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Aerially applied citric acid reduces the density of an invasive frog in Hawaii, USA

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Abstract. The Puerto Rican frog (*Eleutherodactylus coqui*) is an invasive pest in Hawaii. Citric acid is approved for controlling these frogs, but has been limited to terrain accessible by foot or vehicle. We determined the effectiveness of helicopter applications of 16% citric acid and repeated 11% citric acid treatments for eradicating and/or reducing densities of *E. coqui* by monitoring populations before and after treatment using mark–recapture. We also evaluated the effects of the marking technique, toe-clipping, and weather changes on population parameter estimates. We found that a 16% citric acid treatment appears to have reduced adult *E. coqui* density 3-fold in a plot, T1, completely covered with citric acid, but did not reduce adult density in a plot, T2, where 6% of the plot was unintentionally not treated. Preadults were reduced 3- to 5-fold in treated plots. The apparent reduction in adults in T1 lasted at least 5 months. Repeated treatments of 11% citric acid were studied in T2 and likely reduced adults 440-fold while preadults were reduced 9-fold. *E. coqui* that had fewer toe-clips had greater recapture probability and survival estimates, while weather had no effect on parameter estimates. In summary, we found that 16% and 11% citric acid treatments can reduce *E. coqui* density, treatment effects can last 5 months for adults, and repeated treatments appear more effective for reducing density than single applications.

Introduction

The nocturnal terrestrial frog (*Eleutherodactylus coqui* Thomas) is one of 27 amphibians and reptiles that have established in Hawaii, USA, where there are no native reptiles or amphibians (Kraus 2003). *E. coqui* was introduced in the late 1980s via the horticulture trade (Kraus *et al*. 1999). This species has become a notable invader and is considered an invasive pest because it threatens private property value (Kaiser and Burnett 2006) due to its loud (80–90 dB at 0.5 m: Beard and Pitt 2005) mating calls, and floriculture and nursery industries due to decreased sales and rejected shipments (Kraus and Campbell 2002; Kaiser and Burnett 2006). It has been estimated that on the island of Hawaii, complaints about *E. coqui* reduce residential property value and profitability for floriculture and nursery products by as much as 0.16%, which could result in a minimum annual loss of US$7.6 million in property value damages and US$81 000 from decreased floriculture and nursery product sales (Kaiser and Burnett 2006).

In addition, *E. coqui* could have ecological impacts. Research suggests that *E. coqui* can attain extremely high densities in Hawaii, up to 890–910 frogs $(100\ m^2)^{-1}$ (Woolbright *et al*. 2006; Beard *et al*. 2008), consume more than an estimated 690 000 prey items $ha^{-1}\ night^{-1}$ (Beard *et al*. 2008), and reduce arthropod prey abundances (Sin *et al*. 2008). More specifically, it is thought that *E. coqui* may reduce endemic arthropod prey (Beard 2007). It has also been suggested that they may compete with endemic birds and the endangered Hawaiian hoary bat for arthropod prey, and serve as another food source for bird predators (Kraus *et al*. 1999; Beard and Pitt 2005). Interactions between *E. coqui* and endemic birds and bats have not yet been studied, but their impacts on arthropods suggest that they could have these impacts in at least some locations. Furthermore, research in Hawaii shows that *E. coqui* can influence ecosystem processes, for example, by reducing herbivory rates, and increasing leaf litter decomposition and new leaf production rates, particularly through increased nutrient availability (Sin *et al*. 2008). This might have implications for non-native plants that increase in abundance with increased soil nutrient availability (e.g. Ostertag and Verville 2002).

Not only can the densities of *E. coqui* be great, but since its introduction *E. coqui* populations have spread rapidly with human assistance (Kraus and Campbell 2002). In 1998 there were eight documented populations (Kraus *et al*. 1999), and by 2001 *E. coqui* had spread to over 275 locations throughout the Hawaiian Islands (Kraus and Campbell 2002). In part due to eradication efforts on Maui, Oahu, and Kauai, *E. coqui* populations are most concentrated in lowland (0–500 m) forests on the eastern side of the island of Hawaii (Beard 2007). Accidental introductions occurred mostly as a result of moving infested nursery plants, while intentional introductions were made by members of the public who mistakenly believed that *E. coqui* could control mosquitoes (research has since shown that *E. coqui* do not consume mosquitoes: Beard 2007) and/or the presence of *E. coqui* would justify campaigns for water features (although *E. coqui* do not need ponds because they have direct development). The more nefarious releases were a response to scientists reporting that the frogs were restricted to a handful of

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locations in the late 1990s. There are now laws that prohibit movement of *E. coqui*, as well as substantially more public support to restrict frogs and their movement.

More than 50 chemicals have been evaluated as a method for controlling *E. coqui* (Campbell 2002; Pitt and Sin 2004*a*) but, of these chemicals, citric acid is the only one that is both effective and considered a minimum-risk pesticide by the USA Environmental Protection Agency (EPA); as such, citric acid is readily available to natural resource managers, private businesses, and the public for control of *E. coqui* (Ohashi 2004). The use of citric acid to control *E. coqui* is considered humane because a laboratory study shows that a 16% citric acid solution results in mortality due to skin absorption and subsequent osmotic shock for 97% of treated *E. coqui* within 0.5 h, and 100% within 1 h (R. Doratt, pers.comm.). Other control techniques for *E. coqui* that have been evaluated include hot water treatment, hand-capture, habitat modification, and use of traps. Hot water (>45°C) is effective in nursery settings (A. Hara, unpubl. data), but it is not feasible to maintain water at this temperature for the period of time required to transport it and apply it on a large scale. Hand-capturing these frogs is not practical for large populations (Beard 2001). Traps made of bamboo or PVC pipe have had limited success in capturing *E. coqui* (Sugihara 2000). Biological control, such as the introduction of disease, predators and parasites, has been considered, but has not been fully evaluated yet. Thus, at the present time, chemical control is the most effective method for controlling *E. coqui* populations.

Citric acid is 100% lethal to *E. coqui* at a concentration of 16% in the laboratory (Pitt and Sin 2004*a*) and in the field, with no significant impacts on non-target invertebrate density (Pitt and Sin 2004*b*), and only minor damage to plants (Pitt and Sin 2004*c*). Citric acid treatments are typically applied from the ground, which limits its application to terrain accessible by foot or vehicles. However, many *E. coqui* populations are located in areas that are not accessible from the ground, and aerial application may be the only way to effectively control *E. coqui* in these areas.

The *E. coqui* population at Manuka State Park, located within the Manuka Natural Area Reserve (NAR) on the south-west side of the island of Hawaii, USA, was discovered in 2000, and was estimated to cover 10 ha in 2005 (L. Hadway, pers. comm.). Evidence was found that suggested that *E. coqui* was intentionally introduced to this area by residents (L. Hadway, pers. comm.). Although there is a great need for additional research on the ecological impacts of *E. coqui* in Hawaii, controlling the population of *E. coqui* in the Manuka NAR is a management priority due to the potential for *E. coqui* to impact populations of numerous rare animals and plants in the park (Beard and Pitt 2005; Sin *et al*. 2008), and to stem its spread. The *E. coqui* population at Manuka NAR is isolated from the few relatively close populations by many kilometres of inhospitable terrain (bare lava fields with relatively low rainfall), and thus has greater potential to be eradicated than populations in close proximity to each other.

Because an aerial citric acid application is very costly, it is critical to determine the effectiveness of this treatment. Pitt and Sin (2004*b*) examined the effects of ground-sprayed citric acid on the abundance of *E. coqui* by measuring sound levels as an index of abundance; however, this index measures male calling activity, which can be affected by daily weather, and does not necessarily approximate true abundance or density (Fogarty and Vilella 2001). Funk *et al*. (2003) and Fogarty and Vilella (2001) determined that mark–recapture methods are the best known method for monitoring population trends in *Eleutherodactylus* species, because they generate more precise and less biased abundance estimates, and have the ability to estimate key vital rates (i.e. survival). No studies conducted have determined the effectiveness of citric acid for controlling *E. coqui* using mark–recapture analysis.

The purpose of this study was to evaluate the effectiveness of aerial applications of 16% and 11% citric acid solutions for controlling *E. coqui* in Manuka State Park by monitoring *E. coqui* before and after treatment with mark–recapture analysis. We also viewed this study as an opportunity to determine how a marking technique and weather influence population parameter estimates. We used toe-clipping to individually mark *E. coqui*, and some studies suggest that toe-clipping may reduce recapture probability and survival of amphibians (McCarthy and Parris 2004). Studies conducted in Puerto Rico suggest that weather affects the activity of *E. coqui*, and thus might influence parameter estimates (Fogarty and Vilella 2002; Woolbright 2005). Because accurate assessment of *E. coqui* populations is crucial for determining the effectiveness of control efforts, we determined how toe-clipping and weather influence population parameters, such as recapture probability, survival, and population size estimates.

Methods

Study area

The experiment was conducted in the tropical lowland mesic forests of Manuka State Park, located within the Manuka NAR, Hawaii, USA (19°06′N, 154°54′W; elevation: 540 m). Mean annual precipitation is 1000 mm (Giambelluca *et al*. 1986), which is received through the action of sea–land breezes (maximum during May–September) and during winter storms (October–April) (Price 1983). Mean annual temperature is 22°C (Nullet and Sanderson 1993) with little seasonal variation (Price 1983). Dominant vegetation included *Psidium cattleianum*, *Metrosideros polymorpha*, and *Cecropia obtusifolia*, and dominant understorey consisted of *Psidium cattleianum* and *Ochna serrulata*. The park is on an a‘a lava flow substrate (the surface is broken into rough angular fragments) that is 750–1500 years old (Wolfe and Morris 1996).

In the park, there are numerous rare plants, including the endangered Hawaiian grape (*Gouania vitifolia*) and mehamehame (*Flueggea neowawraea*), and rare animals, including Hawaiian hoary bat (*Lasiurus cinereus semotus*), and Hawaiian hawk (*Buteo solitarius*). Some non-native species besides *E. coqui* have been observed in the park, including Jackson’s chameleon (*Chamelaeleo jacksonii*) and cane spiders (*Heteropoda venatoria*). No other non-native frogs were observed at the study site; therefore, ‘frogs’ refers to *E. coqui* throughout.

Sampling design, surveys and treatment

We randomly selected four 20 × 20-m plots [two treatment (T1 and T2) and two control (C1 and C2) plots] within an 80 × 60-m

area (4800 m^2). There was 7–8 m between plots. We deemed this distance appropriate because *E. coqui* have small territory sizes ($5 \times 5\ m^2$) and often do not disperse over the course of several years (Woolbright 1985). Control plots were 15–50 m from treatment plots, which was close enough to have similar vegetation but far enough away to ensure they were not treated.

From 1300 to 1600 hours on 25 May 2005, the State of Hawaii NAR System aerially (by helicopter) applied a 16% citric acid solution to a 1700-m^2 area of Manuka State Park, which included the two treatment plots. A firefighting Bambi bucket was used to dump 303 L of citric acid 30 times to the area, a rate of 1.12–2.24 L m^{-2}. Turf Mark (a blue dye) was mixed with the citric acid to indicate where the pesticide was applied. Control plots were not treated with citric acid.

We used standardised census and marking techniques, techniques that have previously been used on this species (Woolbright 2005), to monitor these populations. Previous mark–recapture surveys conducted in seven locations in Hawaii suggested that it typically requires 5–9 consecutive days to obtain a 60% recapture rate and abundance estimates with adequate precision (Beard *et al*. 2008). Therefore, to evaluate treatment effects, we surveyed plots (pre- and post-treatment) over 16-day periods. The pretreatment sampling period was 9–24 May 2005, and the post-treatment sampling period was 27 May–12 June 2005.

Each night, three researchers searched one pair of plots (C1 and T1 or C2 and T2), and alternated between sets of plots, so that we searched each plot for eight nights. Within each plot, we established four 5-m transects. We used headlamps to search each transect for 15 min, excluding handling time. Plot surveys began after *E. coqui* had sufficient time to move to nocturnal perches (~1930 hours). We began the first survey in the first transect of each plot, and alternated between starting in the first or last transect during subsequent nights.

Five months after the post-treatment sampling period (15–19 October 2005), we resurveyed C1 and T1 to determine the magnitude of population change since treatment. On 23–28 October 2005, from 1300 to 1600 hours, the previously treated area and additional surrounding area (total of ~30 028 m^2, but not including the control plots) were treated, and on 7–10 November 2005 re-treated, using methods similar to the initial treatment (except these treatments consisted of an 11% citric acid solution to reduce mixing time and overall cost). One month after this set of repeated 11% treatments (5–9 December), C2 and T2 were resurveyed. We surveyed only one control and one treatment plot in the 5-month post-treatment survey and in the repeated 11% treatment survey, so that we could visit plots on consecutive, as opposed to alternating, nights, and potentially achieve greater recapture rates.

When frogs were captured, we measured and marked adults ≥25 mm in snout–vent length (SVL; Woolbright 2005). We measured SVL to the nearest 0.1 mm with dial calipers, and sexed and marked frogs by clipping 1–4 toes (one clip per foot) in unique combinations. We did not mark juveniles and subadults (6–24 mm SVL), hereafter preadults, because toes are often too small for clipping, but we counted these frogs each night (Woolbright 2005). To calculate initial total density (frogs $(100\ m^2)^{-1}$), we calculated nightly preadult to adult ratios for the sampling period and multiplied the mean ratio by the estimated adult density (as in Woolbright *et al*. 2006). This ratio assumes that encounter probabilities are equal for adults and preadults. Finally, we measured ambient temperature and relative humidity hourly during survey periods using a HOBO data logger (Onset Computer Corporation, Bourne, MA).

Analyses

We estimated population size and survival rates using a robust-design approach with Huggins closed-capture models in Program MARK (White and Burnham 1999). We then assessed the degree to which citric acid application influenced these parameters using *post hoc* comparisons of the parameter estimates. We selected this approach because robust design models can reduce bias associated with heterogeneous capture/recapture probabilities (Pollock 1982) often found in *E. coqui* populations in Hawaii (Woolbright *et al*. 2006). We estimated the probability of initial capture (p) and recapture (c), survival (S), and population size ($\hat{N}$) for adult *E. coqui*. For these analyses, we were interested in the abundance and survival estimates of frogs in control and treatment plots before and after citric acid application. We established a set of *a priori* models where we included sampling period and the number of toe-clips as an individual covariate. We also considered two environmental covariates, relative humidity and temperature, which were modelled separately and as an index [temperature–humidity index (THI) = (0.8 × ambient temperature) + (% relative humidity/100) × (ambient temperature – 14.3) + 46.3: e.g. Thom 1959] for each sampling period. We assumed that γ'' and γ', the probability that an individual frog is unavailable for detection (i.e. outside the study area) during a given time, given that it was previously available or unavailable, respectively, were random, and modelled $\gamma'' = \gamma'$ (Kendall *et al*. 1997). Similar to Franklin *et al*. (2004), we first modelled p and c similarly, while the structure of survival was general. We used program MARK to generate the likelihood function value and estimate the appropriate Akaike's information criterion value (AIC_c: bias-adjusted for small sample size) for each model we evaluated. We used the minimum AIC_c model for p and c from the initial models, and fitted additional models for survival. Additionally, we conducted a *post hoc* analysis in which we modelled the number of toe-clips as groups to determine the effects of the number of toe-clips on recapture probability and survival estimates in control plots only (i.e. no toe-clip × treatment interaction).

To maximise the information gained within a multimodel approach, we used model averaging, where we used Akaike weights to compute a weighted estimate for each parameter (Burnham and Anderson 1998). Under a model-averaging approach, models with different structures can be considered simultaneously; however, those models with larger Akaike weights will have greater influence on the overall model-averaged estimates. We calculated model-averaged parameter estimates and associated 95% confidence intervals for each plot.

We analysed preadult counts and mean recapture probabilities for adult frogs with one, two, three or four toes clipped using SAS/STAT ver. 9.1.3 (2006). We considered $P < 0.05$ significant for all statistical tests. We tested the counts for normality using Shapiro–Wilk's W (PROC UNIVARIATE). Because the counts deviated

from a normal distribution, we used a non-parametric Kruskal–Wallis test (χ^2) to compare means (PROC NPAR1WAY). We used a one-way ANOVA with repeated measures and *post hoc* multiple comparisons with a Tukey–Kramer adjustment in PROC MIXED to determine differences in mean recapture probability across sampling periods (in all plots before treatment and in control plots across all sampling periods) for frogs with one, two, three or four toes clipped. Results are presented with ± 1 s.e. throughout.

Results

During the course of this experiment, we marked 1399 frogs. Although *E. coqui* populations typically have a 1 : 1 sex ratio (Stewart 1985), of the marked frogs, 1138 were male (mean SVL = 29.4 ± 0.1), 136 were female (SVL = 33.9 ± 0.3), and sex was indeterminable for 125 frogs. Male bias often occurs in studies of *E. coqui* because of the ease of detection of male frogs. The closed-population assumption for each sampling period was generally met in this study, as we observed only 4% of marked adults moving between adjacent plots.

We achieved a recapture rate of 17–75% for all plots during a given sampling period (except T2, during the period after the repeated treatments, when only one frog was captured). During the sampling period 5 months after the post-treatment survey, we sampled for only five consecutive nights, because recapture rates were ≥60%. During the sampling period following the repeated treatments, we ceased the survey after seven nights, because capture rates were very low in C2 (a total of 14 captures during the last five nights), and almost zero in T2.

Citric acid application

After the first treatment, observation of surfaces covered with the dye revealed that T1 was thoroughly covered with citric acid, but there was no dye visible in a 50-m^2 section at the plot boundary of T2 (50% of the section was within the plot), suggesting it was not covered with citric acid. On the first night of sampling in T2 following treatment, 15 of 18 adults captured were found in this untreated section. During additional treatments, treatment plots were completely covered.

Adult density

Across plots, pretreatment adult density estimates were 110–200 adults $(100\ m^2)^{-1}$. After the initial treatment, density estimates of adults were lower than pretreatment estimates in T1, but post-treatment estimates were similar to pretreatment estimates in control plots and T2 (Fig. 1). During the sampling period 5 months after the initial 16% citric acid treatment, $\hat{N}$ for both C1 and T1 plots increased, but both were still similar to post-treatment estimates, suggesting that T1 did not recover to pretreatment levels. During the sampling period following the repeated 11% citric acid treatments, when surveys were conducted in C2 and T2, $\hat{N}$ for C2 did not change, and we were not able to estimate $\hat{N}$ for T2 because we found only one adult during that period (Fig. 1). If this one individual represents the size of that population, it would be drastically lower than previous estimates.

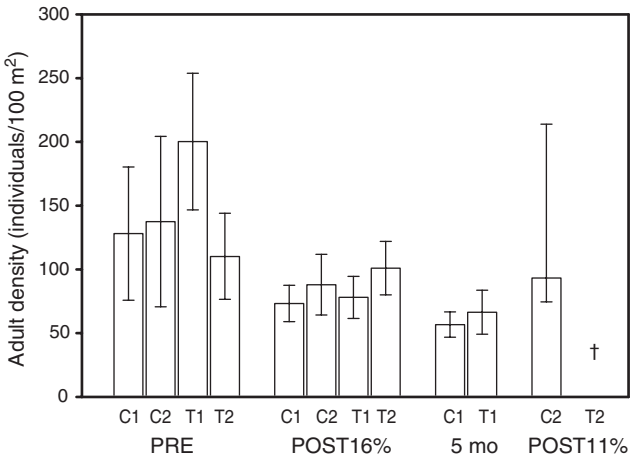

Fig. 1. Model-averaged population density estimates for adult *Eleutherodactylus coqui* and 95% confidence intervals for four sampling periods: pretreatment (PRE), post-treatment (POST16%), 5 months after post-treatment (5 months), and after repeated treatments (POST11%) in control plots (C1 and C2) and plots treated with citric acid (T1 and T2). † = estimate is 0.25, based on maximum count.

Preadult counts and total plot density

Before treatment, maximum preadult counts ranged from 16 to 65. Total plot densities ranged from 223 to 983 frogs $(100\ m^2)^{-1}$ during the pretreatment period. In C1, mean preadult counts were not significantly different across sampling periods ($\chi^2 = 3.33$, d.f. = 2, $P = 0.19$), and in C2 mean preadult counts were not significantly different between the pretreatment period and after the 16% citric acid treatment ($\chi^2 = 0.34$, d.f. = 1, $P > 0.05$). After the 16% treatment, preadult counts were 5.4 times lower in T1 ($\chi^2 = 7.8$, d.f. = 1, $P < 0.01$) and 3.0 times lower in T2 ($\chi^2 = 8.1$, d.f. = 1, $P < 0.01$) than in the pretreatment period. Five months after the 16% treatment, preadult counts were not different in C1, but increased 4-fold from the post-treatment survey in T1 ($\chi^2 = 8.6$, d.f. = 1, $P < 0.01$). After the repeated 11% citric acid treatments, preadult counts increased 4-fold in C2 ($\chi^2 = 7.0$, d.f. = 1, $P < 0.01$) and decreased 9-fold in T2 ($\chi^2 = 7.4$, d.f. = 1, $P < 0.01$) from the previous survey period.

Across sampling periods, capture and recapture probabilities were generally low (mean capture and recapture probabilities were both 0.07 ± 0.01). However, mean capture (C1: 0.18 ± 0.02; T1: 0.12 ± 0.02) and recapture (C1: 0.18 ± 0.03; T1: 0.14 ± 0.02) probabilities were higher during the 5-month post-treatment survey than during the pretreatment survey and following the 16% post-treatment survey, probably because we surveyed on consecutive nights during the post-treatment survey. Low capture and recapture probabilities limited our ability to calculate robust estimates of survival in all plots. We did find that the survival estimate in C1 was 0.94 ± 0.07 between the first two sampling periods, and 0.53 ± 0.09 between the post-treatment survey and 5 months later, and 0.37 ± 0.20 in C2 between the post-treatment survey and 6.5 months later. We were also able to determine that survival estimates were greater in C1 (0.53 ± 0.09) than in T1 (0.35 ± 0.07) between the post-treatment survey and 5 months later.

Table 1. Akaike's information criterion adjusted for small sample size (AIC_c), AIC_c weights, number of parameters, and deviance for robust design models examining survival estimates (*S*) and capture (*p*) and recapture (*c*) probabilities of adult *Eleutherodactylus coqui* in control plots (C1 and C2) and plots treated with citric acid (T1 and T2) during pretreatment and multiple post-treatment periods

The number of frog toes clipped (toe) and a temperature–humidity index (THI) were included as covariates. t = sampling night; γ'' and γ' = the probability that an individual frog is unavailable for detection during a given time, given that it was previously available or unavailable, respectively; and $p = c$ indicates that *p* and *c* were modelled similarly

Plot	Model	ΔAIC_c	AIC_c weight	No. of parameters	Deviance
C1	$S_{(t+toe)}\ p = c_{(period*t)c+toe}\ \gamma' = \gamma''_{(.)}$	0.00	0.7424	27	2667.36
C1	$S_{(t+toe)}\ p = c_{(period*t)c+toe+THI}\ \gamma' = \gamma''_{(.)}$	2.21	0.2463	28	2667.36
C2	$S_{(t+toe)}\ p = c_{(period*t)c*toe}\ \gamma' = \gamma''_{(.)}$	0.00	0.2788	30	1914.13
C2	$S_{(.)}\ p = c_{(period*t)c*toe}\ \gamma' = \gamma''_{(.)}$	1.20	0.1539	28	1919.97
C2	$S_{(t+toe)}\ p = c_{(period*t)}\ \gamma' = \gamma''_{(.)}$	1.32	0.1443	27	1922.41
C2	$S_{(t)}\ p = c_{(period*t)c*toe}\ \gamma' = \gamma''_{(.)}$	2.03	0.1011	29	1918.50
T1	$S_{(t)}\ p = c_{(period*t)c+toe}\ \gamma' = \gamma''_{(.)}$	0.00	0.4507	26	3234.75
T1	$S_{(t+toe)}\ p = c_{(period*t)c+toe}\ \gamma' = \gamma''_{(.)}$	0.46	0.3578	27	3233.04
T2	$S_{(.)}\ p = c_{(period*t)c+toe}\ \gamma' = \gamma''_{(.)}$	0.00	0.3591	25	2129.05
T2	$S_{(toe)}\ p = c_{(period*t)c+toe}\ \gamma' = \gamma''_{(.)}$	1.57	0.1637	26	2128.38
T2	$S_{(.)}\ p = c_{(period*t)c+toe}\ \gamma' = \gamma''_{(.)}$	1.81	0.1451	23	2135.33
C1,C2[A]	$S_{(t*toe)}\ p = c_{(period*t)\ (t+toe)}\ \gamma' = \gamma''(.)$	0.00	0.9694	50	4642.91

[A]*Post hoc* model for control plots combined.

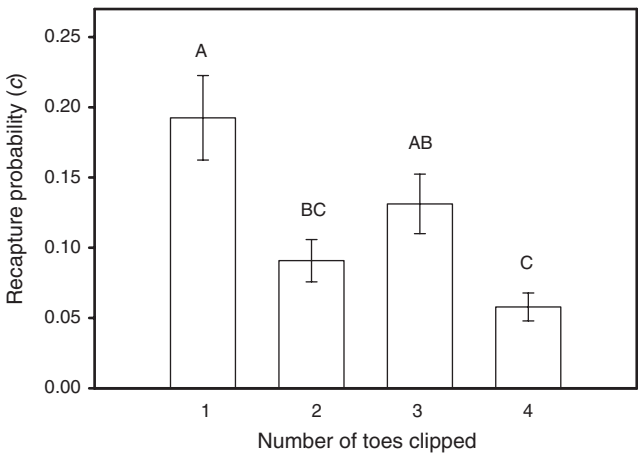

Fig. 2. Mean recapture probability (±s.e.) for adult *Eleutherodactylus coqui* for frogs with one, two, three or four toes clipped during the pretreatment sampling period in two control and two citric acid treatment plots. Mean recapture probabilities with the same letter are not significantly different ($P < 0.05$).

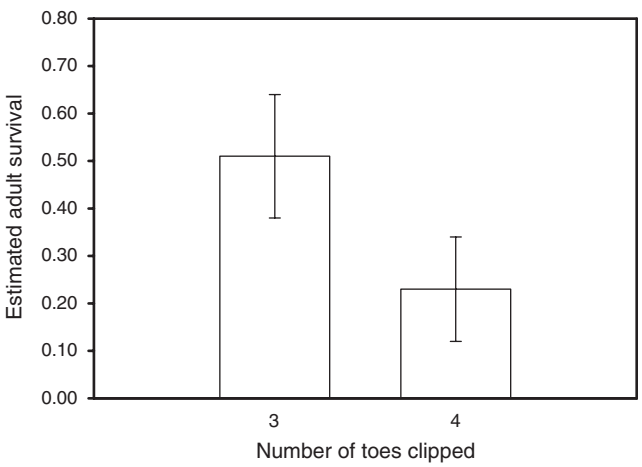

Fig. 3. Survival estimates (±s.e.) for adult *Eleutherodactylus coqui* for frogs with three or four toes clipped in one control plot (C1) between two sampling periods (post-treatment and 5 months after post-treatment). Robust survival estimates could not be determined for frogs in a second control plot, for frogs with one or two toes clipped, for the time interval between pretreatment and post-treatment, or for the time interval between 5 months after post-treatment and after repeated treatments.

Effects of monitoring methods

Overall, model structure of the top *a priori* models was similar across plots (Table 1). None of the top models contained either of the environmental covariates or THI (ΔAIC_c scores >2). The top model for all plots included the toe-clip covariate. The number of toe-clips had a negative effect on recapture probability across plots, and on survival estimates in control plots (Table 1). ANOVA results showed that recapture probabilities were different among frogs with different numbers of toes clipped across all plots before treatment (toe, $F_{3,40.1} = 8.01$, $P < 0.001$). Pairwise comparisons showed that frogs with one toe clipped had a higher recapture probability than frogs with two or four toes clipped (Fig. 2). Similarly, for control plots across all sampling periods, ANOVA results showed that recapture probabilities were different among frogs with different numbers of toes clipped (toe, $F_{3,35.8} = 3.47$, $P = 0.026$), and pairwise comparisons showed that frogs with two toes clipped had a higher recapture probability than frogs with three toes clipped (toe, $t_{34} = 3.05$, $P = 0.021$). We were also able to determine that survival estimates were greater for frogs with three toes clipped than for those with four toes clipped in C1 between the post-treatment survey and 5 months later (Fig. 3).

Discussion

At Manuka NAR before treatment, adult *E. coqui* densities (110–200 adults $(100\,m^2)^{-1}$) were 3–6 times greater than mean long-term, high-end estimates from Puerto Rico (33 adults $(100\,m^2)^{-1}$: Stewart 1985; Stewart and Woolbright 1996). In addition, estimates of total frog density (223–983 frogs $(100\,m^2)^{-1}$) were 1.1–5.0 times greater than mean long-term estimates from Puerto Rico (206 frogs $(100\,m^2)^{-1}$: Stewart and Woolbright 1996). Both of these results suggest that *E. coqui* densities in Hawaii can be 5 times greater than long-term density estimates from Puerto Rico (Woolbright *et al*. 2006).

Citric acid effects

We found that aerial application of 16% citric acid caused what appears to be a 3-fold reduction in adult density in T1, while no change was observed in control plots. We also found that 16% citric acid caused a 5-fold reduction in preadult density in T1 and a 3-fold reduction in T2, while no similar change was observed in control plots. The lack of a decrease in adult density in T2 with the first application was likely due to incomplete coverage with citric acid. This result was expected because on the first night following treatment, 83% of the frogs captured in this plot was found in the untreated area, and highlights the importance of completely covering areas targeted for control.

Because only 2% of the 10-ha infestation at Manuka NAR was treated with the 16% citric acid application, it is likely that *E. coqui* would completely reinfest this area over time. We found that 74% of the adults in T1 during the 5-month post-treatment survey were not previously marked, suggesting that some adults had begun to migrate into the treated area. However, our study suggests that adult *E. coqui* were not able to significantly reinfest the treated area over 5 months, because adult density following the treatment and 5 months later were similar. Numbers of preadults increased 4-fold by 5 months after the treatment in T1. It is unclear what proportion of these preadults was in the plot during the treatment, what proportion was new hatchlings, and what proportion migrated from untreated areas. However, the results suggest that preadults are the most likely individuals to reinfest treated areas. This result is not surprising because adult *E. coqui* are known to be territorial (Townsend *et al*. 1984), but highlights the importance of treating the entire infested area.

While laboratory research suggests that 16% citric acid is the lowest concentration that is 100% lethal to *E. coqui* (Pitt and Sin 2004*a*), 11% was attempted in the field because it greatly reduced mixing time and hence overall cost. When a much larger area (30% of the 10-ha infestation) was treated with 11% citric acid twice within 2.5 weeks, we found only one adult in the treatment plot studied (an apparent 440-fold reduction) and a 9-fold reduction in preadult counts following the treatment. These findings, in addition to the lack of change in adult density and an increase in counts of preadults in the control plot, suggest that these treatments caused an even greater reduction in localised frog abundance than the single application of 16% citric acid. Repeated treatments are likely to be more effective than a single treatment because some frogs do not emerge from retreat sites each night (Stewart 1985; Townsend and Stewart 1994) and retreat sites, which are often curled leaves, under rocks, or in bark (Stewart 1985), may be sheltered from treatment. During survey nights when capture rates were low, we observed a large number of frog calls originating from subterranean passages in the lava rock; the use of subterranean passages may also reduce the effectiveness of citric acid treatments.

Effects of monitoring methods

While some studies have reported a reduction in recapture probability and survival estimates of animals marked with toe-clips (Clarke 1972; Bull and Williamson 1996; McCarthy and Parris 2004), others have found no significant effects (Lemckert 1996; Reaser and Dexter 1996; van Gelder and Strijbosch 1996; Ott and Scott 1999), although the absence of significant effects in at least Lemckert's (1996) study may be due to a lack of statistical power (Parris and McCarthy 2001). Parris and McCarthy (2001) also found that amphibian recapture probabilities generally decrease with increasing number of toes clipped. We found that the number of toes clipped generally has a negative effect on recapture probability and survival estimates in *E. coqui*. We found that in all plots before treatment, frogs with two or four toes clipped had lower recapture probabilities than frogs with one toe clipped. We also found that in control plots across all sampling periods, frogs with two toes clipped had higher recapture probabilities than frogs with three toes clipped. Our results suggest that the fewer toes clipped, the greater the recapture probability, and thus caution should be exercised when using the toe-clip marking method because it may influence parameter estimates, especially in the frog's native range, where montane populations are declining (Burrowes *et al*. 2004). If toe-clips are used, we recommend no more than one toe-clip per frog. Fluorescent marking is another method that has been used in mark–recapture studies of *E. coqui* (Fogarty and Vilella 2001, 2002); future research should compare survival and recapture probability estimates for toe-clipping, fluorescent marking, and other potential methods of marking *E. coqui* (Phillot *et al*. 2007).

We have two potential explanations for the low recapture probabilities observed in this study. First, we found that recapture probabilities were higher when surveys were conducted on consecutive nights, which suggests that future surveys of *E. coqui* should be conducted on consecutive nights when possible. Second, our observation of a large number of frog calls originating from subterranean passages in the lava rock during nights when capture rates were low suggests that use of such passages by frogs limited our ability to locate individuals and may, in part, explain the low recapture probabilities. This suggests that, at similar sites in Hawaii, most *E. coqui* may not be observed in a given night, and that recapture probabilities may be low (Woolbright *et al*. 2006).

Future research

The studies examining the effects of citric acid on non-target invertebrates and plants (Pitt and Sin 2004*b*, 2004*c*) were conducted on relatively short time-scales. During our study, we noted that, other than *E. coqui*, the only observed mortalities were non-native earthworms. We also noted some discolored leaves of non-native vegetation. The USA EPA believes that citric acid poses a negligible long-term effect on

the environment (a minimum-risk pesticide) because (1) it occurs naturally in soil, water, plants, citrus fruits, and animal tissues and fluids, (2) it is a common food additive, (3) it is a well known product of carbohydrate metabolism in living organisms, and (4) it degrades readily (US Environmental Protection Agency 1992). Nonetheless, future studies examining the long-term impact of citric acid application to the environment would be of interest.

Since the conclusion of this study, the State of Hawaii NAR System has treated the infestation at Manuka with aerial application of 13% citric acid solution on three different occasions in 2006 (March, August, and December), and with a 14% citric acid solution on two different occasions in 2007 (March and May). For each of these treatments, the acid concentration was below 16%, the entire infested area was not treated, and one application was conducted during each period (unlike the last treatment in 2005) due to high costs and logistical constraints. Following each of these treatments, reinfestations of treated areas have been observed, making continual treatment necessary (H. Sin, pers. comm.). Thus, it appears that to justify the cost and effort of controlling *E. coqui* with citric acid, the entire infested area should be treated multiple times within a short period, and then the reduction in abundance of *E. coqui* should be weighed against the cost. If the population is contained or eradicated, then the cost of applying the acid may be justified.

E. coqui is not considered eradicable on the island of Hawaii, and control efforts are now focussed on treating small isolated populations to contain spread (HDLNR/HISC 2006). The cost of current detection and control efforts on the island of Hawaii is US$2.8 million annually, and it has been estimated that it would cost another US$6.0 million annually to reach a more desirable level of detection and control; however, funding for this additional amount is not typically available (HDLNR/HISC 2006). Other Hawaiian islands also have populations of *E. coqui*. Kauai has one small population; Oahu has no wild populations (populations outside of nurseries); and Maui has relatively few populations (one population is relatively large). Island-wide eradication for these other islands is the focus and is thought to be possible. We recommend the use of citric acid for these control efforts, especially if the results of long-term studies on environmental impacts are favourable; however, we do not recommend its use in geographical locations outside Hawaii where native amphibian species and/or amphibian species of concern occur.

Conclusions

Daytime aerial application of a 16% citric acid solution and repeated 11% citric acid solution appears to be effective for temporarily reducing the abundance of *E. coqui*. In areas inaccessible to ground spraying equipment, we recommend that this method of pesticide application be used. We especially recommend its use in small and/or isolated areas, where containing the spread of *E. coqui* or its eradication is considered likely. Managers should first identify the extent of the infestation on the landscape, and then treat the entire frog-infested area, along with a buffer zone in the surrounding area as quickly as possible. We recommend using dye to identify whether sections of the treatment area were missed. To justify the cost and efforts of control, the entire frog-infested area should be treated multiple times within a short period. Current knowledge suggests that the citric acid treatment is ethically and environmentally justifiable for control, but future studies examining long-term environmental impacts would be beneficial. Furthermore, when monitoring *E. coqui* populations, the effects of the marking method on recapture probability and survival should be considered when estimating population parameters.

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