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EVALUATION OF THE PHOTOGRAPHIC IDENTIFICATION METHOD (PIM) AS A TOOL TO IDENTIFY ADULT *LITORIA GENIMACULATA* (ANURA: HYLIDAE)

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***Abstract.*—Marking anurans by invasive methods has been strongly debated on the grounds of ethics, public opinion, the potential for infection, and potential impacts on behavior and survival of marked animals. One possible alternative is the use of photographs to enable identification of individuals by their patterns. This requires that individuals have distinguishable markings that remain constant through time. We compared the accuracy of the photographic identification method (PIM) to marked frogs in the field to evaluate whether PIM can be used to identify adult Green-eyed Tree Frogs, *Litoria genimaculata*. We captured 59 animals on second and subsequent nights of trips. Thirteen were correctly identified as recaptures; 61.5% of them by using the PIM. This was a substantial (39.5%) improvement over chance (expected rate if frogs assigned as recaptures at random was 22%), but was less accurate than the toe-tipping method, with which only one recapture was misidentified (92.3% correct). The majority of frogs, including all of the individuals that were not correctly identified, lacked a distinct dorsal "hourglass" pattern. This may have contributed to the relatively high error rate. We found that the PIM was slower than toe-tipping animals in both time taken for application and identifying individuals. We conclude that the PIM needs to be carefully validated before it is applied to a new species. For *L. genimaculata* it may be of use in short-term studies when the goal is to reduce the chance of double-sampling individuals. Even then, it will probably only be worth using when a relatively high proportion of the population is being sampled on each occasion, so that a 60 to 70% reduction in double-sampling rates over random is worth achieving.**

Key Words.—anuran; field method; Green-eyed Tree Frog; *Litoria genimaculata*; photographic identification method; recapture

INTRODUCTION

Mark-recapture studies of amphibians often use techniques that apply a physical mark, allowing the identification of individuals, including toe-tipping (removing the toe-pad only) and -clipping (removing larger proportions of the digits; Clarke 1972; Waichman 1992; Richards and Alford 2005; Simoncelli et al. 2005), inert fluorescent polymer (elastomer) subcutaneous marking (Anholt et al. 1998; Schlaepfer 1998), and passive integrated transponder (PIT) tagging (Brown 1997; Schulte et al. 2007). All of these techniques are invasive, but some can provide valuable, additional information on animal age and population genetics (Halliday 1995; Funk et al. 2005; Phillott et al. 2007). Researchers have to balance the possible negative effect of the marking procedure on individuals and populations against cost, time efficiency, and the value of information collected (Phillott et al. 2007). Marks are also used to avoid repeatedly collecting samples, such as diagnostic samples for determination of infection, from the same animals.

To date, the photographic identification method (PIM), which uses variation in skin patterns to distinguish individuals, is the only non-invasive technique for individual identification of amphibians (Hagstrom 1973; Bradfield 2004). Pattern recognition software, used in association with PIM, has a high accuracy of identification and greatly reduces the time required to manually search through hard-copies of images (Gamble et al. 2007; Speed et al. 2007). However, pattern recognition software often cannot be used when it is necessary to identify individual animals in the field at the time of capture to avoid sampling them multiple times; for example, when taking samples to determine infection prevalence or samples of antimicrobial peptide secretions over more than a single night at one locality (Woodhams et al. 2005).

One limitation of the PIM is that its application is restricted to species with natural features or markings (Bradfield 2004). Though a species may have a pattern, in order to be acceptable as a natural mark, pilot studies must indicate that all individuals have unique patterns and that these patterns are permanent through time. *Litoria genimaculata* (the Green-eyed Tree Frog, Fig. 1)

FIGURE 2. A Green-eyed Treefrog (*Litoria genimaculata*) from Frenchman Creek (Queensland, Australia) showing its dorsal pattern. (Photographed by Ross Alford).

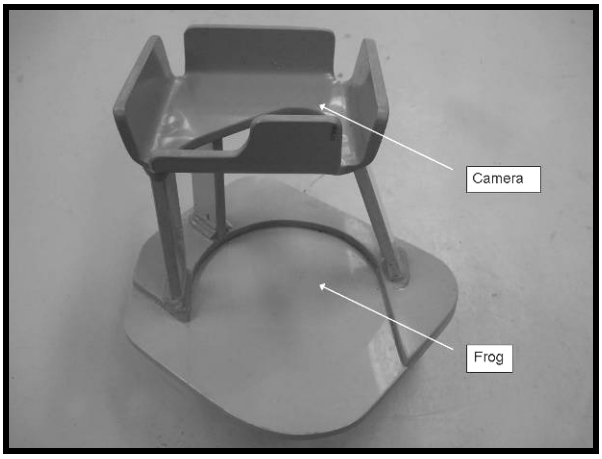

FIGURE 2. Stage, allowing constant focal distance, used to take digital images of *Litoria genimaculata*. Arrows indicate where camera and frog are placed. (Photographed by Nicole Kenyon.)

is a stream-dwelling rainforest anuran that occurs between Townsville and Cooktown in Queensland, Australia. The dorsal coloration and pattern vary among individuals. Overall coloration varies from green to brown, and there is usually a broad russet/brown pattern, with variable hourglass-shaped lines along the dorsal area and circular- and triangular-shaped marks between the eyes and nares, that might be specific to individuals, allowing identification of individuals using the PIM. The ventral surface of this species is a consistent cream color and, therefore, unsuitable for this method (Barker et al. 1995).

Highland populations of *L. genimaculata* showed declines during initial outbreaks of chytridiomycosis, a global amphibian disease caused by the fungus *Batrachochytrium dendrobatidis*, but have subsequently recovered (McDonald and Alford 1999; McDonald et al. 2005). The recovery of *L. genimaculata*, but not of some other sympatric species, makes it an interesting species to investigate population structure and behavior, and PIM would allow non-invasive recognition of individual frogs. Our aims were to determine whether the PIM can be used to identify individual adult *L. genimaculata* based on their dorsal pattern, and to compare the accuracy and time requirements of the procedure with that for traditional toe-tipping.

MATERIALS AND METHODS

We used a population of toe-tipped *L. genimaculata* in Murray Upper National Park, Queensland, Australia, to test the accuracy of the PIM. We decided not to include the use of recognition-assistance software, as we wanted to examine an identification method that is exclusively field based.

A team of three people, investigator 1 (responsible for toe-tipping), investigator 2 (responsible for PIM), and one volunteer, conducted three field trips: the first in December 2006 for two nights, the second in February 2007 for two nights, and the third in October 2007 for four nights. Investigator 1 and 2 were present during all field trips. The sampling location was the site of a long-term mark-recapture study by Investigator 1, who was collecting population data, measuring frogs, and swabbing some individuals for diagnostic quantitative PCR to detect infection by the amphibian chytrid fungus. We handled all frogs using a new pair of latex gloves for each individual, and we cleaned instruments that touched frogs using alcohol between animals. Each night we surveyed the same 200-m transect along a rainforest creek. Each frog located was initially captured by Investigator 1, who examined the frog and read its existing toe-tipping code or performed toe-tipping. Investigator 2 waited at a distance, did not watch Investigator 1, and did not examine the feet of the individual once it was passed to her, and thus did not know whether the frog was a recapture or newly toe-tipped. Investigator 2 took digital photographs of each individual.

During the first survey night on each field trip, up to three digital photographs were taken of the dorsal pattern of every *L. genimaculata* captured. We placed a Pentax Optio 33WR camera (3.1 megapixels) on a fixed stage (Fig. 2) to standardize focal distance. We held each frog by its left hind foot and placed it on a small plastic bag (changed between frogs to avoid pathogen transfer) on the stage. One head lamp was used to give additional light, as flash alone was insufficient. We recorded the digital image number or numbers and the toe-tip number for each individual on site. The next day, the best image for each frog was printed (Photosmart 325, Hewlett-

TABLE 1. Recognition of new and recaptured *Litoria genimaculata* in Murray Upper National Park, northern Queensland, Australia using the PIM (photographic identification method). The night count was restarted for trip 3 due to the long interval between trips 2 and 3. Note that much of the contribution to the chi-squared test of the null hypothesis that PIM classification was equivalent to random assignment of individuals as new or recaptured comes from the large excess of recaptured individuals correctly identified as such. The chi-squared test was significant (χ^2 = 20.19, df = 3, $P < 0.001$).

Trip	Night	New capture, identified as		Recapture, identified as	
		New capture	Recapture	New capture	Recapture
1	1	10	--	--	--
	2	14	0	0	1
2	3	5	2	1	1
	4	4	0	1	2
3	1	7	--	--	--
	2	5	0	--	--
	3	6	0	1	2
	4	10	0	2	2
Total		61	2	5	8
Total excluding nights 1		44	2	5	8
Expected		35.86	10.14	10.14	2.86
Contribution to chi-square		1.85	6.53	2.60	9.21

Packard, Sydney, Australia) and labeled on the reverse with the toe-tip number. We sorted images by capture location and dorsal pattern (presence or absence of dorsal hourglass shaped lines, circular-shaped marks between the eyes, and triangular-shaped marks between the nares) and placed each photo in a small album. On the second or third night of each field trip, and the first night of field trip 2, Investigator 2 compared each frog with the images in this album and either identified it as a recapture or classified it as a new individual. She then consulted Investigator 1, and they determined the true identity of each individual via toe-tip method. On these nights, we photographed only newly captured individuals, using the techniques already outlined.

On the second field trip (February 2007), we compared frogs with photographs from the first field trip (December 2006) to determine whether dorsal patterns changed during the two-month recapture interval. We did not include existing images from previous field trips during the last field trip in October 2007 due to the long interval. We recorded the time taken to photograph frogs new to the study and to compare captures with existing images (from second, third, and fourth night). These were compared with times required to toe-tip or recognize previously toe-tipped frogs of a number of species (*L. genimaculata*, *L. nannotis*, *L. rheocola* and *Nyctimystes dayi*; Andrea Phillott, unpubl. data) using two-sample t-tests.

We used Fisher's exact tests to determine if there was a difference between the accuracy of recognizing recaptures using PIM and toe-tipping; as well as, if there was a difference between rates of correct and incorrect identification of recaptured *L. genimaculata* within and across field trips. We also tested the hypothesis that PIM performed better than random classification of frogs. We used the overall proportions of previously captured and not-previously captured frogs to establish the proportions of frogs in each category if decisions were made at random. We used these expected proportions to calculate expected numbers, and then compared the observed and expected numbers using a chi-squared goodness-of-fit test. For all tests, $\alpha = 0.05$.

RESULTS

We captured 63 individual adult *L. genimaculata* a total of 76 times during the study (Table 1). Seventeen of these were captured and photographed during the first visits to the sites on trips 1 and 3, and were thus automatically regarded as new captures for the PIM. Of the remaining 59 captures, we correctly identified 44 as new captures using the PIM (Table 1). We misidentified two new captures as recaptures using the PIM, a misclassification rate of 4.3%. Both individuals that were incorrectly regarded as previously captured lacked a dorsal hourglass pattern (Fig. 3).

There were 13 recaptures of 12 individuals (Table 1). The rate of correct identification of these individuals using PIM was low (8 out of 13; 61.5%). The toe-tip number was read correctly on 12 of the 13 recapture events (92.3%); this error was detected by comparison with the PIM result. The accuracy of PIM in recognizing recaptures was lower, but not significantly

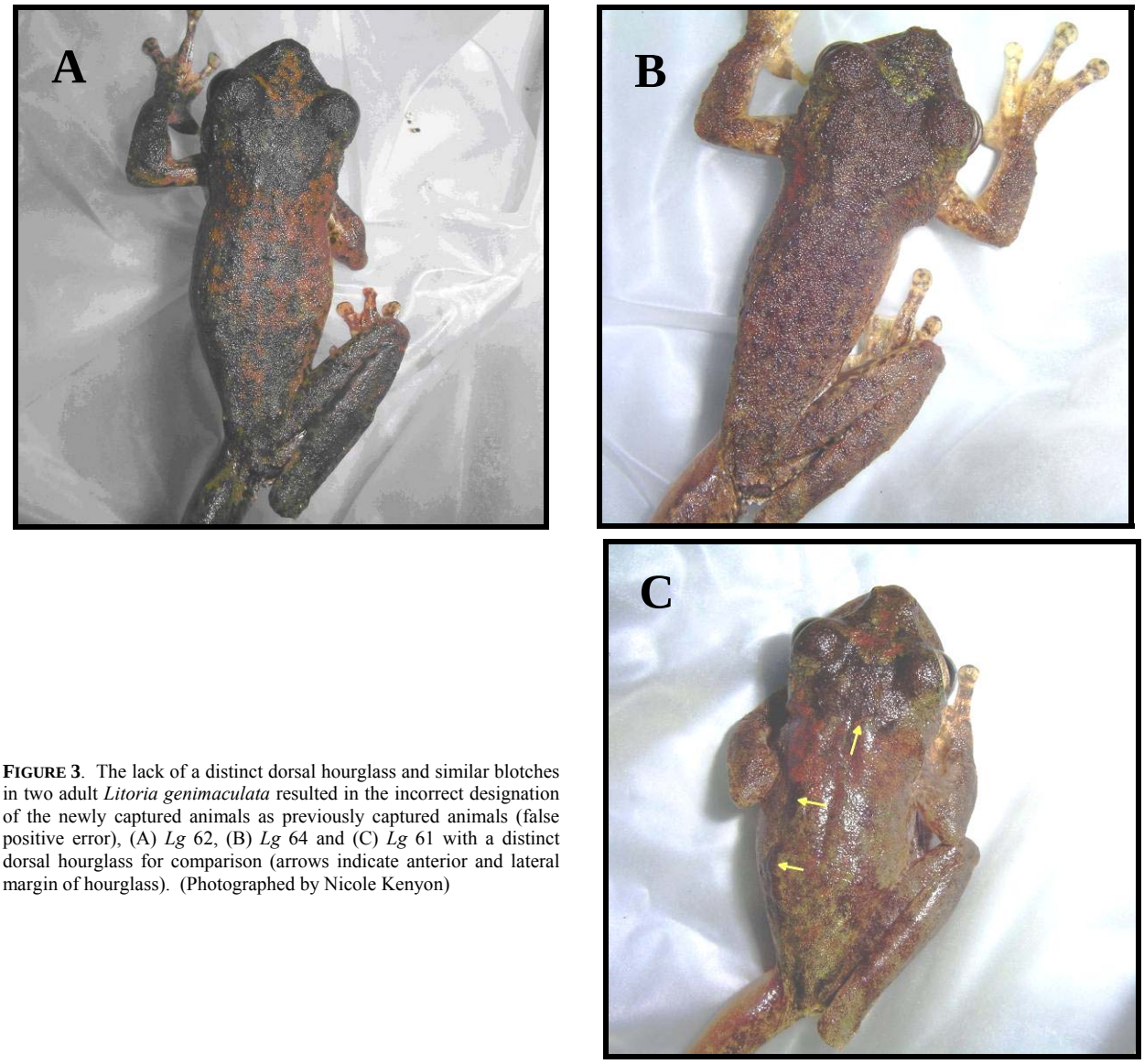

FIGURE 3. The lack of a distinct dorsal hourglass and similar blotches in two adult *Litoria genimaculata* resulted in the incorrect designation of the newly captured animals as previously captured animals (false positive error), (A) *Lg* 62, (B) *Lg* 64 and (C) *Lg* 61 with a distinct dorsal hourglass for comparison (arrows indicate anterior and lateral margin of hourglass). (Photographed by Nicole Kenyon)

lower, than the toe-tip method (Fisher's exact test, P = 0.16). Overall, using PIM resulted in significantly better identification of individuals than would have random assignment of frogs to groups (61.5% versus 22% [i.e., 2.86/13]; Table 1).

The incorrect identification of one frog, using PIM, was due to a change in dorsal pattern when a black circular-shaped marking on the left flank was lost and a month recapture interval (Fig. 4). Incorrect identification of the remaining individuals was due to difficulty in recognizing the patterns of individuals from photographs; however, the majority of those individuals had no distinct hourglass pattern and instead possessed only random blotches (Fig. 3). Overall, 53% of the frogs captured during the study had no hourglass shape within their dorsal pattern.

We recaptured three individuals, of which two were incorrectly identified, across field trips. We correctly identified seven of the 10 individuals that were recaptured within a field trip. There was no significant difference between the accuracy of identifying recaptures using PIM within and across field trips (Fisher's exact test, P = 0.51).

The time taken to search through photographs and identify an individual as new to the sample or previously photographed, took the longest for 18 images (Fig. 5). Time required to take a digital image was significantly greater than time to toe-tip a frog (T = 3.549, df = 31, P < 0.001, Levene's tests for equality of variances satisfied). When all groups were considered, searching images to identify an individual was significantly slower (T = 10.052, df = 29, P < 0.001, equal variances not

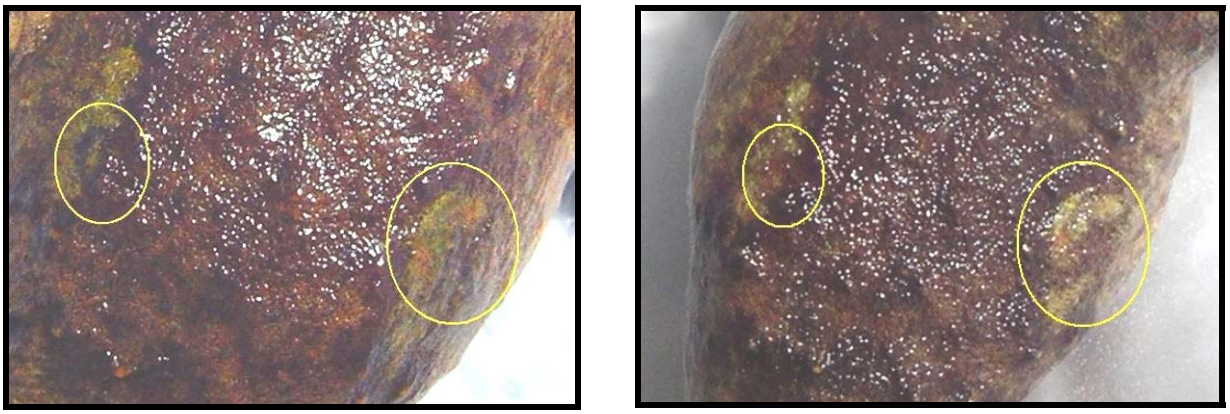

FIGURE 4. Dorsal pattern change of a *Litoria genimaculata* individual within two months, December 2006 (left) where a clear black circle is seen on the left flank and no black marking on the right flank (yellow circles), and February 2007 (right) with no black circle on the left flank and a black marking on the right flank (yellow circles). (Photographed by Nicole Kenyon)

assumed) than determining the unique number of a previously toe-tipped animal.

DISCUSSION

In this study, the rate at which we misclassified newly captured animals as recaptures using PIM was low (4.3%). This is a five-fold reduction compared to the error rate that would have occurred if individuals were randomly classified as new captures or recaptures (22.0%, Table 1). The two individuals misclassified as previously captured both lacked a distinct dorsal hourglass pattern, making them more difficult to identify and contributing to this error. However, the reliability of the PIM as a method to identify previously captured individuals was lower (61.5% correctly identified) than using the toe-tipping method (92.3%). Although this difference was not significant, we believe it is likely that the error rate of reading toe-tipping markings is generally lower than 7.7%. One reason is that because reading a toe-tip mark produces a unique identification code, many misreadings will produce a nonexistent code, prompting re-examination of the animal and reducing the error rate. There are no comparable means of self-correction in the PIM, and it thus seems likely that it truly has an intrinsically higher error rate.

We recaptured three *L. genimaculata* individuals across field trips. One was not identified as previously captured due to a change in dorsal pattern during the two-month recapture interval. Other studies have found both substantial (Reaser 1995) and only minor (Stephenson and Stephenson 1957; Hagstrom 1973; Healy 1975; Denton and Beebee 1993; Doody 1995) changes in adult markings over time. The causes of these pattern changes are unknown. There was no significant difference between the accuracy of recognizing recaptures, using PIM, across and within field trips. However, the fact that we found that dorsal pattern can change over a two-month interval suggests that in *L. genimaculata*, the accuracy of PIM may decrease with increasing time.

The remaining four instances in which recaptures within field trips were not correctly identified were caused by the difficulty of comparing and recognizing complex patterns from photographs in the field. This contributed to the PIM taking significantly longer than the toe-tipping method, which may increase handling stress, although studies have not yet shown a negative impact of stress in amphibian responses to extensive handling (Cabanac and Cabanac 2000; Cabanac and Cabanac 2004; Kinkhead et al. 2006).

The low time efficiency of the PIM and the high rate of misidentification of recaptured individuals raise concerns about the application of this method to *L. genimaculata*. Photographing the frog from different angles would not have increased the accuracy as the distinctive pattern is dorsal. It is possible that accuracy could have been improved using some form of computerized pattern matching, but that would not have been practical given that our objective was identification of frogs in the field to prevent double sampling.

The suitability of the PIM for any species depends on the presence of individual skin patterns that can be reliably identified (whether by human operators or by pattern-recognition software) and that persist over time. For any species, the existence of such patterns should be established before employing the PIM, and ideally, trials should be conducted using a second marking technique to determine the accuracy of the PIM before it is applied to a new species. The difficulty of identifying *L. genimaculata,* particularly individuals without distinct dorsal hourglass patterns (in this study 53%), and the

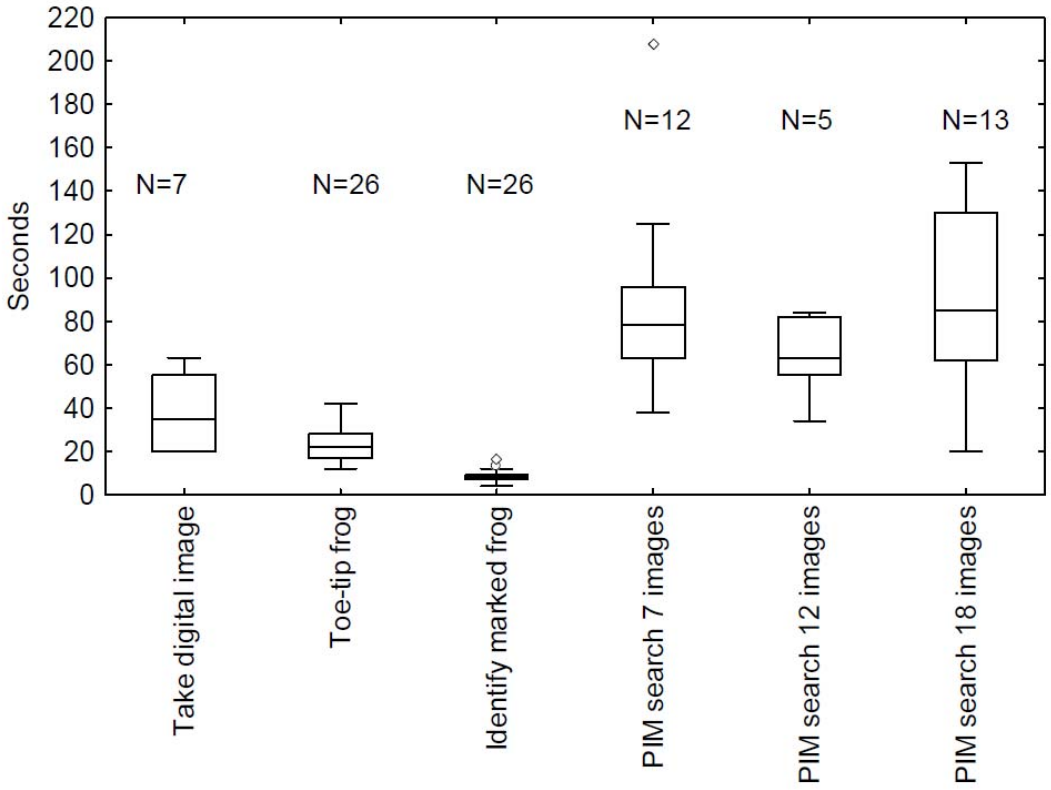

FIGURE 5. Time taken to apply toe-tipping and PIM (Photographic Identification Method) to adult frogs (vertical bars indicate ranges, horizontal bar indicates median, boxes indicate upper and lower quartile boundaries, ◇ indicates extremes).

fact that their patterns can change over relatively short periods of time, means that the PIM is of limited use for this species. If the investigator is sampling from a relatively small population, then it may be worth using the PIM to reduce rates of double sampling, particularly if it is combined with other approaches, such as avoiding sampling frogs occupying previously sampled perches or sampling non-overlapping areas of habitat on different nights. The PIM is clearly not of use in *L. genimaculata* when all recaptures must be identified in the field with a high degree of certainty. Similarly, if small samples are being taken from a large population, the extra effort may not be worth the relatively small reduction it would provide in rates of double sampling.

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ROSS A. ALFORD (left) received B.S. and M.S. degrees from the University of Florida, and a Ph.D. from Duke University. He is presently Professor (Personal Chair) in Marine and Tropical Biology at James Cook University in Townsville, Australia. His research interests include population and community ecology, behavior, and conservation biology, particularly of amphibians and reptiles, and the host-pathogen biology of amphibians and its relationship to global amphibian declines. (Photographed by Karen Lips)