets < 5 mm are fed on Fridays.

A3.4 Hamilton Zoo

Contact

Kara Goddard, zookeeper (email: kara.goddard@hcc.govt.nz).

Species

Leiopelma hochstetteri ex University of Canterbury.

Group housing

All frogs are kept in an outdoor enclosure unless they are sick or in quarantine. The enclosure is wooden, with Perspex-lined walls and plastic liner in the pools and streams. The waterways contain small, smooth gravel as well as rocks, soil and leaf litter. The enclosure has a roof, and there are native trees on one side of the enclosure and another enclosure on the other side; however, there is natural patchy sunlight on the ground inside. There are three habitat cells in the enclosure, each of which is 1.7 m × 2 m and houses a maximum of 15 individuals. The substrate is gravel, rocks, screened topsoil and leaf litter, all of which were purchased or obtained on site. All rocks and gravel were rinsed and/or scrubbed and then thoroughly dried, and all soil, logs and leaf litter was dried in a hot shed (over 20°C ambient temperature) for 2 weeks before being used in the enclosure.

Individual housing

Individual housing is currently only used for quarantine or disease isolation purposes for a limited amount of time. The housing is a plastic container ('terrarium') that contains moistened, unbleached paper towels. It is sprayed with filtered water and paper towels are changed twice a week. The temperature is kept as cold as possible with an air conditioner. The terraria are kept in a darkened room with only a small amount of light allowed in during daylight hours.

Temperature

Natural Hamilton conditions.

Lighting

Natural Hamilton conditions.

Water/humidity

Natural Hamilton conditions. There is a stream system and seepage in each habitat cell, and irrigation in the roof, which comes on once or twice a day at variable times and for different lengths of time.

Hygiene

Rubber gloves (Lab Serv Nitril, powder-free) are used for handling frogs and equipment. A variety of disinfectants are used for cleaning equipment and bench tops depending on the stock available: Virkon (1%) solution, Trigene (1%) solution, bleach (5%) solution and clear methylated spirits.

Observations/handling

Each group-housed frog is weighed, measured and examined every 2 months. Individually housed frogs are checked daily and weighed weekly. The outdoor enclosure is checked daily for any problems and a nocturnal frog count survey is done every 2 weeks.

Feeding

All individuals are fed crickets less than 5 mm, wax moth larvae (small) and *Drosophila* three times a week.

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