Testing tools for eradicating the invasive toad *Duttaphrynus melanostictus* in Madagascar

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SUMMARY

In 2014, the Asian toad *Duttaphrynus melanostictus* was first recorded as an invasive species in Madagascar. A feasibility study identified an urgent need to test eradication tools. This study attempts to refine estimates of the toad population and test four potential eradication tools: 1) pitfall trapping and drift fencing, 2) hand-capture removal, 3) citric acid sprays, and 4) tadpole trapping. Using delimited searches and removal trials we estimate that the Asian toad population exceeds seven million post-metamorphic toads within the incursion. Pitfall trapping and drift fencing appeared to function well as control strategies, considering the challenges of operating in a rural working environment. Capture rates suggested that, at the spacing used, a minimum of 14 nights of trapping was needed to see a strong decline in capture rates. Hand-capture of toads demonstrated the potential of local labour to deplete a free ranging toad population, but also showed that the duration of effort would need to be extended as capture rates did not decline strongly over time. Citric acid spray trials showed that this topical toxicant can be very effective for toad control, especially for juveniles. Phytotoxicity trials suggest crop and vegetation damage was not prohibitive to its broader use. Tadpole traps did not work, but we are uncertain of the influence of tadpole developmental stages on this result. This study suggests that an eradication strategy may be possible and should be tested in carefully ordered trials within a delimited area. However, the prospects of employing the best methods over the entire incursion area is likely to be cost-prohibitive and extremely high risk.

BACKGROUND

Invasive species are acknowledged as amongst the primary drivers of biodiversity loss and degradation of ecosystem function (Butchart *et al*. 2010, Lowe *et al*. 2000, Mack & Antonio 1998). Whilst tools are available to eradicate the more common invasive vertebrates, procedures to eradicate a broader taxonomic range of invasive animals remain less well developed. In the case of invasive reptiles and amphibians, only a handful of established frog populations have been eradicated around the globe (Kraus 2009, Beachy *et al*. 2011, Wingate 2011). Established populations of other reptiles and amphibians have not yet been successfully eradicated, although an incipient incursion of four Italian wall lizards *Podarcis siculus* was wisely removed from Great Britain prior to allowing a population to establish (Hodgkins *et al*. 2012). Despite a paucity of tools and experience, there have been some efforts to remove a small number of other amphibian incursions, namely the cane toad *Rhinella marina* in Australia and Bermuda; the north American bullfrog *Lithobates catesbeianus* in the US, British Columbia, and parts of Europe; and the coquí *Eleutherodactylus coqui* in Hawaii (Kraus 2009, Beachy *et al.* 2011, Orchard 2011, Wingate 2011). Research and management of cane toads in Australia have largely focused on ecological impacts and invasion biology.

There have also been attempts to develop methods to limit the spread and control populations of invasive amphibians. Chief amongst these is the development of techniques that exploit the parotoid gland secretions as a tadpole attractant (Crossland & Shine 2011) and acoustic luring of adult toads (Schwarzkopf & Alford 2007). In Hawaii, techniques had been developed to remove coquí populations using citric acid sprays (e.g. Beachy *et al.* 2011), and this technique is the only easily scalable tool for removing terrestrial amphibians without the need for individual capture or significant habitat alteration. Although some evidence from Hawaii exists for phytotoxicity of 16% w/v citric-acid solution, impacts on crop health were minimal (Pitt & Sin 2004).

In March 2014, the Asian toad *Duttaphrynus melanostictus*, was first recorded in Madagascar (Kolby *et al.* 2014). This species is a large, robust bufonid anuran already known as a successful invader in Indonesia, the Maldives, New Guinea, and Timor-Leste (Kraus 2009, Trainor 2009). For this reason, and by analogy with ecological damage created by the cane toad in Australia (Lever 2001, Kraus 2009, Shine 2010), concerns were raised that this species could have a serious impact on Madagascar's biodiversity by means of predator poisoning, competition, or disease transmission to native wildlife (Kolby 2014). Work was quickly conducted to establish the range and circumstances of the incursion, which was determined as covering approximately 108 km^2 near the eastern port city of Toamasina, centred around the large Ambatovy nickel refinery as of November 2014 (Moore *et al.*

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2015). An eradication feasibility study stated it was immediately clear that the scale of the incursion was at a point where successful eradication was a rapidly diminishing possibility (McClelland *et al*. 2015). This study identified an urgent need to identify and test potential eradication tools as there was no precedent for the successful eradication of an amphibian on such a scale, nor are there any examples of attempted eradication of the Asian toad.

Along with novel techniques it was necessary to test the effectiveness of more established tools, such as drift fences and pitfall traps. We therefore used these methods to aid estimation of toad densities and contribute to an eradication strategy (Corn 1994, Greenburg *et al*. 1994). Early discussions of possible eradication methods often returned to the large and inexpensive labour pool available in the incursion area, since Toamasina has a human population of approximately 250,000, many of whom are subsistence farmers and labourers in one of the poorest economies on earth (Minten & Zeller 2000). We therefore also tested hand capture in a clearly demarcated area to evaluate the logistics, efficacy and sustainability of using local labour for toad removal, as well as to evaluate capture rates. Density estimates from this trial were also used to update our estimates of the total toad population, which in turn could influence the chosen control strategy.

ACTION

Population densities: In January 2015, we deployed small teams of labourers to determine the total number of Asian toads in plots selected from urban, rural/agricultural, and non-production forest habitat within the incursion area, the last of which largely consists of regenerating exotic woody species dominated by *Eucalyptus* spp. For each of these three ecotypes, we arbitrarily selected three 20 × 20 m plots for trials, making nine plots in total. We demarcated these plots with a string boundary marker, and a team of five individuals systematically searched through the habitat, disturbing surface vegetation, lifting all objects capable of concealing toads, and removing all individuals detected. Toad populations remained open during searches but the daytime timing (09:00-15:00 h) of the searches means that toads were all sedentary in refuges. Searches took 2–3 hours or 10–15 man-hours/plot, and each plot was searched three times on different days.

Pitfall trapping and drift fencing: We selected three sites near villages (Farafaty, Tanandava and Ampasimaneva) to test toad eradication tools in habitat typical of the incursion area. These sites were used to conduct a series of trials, including obtaining a population estimate. At each site a delimited area of 1,600 m^2 (Figure 1), enclosed with a 60 cm tall plastic drift fence, was set with twenty-one 20 litre pitfall traps of depth 50 cm, set 10 m apart. We drilled buckets with a 5 mm bit to allow drainage. These enclosed areas also had two 40 m drift fences set in a cross. We serviced all pitfall traps daily, removed all captured toads and measured snout-vent length (SVL), and recorded and released all non-target species. We continued trapping until there were three consecutive days with no capture of toads.

On capture, we humanely dispatched all toads, using a sharpened reinforcing rod to sever the spinal cord by stabbing the toads between the parotoid glands.

Hand capture removal: In January and February 2016, we selected one plot of 9 ha at each of our three village study sites

Figure 1. Aerial view of the fenced enclosure at Farafaty, Madagascar, encompassing homes, gardens and trees, which was sampled with drift fences and pitfall traps. Plots in the other two villages had the same design.

to include standing and flowing water bodies, inhabited areas, rice paddy, gardens, forest, and grazing land. We demarcated these plots with flagging tape at 10 m intervals tied to vegetation and boundary structures. From the local community, we employed teams of ten individuals tasked with working 8 h/day collecting toads within each plot; toads were gathered in barrels each morning for processing by the field teams (Figure 2.). Search teams focused their efforts between 04:00–08:00 h and again between 18:00–22:00 h. We trained teams to search areas of natural cover and to identify toad calls to aid in locating calling males. Each team worked daily for 21 days.

At each site, six man hours were devoted to conducting abundance surveys, recording catch per unit effort prior to the removal of toads, again after two weeks of removal, and again at the end of the catching period.

Citric acid trials: We tested the toxicity of both 16% and 25% w/v citric acid solution on different size classes of the Asian toad during January and February 2016. We prepared two treatment and one control containers (plastic drums of 70 cm diameter) each to test small (< 35 mm SVL), medium (35–70 mm SVL), and large (>70 mm SVL) toads (a total of 9 containers). We used 10 toads for each test. Each set of ten toads was sprayed with 50 ml 16% citric acid solution, 50 ml 25% citric acid solution, or 50 ml water (control). We applied

Figure 2. A barrel of Asian toads *Duttaphrynus melanostictus* collected by hand in one night from the unfenced 9 ha plot at Farafaty, Madagascar.

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the citric acid solution using a 500 ml hand-pump spray bottle. We recorded toad condition after 30 minutes of exposure, with blink response, limb retraction, and laboured breathing scored as measures of stress and morbidity. We then washed the containers to remove solution residue, placed the toads back in the cleaned containers, and checked them 24 hours later. Dead toads were removed after each treatment. We repeated this treatment on the remaining toads in each treatment for a maximum of three days (i.e. three treatments).

Following establishment of citric acid toxicity to toads we constructed a series of enclosures to test the dosage (both amount and concentration of solution) needed to kill toads in both structurally simple and complex habitat. We made twelve toad-proof 10 × 10 m enclosures using 1 m tall plastic drift-fencing, including a 1 × 1 m sub-enclosure to hold small toads (< 35mm SVL) so as to make observation easier. We released a total of 480 toads of different size classes into these enclosures 24 h prior to treatment to allow them time to find natural refuges, and then applied all treatments in a single spraying session. Of the twelve enclosures, the first set of six enclosures was placed in relatively simple habitat consisting of open grazed vegetation and low shrubs, the second set was in more complex habitat that included dense herbaceous vegetation and some trees and shrubs.

To test for phytotoxicity, we treated three enclosures with either 50, 100, or 200 ml/m^2 of 16% citric acid solution, with the three control enclosures treated with equal quantities of stream water applied with a backpack sprayer. Twenty four hours after treatment, we searched the enclosures, collected all toads, and recorded their status (living or dead). The enclosures were then left for 5-7 days during which heavy rains occurred before restocked with toads and after 24 hours, repeating the treatment using 25% citric acid solution for treatment enclosures and stream water for control enclosures.

We sprayed plots containing 34 species of crops or common local plants separately with treatments of 16% or 25% citric acid solution and monitored for plant damage or death one, three, and ten days after treatment.

Tadpole traps: To test for the luring effect of parotoid gland secretions on tadpoles, in January and February 2016 we constructed tadpole traps following the design of Crossland & Shine (2011). We identified streams and ponds that contained Asian toad tadpoles, and delimited the streams using mesh screens to create closed populations of tadpoles in 10 m sections for the duration of the trials. Into each section of stream, we deployed paired tadpole traps, one containing a glass microscope slide smeared with parotoid-gland secretions freshly collected post- mortem from toads obtained locally, and the other containing a blank microscope slide. In each pond, we placed six, paired treatment and control tadpole traps at 5 m intervals around the edge of the waterbody. We checked traps daily, replaced toxin slides, and counted all captured tadpoles.

Table 1. Asian toad density estimates from three consecutive daily searches of 20 x 20 m plots in different habitats.

Habitat type	Numbers toads/ search	Mean toads/ha
Forest	0, 0, 0	0
Agricultural	5, 5, 5	500
Urban	18, 15, 5	1266

We also conducted a 20 minute midday abundance survey daily that counted all observed tadpoles either within the delimited section of stream or within 5 m of the paired traps in ponds.

Statistics: We tested the efficacy of baited tadpole traps with a paired-sample t test. We used separate Poisson regression model for each of the three sites separately, with consecutive days of capture effort as predictor and daily toad captures as response variable, to examine the depletion of toad populations using hand-capture techniques.

CONSEQUENCES

Toad population abundance and densities: Surveys of 20 x 20 m fenced areas in urban, rural/agricultural, and non-production forest habitats provided a wide range of abundance estimates (Table 1). On average across these sites we found 5.8 toads/100 m^2 or 588/ha, with a maximum density of 18 toads/100 m^2 or 1,800 toads/ha. Urban habitats had the highest numbers of toads, with no toads found in forest habitats. Enclosed 1,600 m^2 plots sampled using drift fencing and pitfall traps captured a total of 158, 50, and 52 toads prior to three consecutive days of no captures (Figure 3). These numbers translate into densities of 325–987 toads/ha, with an average of 542 toads/ha. Pitfall trapping also caught several non-target species (Table 2).

Hand capture removal and pitfall trapping with drift fences: Manual removal of toads from three 9 ha plots by field

Table 2. Total number of captures of target and non-target species using pitfall traps from Farafaty, Tanandava and Ampasimaneva during January and February 2016

Species	Common name	Order	Provenance and IUCN status*	Number of captures
Tenrec ecaudatus	Common tenrec	Afrosoricida	Endemic, LC	1
Mus musculus	House mouse	Rodentia	Introduced, LC	2
Suncus murinus	Asian house shrew	Eulipotyphla	Introduced, LC	7
Rattus rattus	Ship rat	Rodentia	Introduced, LC	2
Furcifer pardalis	Panther chameleon	Squamata	Endemic, LC	1
Trachylepis elegans	Elegant mabuya	Squamata	Endemic, LC	6
Zonosaurus laticaudatus	Western girdled lizard	Squamata	Endemic, LC	5
Ptychadena madagascariensis	Mascarene grass frog	Anura	Native, LC	47
Hydrothelphusa madagascariensis		Decapoda	Endemic, LC	3
Duttaphrynus melanostictus	Asian toad	Anura	Introduced, LC	260

*****LC**, Least Concern (IUCN 2016)

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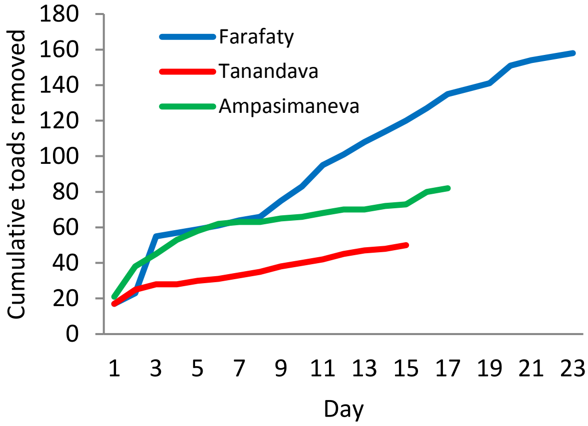

Figure 3. Cumulative numbers of Asian toads caught at three village sites in 1600 m^2 enclosed plots sampled with drift fences and pitfall traps.

teams yielded 1,281, 1,061, and 6,414 toads from three village sites that were broadly comparable in terms of their inclusion of rice paddies, occupied village, crop plantation, and non-production shrub and forest. These translate into densities of 118–713 toads/ha (average of 324 toads/ha), which is lower than densities estimated from pitfall trapping. In the densest population at Ampasimaneva, catch rate showed no sign of reducing over the three weeks of the survey (Figure 4). There was a decline in catch per unit effort during independent monitoring over the period of the study (paired t-test of pre- (average = 3.68 toads/h, S.D. = 1.69) and post- (average = 0.66 toads/h, S.D. = 0.94) capture rates: t = -5.2, p = 0.018), Figure 5). At all sites, depletion of the toad populations was significant in a Poisson regression model with day as covariate (Farafaty: coefficient estimate = -0.06, S.E. = 0.005, t = 14.4, rate = 0.93/day, p < 0.01; Tanandava: coefficient estimate = -0.108. S.E. = 0.008, t = 13.4, rate = 0.89/day, p < 0.01; Ampasimaneva: coefficient estimate = -0.058, S.E. = 0.002, t = 26.0, rate = 0.94/day, p < 0.01). No non-target species were recorded among the anurans captured during the manual removal period, suggesting a high search specificity among the recruited search teams.

The combined results of pitfall trapping and drift fencing, manual removal, and density estimates from habitat surveys all provided comparable estimates of toad density in similar habitats. If we use the density estimate of 542 toads/ha from pitfall trapping and drift fencing as representative of agricultural and village habitat, zero toads/ha for non-production forest and 1,266 toads/ha in urban areas and we apply these estimates to the 10,800 ha incursion area described between April and November 2014 (Moore *et al.* 2015), we obtain a total abundance estimate of 4,178,928 post-metamorphic toads in the estimated 3,308 ha of urban habitat,

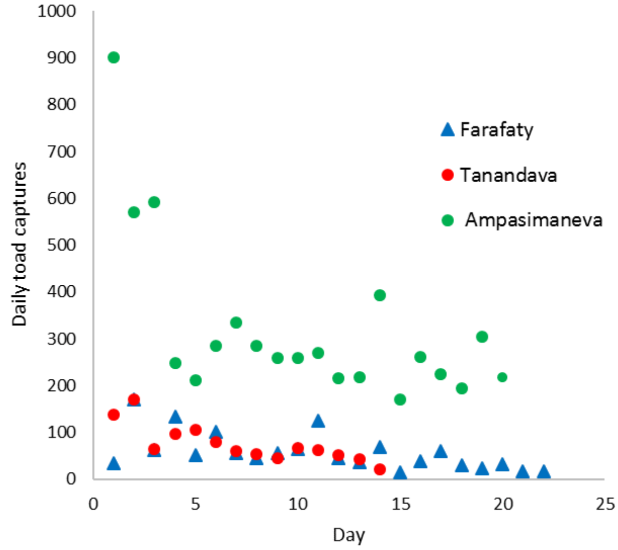

Figure 4. Daily numbers of Asian toads captured at three village sites within 9 ha unfenced survey plots.

3,022,192 post-metamorphic toads in the 5,576 ha of agricultural and village habitat, and an unrealistic estimate of zero toads in the remaining non-production forest habitat.

These estimates offer us a total population abundance estimate of 7,201,120 post metamorphic toads within the incursion area in April–November 2014.

Citric acid trials: Toads exposed to either 16% or 25% citric acid solution demonstrated 100% mortality across all size classes within a 48 h period that included two treatment cycles (Table 3, Figure 6).

Field trials of the spray application of both 16% and 25% citric acid solutions within 10 × 10 m enclosures led to high rates of mortality in all size classes. Treatments were completely effective against the smallest size class at all application rates but requiring higher application rates to achieve moderate to high removal rates for larger toads (Figure 7). For larger toads, spray treatments were less effective in complex habitats than simple habitats but were more effective at higher concentrations.

Phytotoxicity trials of 16% w/v citric acid solutions showed very little effect on the 34 species tested including all common crop plants from the area, and showed mortality for only one species (*Mimosa* spp.) at 25% w/v.

Tadpole traps: Tadpoles were captured at a mean rate of 2.8 tadpoles/trap (S.D. = 4.6) in any 24 h period over 159 trapping days. There was no significant difference in the capture rates between baited (2.96 tadpoles/trap, S.D. = 4.74) and non-baited traps (2.82 tadpoles/trap, S.D. = 4.69, paired sample t (78) = -0.3, p = 0.38).

Table 3. Mortality of three size classes of Asian toads after treatment with 16% or 25% citric acid solution, or a control treatment of water. Two identical treatment cycles were carried out, measuring mortality 30 min and 24 h after citric acid application. Effects were measured on small (< 35mm snout to vent length (SVL)), medium (35–70mm SVL) and large (> 70mm SVL) toads

Treatment cycle	**Exposure time**	**Control**			**16%**			**25%**		
		Small (n=15)	Med (n=21)	Large (n=15)	Small (n=6)	Med. (n=18)	Large (n=8)	Small (n=20)	Med (n=20)	Large (n=20)
1.	30 min	0%	0%	0%	100%	33%	12%	100%	75%	0%
1.	24 hrs	0%	0%	0%	n/a	78%	37%	n/a	85%	70%
2.	24hr 30 min	0%	0%	0%	n/a	78%	50%	n/a	95%	80%
2.	48 hrs	0%	0%	0%	n/a	100%	100%	n/a	100%	100%

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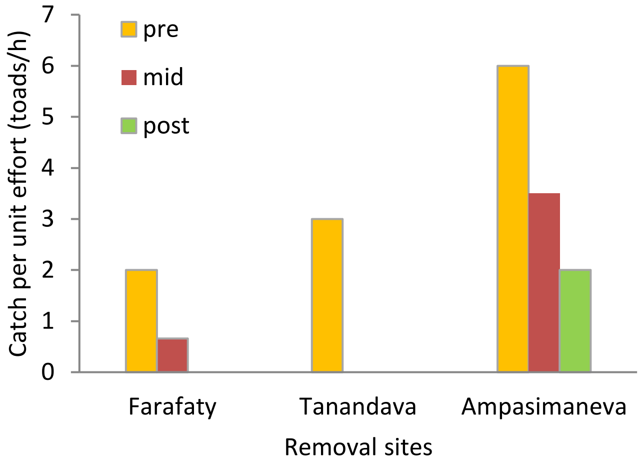

Figure 5. Toad numbers manually removed from three 9 ha unfenced survey plots, measured as catch per unit effort, before (“pre”), during (“mid”), and after (“post”) the removal period

Figure 6. Toads killed by 16% citric acid solution exposure 30 min after a single treatment. Ruler is 30 cm.

DISCUSSION

Our estimates of density and total abundance of Asian toads generated from pitfall trapping, drift fencing, and manual removal led us to revise an earlier estimate of likely total Asian toad abundance in the incursion area from 3.77 million in January 2015 (McClelland *et al.* 2015) to more than seven million post-metamorphic toads as of February 2016. This estimate is still conservative because our extrapolation used a three-year-old range estimate based on only moderate search effort, which was likely to miss toad populations at low abundance (Moore *et al.* 2015), toads have continued to expand their range since this 2014 range estimate, and it is implausible that Asian toads are entirely absent from non-production forest. Our failure to detect toads in forested habitat probably reflects their lower densities and diminished detection probabilities resulting from the increased structural complexity of the habitat instead of their absolute absence. Regardless, our results make clear that this invasion numbers millions of individuals over an area somewhat larger than that described by Moore *et al.* (2015).

Potential control tools demonstrated variable effectiveness. Pitfall trapping and drift fencing were important for estimating toad densities and for measuring the time required to reduce toads to undetectable levels. These could potentially be useful in curtailing breeding by removing toads trying to access small waterbodies. Buckets with a 20 l capacity and a depth of 50 cm were required to capture toads successfully; shallower buckets allowed toads to escape, as indicated by empty buckets containing toad faeces. The drift fence height used in our trials seemed adequate to prevent toad escapes and yet was low enough to allow adult foot traffic to easily cross fences. Fence damage, however, was created by smaller children crossing fences and from cattle wandering freely in the trial areas. The polyethylene fencing material was not sufficiently robust to be functional beyond a two- to three-week period, and any need to drift fence for longer periods would require a more robust material with greater rigidity and tear resistance.

Our hand-capture trials were designed to test the efficacy of using local contract labour to remove Asian toads from the environment and to evaluate the social and logistical feasibility of the technique. For hand capture to be an effective control tool, consistent availability of labour and willingness to follow operational instructions are critical. Our experience was

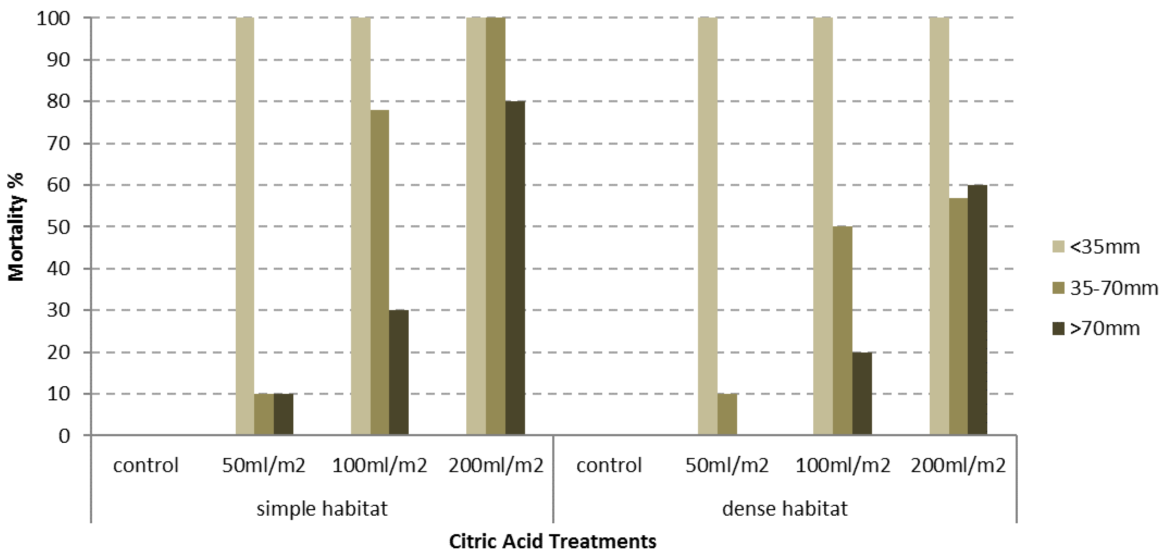

Figure 7. Mortality rate of different-sized Asian toads in simple and complex habitats 24 h after spraying with two different citric-acid solutions.

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that there was a willing labour force for this work because work could easily be accommodated around other commitments due to the crepuscular nature of toad activity. However, there was difficulty obtaining longer-term time commitments from hired collectors due to other responsibilities and unknown variables. Although it was our intention to maintain the manual removal trials for 21 days at each site, this was only achieved at one location; the two other study sites were only exposed to manual removal for 15 and 17 days respectively due to the field team logistics. As far as we could ascertain, our instructions were followed, and labour organisation was greatly assisted by the social hierarchy of village populations, where elected village chiefs play a strong management and coordinating role in social and economic affairs.

Despite our general success in organising repeated surveys to remove toads from clearly delimited areas near villages, removal rates showed no signs of declining (Figure 3). This suggests that either considerable numbers of Asian toads remained within the 9 ha trial plots or large numbers of toads continued to disperse into the area during the trials. It is likely that both factors contributed to the unabated rates of toad removal. Furthermore, total numbers removed were usually lower than for pitfall traps: at Farafaty manual removal yielded 142 toads/ha compared to 987/ha for pitfall trapping; at Tanandava 118 toads/ha compared to 312/ha; but at Ampasimaneva manual removal yielded 712/ha compared to 518 toads/ha removed with pitfall trapping. These results are challenging to interpret but suggest that a manual removal programme would potentially need to be run for much longer than our trial to severely depress a population, an extrapolation that does not account for diminishing capture success with time and its consequence on morale and commitment of hired labour. Therefore, this tool probably has value only as a method for initial culling of a population as an early phase eradication tool, or as part of a control or containment program. Such circumstances may justify the effort to reduce density-dependent dispersal and recruitment rates in synergy with other methods.

The most important finding of our trials is that Asian toads appeared highly susceptible to citric acid spray. In simple habitat, there was only negligible advantage to using a higher-concentration solution; in contrast, in more complex habitat dosage rate had greater influence on mortality rates (Figure 7). Whilst our sample sizes were not large, the results are unambiguous, encouraging confidence in its efficacy. In simple habitat, 200 ml of either 16% or 25% solution killed a similar proportions of toads of all size classes, but the stronger solution was more efficacious for large animals. Considering the small samples tested and the lack of strong phytotoxic effects, we propose the use of lower amounts of the stronger solution in open habitats, because of its greater efficacy against adult toads. A treatment of three applications of a 25% w/v solution at a rate of 200ml/m^2 to an area within a period of one to two weeks would appear optimal for eradicating toads.

In complex habitats and at lower delivery rates the stronger 25% solution performed significantly better than the weaker solution, but these differences diminished as the application rates increased. Considering the elevated risk of limited penetration of solution to Asian toads hiding under cover, we believe that the higher concentration and dosage would be the preferred treatment and the treatment should be repeated within a one to two-week period. Hence, for both simple and complex habitats we find that a 25% citric acid spray is preferred. This contrasts with the 16% solution used in controlling coquí populations in Hawaii (Beachy *et al.* 2011). It is beyond the scope of this study to assess, but it may be that the drier skin of toads provides greater resistance to penetration of citric acid solution than the moist skin of the coquí. If these methods are to be used effectively, it will be critical to account for the surviving toad population. Delimitation and secondary capture techniques with greater probability of detecting individuals will be vital to achieve eradication after knockdown with citric acid.

During our trials, we prepared citric acid solution by hand and deployed it with backpack spray units. To use this tool at the scale required in Madagascar, much more efficient methods need to be developed. Treating one hectare with 200 ml/m^2 using a backpack sprayer would take 100 man hours, extrapolating from the time taken to treat 100 m^2 enclosures in this study. The volume required would be 200 l/ha, which would require 50 kg of citric acid monohydrate/ha. In considering such a treatment, we would clearly have to make use of mechanical mixing, storage, and application methods. For application, boom or lance-spray application from helicopters is probably the most efficient method. To treat the entire known incursion area as per the area estimates of Moore *et al.* (2015) would require approximately 6000 metric tons of citric acid, which, at an estimated cost of US$600/metric ton, would cost US$3.6 million for the chemical alone. Helicopter time is likely to run US$3000/h or more, so it is obvious that costs for any toad eradication or control programme range would be large. It would also require scaling up the solution preparation and storage, and this would likely require that buffering agents and water conditioners be added to retain acidity. The inclusion of wetting and spreading agents, such as a blend of organosilicone and organic surfactants, may also improve efficacy and should be investigated for their potential to reduce effective concentrations of the citric acid and therefore non-target risks and the cost of preparation. Spray application also requires careful calibration of equipment and droplet size to optimise coverage: too coarse a droplet (>500 microns) can result in poor swathe coverage whereas too fine a droplet (<300 microns) can result in spray drift and variable application rates. There are obvious concerns about non-target impacts of such a tool and we regard citric acid as viable for consideration only in areas of extreme ecological degradation, such as the current incursion area, and would caution its use in more ecologically intact environments as all other anurans within the treatment area will potentially be killed.

Tadpole traps were not effective for capturing tadpoles during our trial period. This could be because parotoid gland secretions do not act as an attractant for Asian toad tadpoles, as has been observed in canes toads by Schwarzkopf and Alford (2007). However, an alternative explanation could be that the stages of tadpoles present were inappropriate for the lure to be effective. After our trials, we learned that in *Rhinella marina*, only tadpoles at Gosner stage 28 (Gosner 1960) and older are effectively trapped using parotoid gland secretions and that the freshness of secretions and wind conditions can have a strong influence on trapping effectiveness (Samantha McCann, personal communication). It may therefore be worth repeating tadpole trapping trials across a wide and known range of developmental stages. We suggest this because initial trials (unreported here), prior to our field trials, appeared to effectively trap many toad tadpoles (Devin Edmonds, personal communication).

Collectively, our results appear promising for inclusion in a strategic eradication effort. However, the formidable extent of the toad incursion in Madagascar, which is likely to now be

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considerably beyond the area described by Moore *et al*. (2015), means that scaling a programme to manage the issue would be both extremely expensive and at high risk of failure. Delimitation of the toad incursion and preventing toad movement and reinvasion within a rolling front of an eradication effort would require extensive use of barriers such as drift fences and controls on the movement of materials between operational areas. This is difficult to envisage as feasible, given the human population, trade, and movement within the area. The relative poverty of the population within the toad incursion area also means that eradication materials would likely be taken for other uses without proper investment in community ownership of the eradication effort. Critically, the methods described here need to be tested together in a strategic attempt to eradicate toads from an ecologically and logistically meaningful area where monitoring can confirm that eradication can be demonstrated.

Sadly, the Asian toads continued expansion through 2017, apparently accelerated by Cyclone Enawo (K. Freeman personal communication), has escalated an already extremely challenging task (due to financial, logistical and political challenges) to almost logistically impossible, as any future eradication effort would need to account for dispersal via tadpole movements within extensive waterways. However, we see value in the development of better tools, as these will be vital for removing new satellite populations vectored by in-country trade, although infrastructure and resourcing means such efforts would be high risk. Whilst it is expected that the Asian toad will eventually spread to all but the most arid regions of Madagascar, it remains possible that biological or environmental barriers to its spread may exist. Responding to new incursions as part of a programme of mitigation may be important for protecting other regions of the country. Moreover, the development of tools for toad control in Madagascar can help provide tested response tools for use against new anuran incursions elsewhere. Above all else, this incursion is a salutary lesson in the need for improved biosecurity standards and the rapid detection and response to invasive species.

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