

# Shot the spots: A reliable field method for individual identification of *Amolops formosus* (Anura, Ranidae)

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## Abstract

Natural body patterns in amphibians are widely used for individual recognition. In this study, we photographed individuals of *Amolops formosus* for four days of sampling without handling them. We processed 301 photographs of dorsal blotch pattern in HotSpotter software and verified them visually for confirmation. We identified 160 unique individuals of *A. formosus* based on the images taken in the field, resulting in an abundance estimate of 180 individuals. The success rate in identifying individuals of *A. formosus* using the HotSpotter software was 94.3%. We tested the effect of image quality and distance on recognition efficiency. Poor image quality reduced the recognition efficiency of the software but with a careful user review it was possible to identify the individual. The difference between using only the software and software plus human confirmation was very small. This protocol is useful for rapid population estimation of frogs with natural body patterns.

## Key Words

Amphibia, mark recapture, pattern recognition software, population estimation

## Introduction

Conservation and management for any species requires explicit information about its demography, population status and dispersal patterns (Morris and Doak 2002; Gamble et al. 2008). Therefore, individual identification is vital to uncover the demographic patterns to understand population dynamics in a mark-recapture framework (Nichols 1992). It also generates information on dispersal, activity pattern, growth, movement, health and behavior (Delany 1978; Osbourn et al. 2011). Traditional methods for individual identification often require physical capture and handling of animals (Williams et al. 2002; Richards and Alford 2005; Courtois et al. 2013; Ringler et al. 2015). This may change the demographic pattern and behavioral responses (Powell and Proulx 2003; McMahon et al. 2005) and is often costly and time consuming (Jonas et al. 2011). On the other hand, identification based on natural color pattern proved to be inexpensive,

reliable and noninvasive, where any kind of physical capture of the animal was avoided (Arntzen et al. 2004). Application of photographic identification, however, is restricted to species with variable natural marking patterns (Bradfield 2004; Kenyon et al. 2009).

Amphibians possess a diverse range of color pattern and body markings (Hoffman and Blouin 2000) that can be used for individual identification (Heyer et al. 1993). For example, natural color patterns were recently used for the individual identification of amphibians such as *Leiopelma archeyi* (Bradfield 2004), *Melanophryniscus cambaraensis* (Caorsi et al. 2012), *Anaxyrus baxteri* (Morrison et al. 2016), *Salamandrina perspicillata* (Romiti et al. 2017) and *Triturus dobrogicus* (Naumov and Lukanov 2018), where the individuals were physically captured and handled for documenting their unique patterns. Our study, however, differs from others because we did not handle any frog and merely remotely photographed them in the natural habitat. Photographing in the field led to

variation with respect to angle of the photograph, image quality and body posture of each animal. These variations may lead to identification error and affect the population parameter estimates. To support identification, we used the semi-automated Computer-Assisted Pattern Recognition Software (CAPRS) HotSpotter, as manual identification is more error prone and time consuming with large datasets (Morrison et al. 2011; Crall et al. 2013). To test the accuracy of individual identification and abundance estimates, we calculated the success rate and error rate of HotSpotter. We also tested the individual recognition capacity of HotSpotter in terms of image quality and photographic distance from the individual.

## Methods

### Study area

We conducted fieldwork in Jamak Stream, near Maneri dam, Bhagirathi River Basin, Uttarkashi, Uttarakhand, India (39°43.8727'N, 78°31.6973'E (DDM); 1300 m asl; Fig. 1). The area is classified as Himalayan Chir Pine Forest, with *Pinus roxburghii* being the dominant tree (Champion and Seth 1968). Forest, agriculture, and settlements are the major land use types (Nautiyal 2010).

### Species identification

*Amolops formosus* is a medium sized (male, maximum SVL 53 mm; female, maximum SVL 75 mm), slender bodied frog (Fig. 2, Schleich and Kästle 2002). The dig-

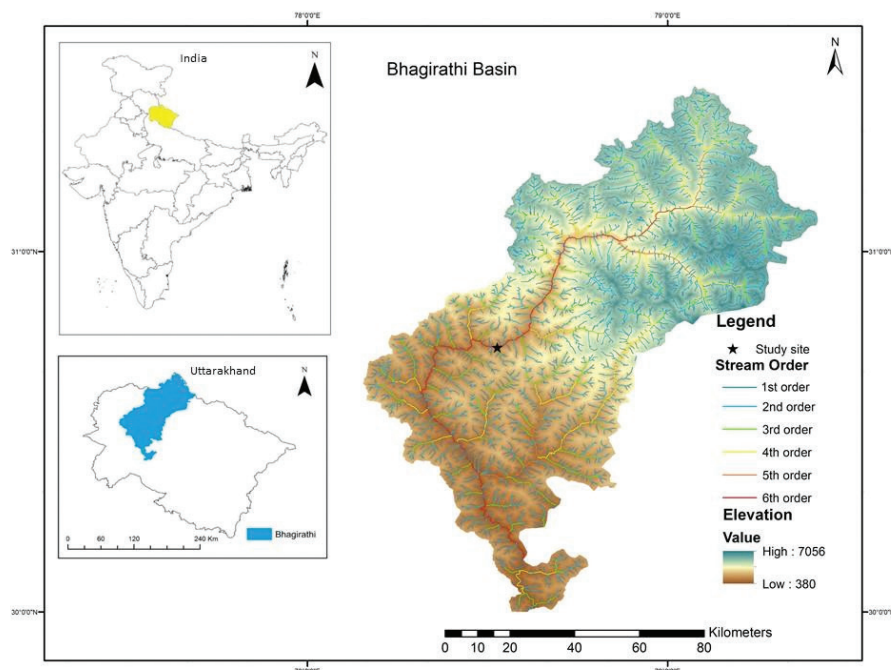
ital pads on the fingers are wider than those of the toes, and individuals have a skin fringe on the third finger (Yang 1991; Schleich and Kästle 2002). The dorsum is bright green with irregular, sharply delimited dark brown blotches (Fig. 2). Small flat warts are present densely on the dorsal body (Schleich and Kästle 2002).

### Data collection

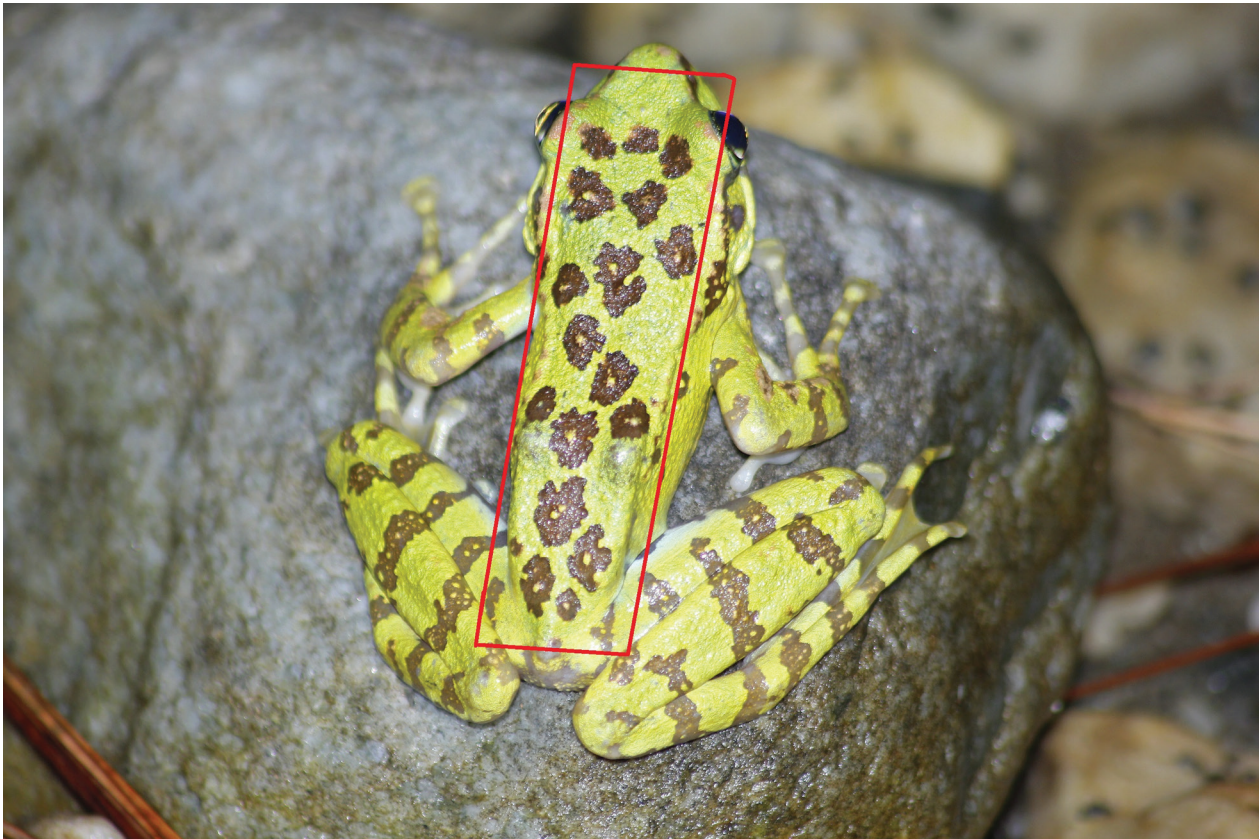
We employed Nocturnal Visual Encounter Surveys (NEVS) from 1900 to 2130 h (Heyer et al. 1993). The study site was sampled four times over a period of 5 days (19, 20, 21, and 23 May 2016) along a belt transect (400 × 50 m) along the stream. We detected frogs from their eye shine against a torch (Ledlenser, Portland, USA). After each detection, we photographed the dorsal side of individuals using a Canon 60D digital camera (Canon inc., Tokyo, Japan) mounted with a 70–300 mm lens (Sigma Corporation of America, New York, USA), or a Nikon D3200 camera with 55–250 mm lens (Nikon Cooperation, Tokyo, Japan). We used the inbuilt flash of the camera to document the frogs. All the frogs were photographed in their natural position maintaining a 1–6 m distance to avoid any disturbance.

### Individual identification by HotSpotter

We assumed that natural marking patterns of the individual adult frogs did not change with time during our study. We included only the dorsal patterns of *A. formosus* as Region of Interest (ROI) for individual identification



**Figure 1.** Map showing the study site location in Bhagirathi River Basin, Western Himalaya.



**Figure 2.** Region of interest (ROI) for natural pattern is marked in red boundary.

(Fig. 2). Individual frogs were identified using pattern recognition software, HotSpotter (Crall et al. 2013). We created a new directory and uploaded all the photographs for identification in the software, before defining the ROI and orientation of each photograph. This step converts each photograph into a chip. Then we selected one chip and ran a query option. HotSpotter computes its hotspots (unique individual features) within these chips and provides a similarity score before ranking the chips in order of the most similar to the least similar one (Fig. 3). When the matching chips belonged to the same individual, it was recorded as a successful identification.

### Individual recognition efficiency

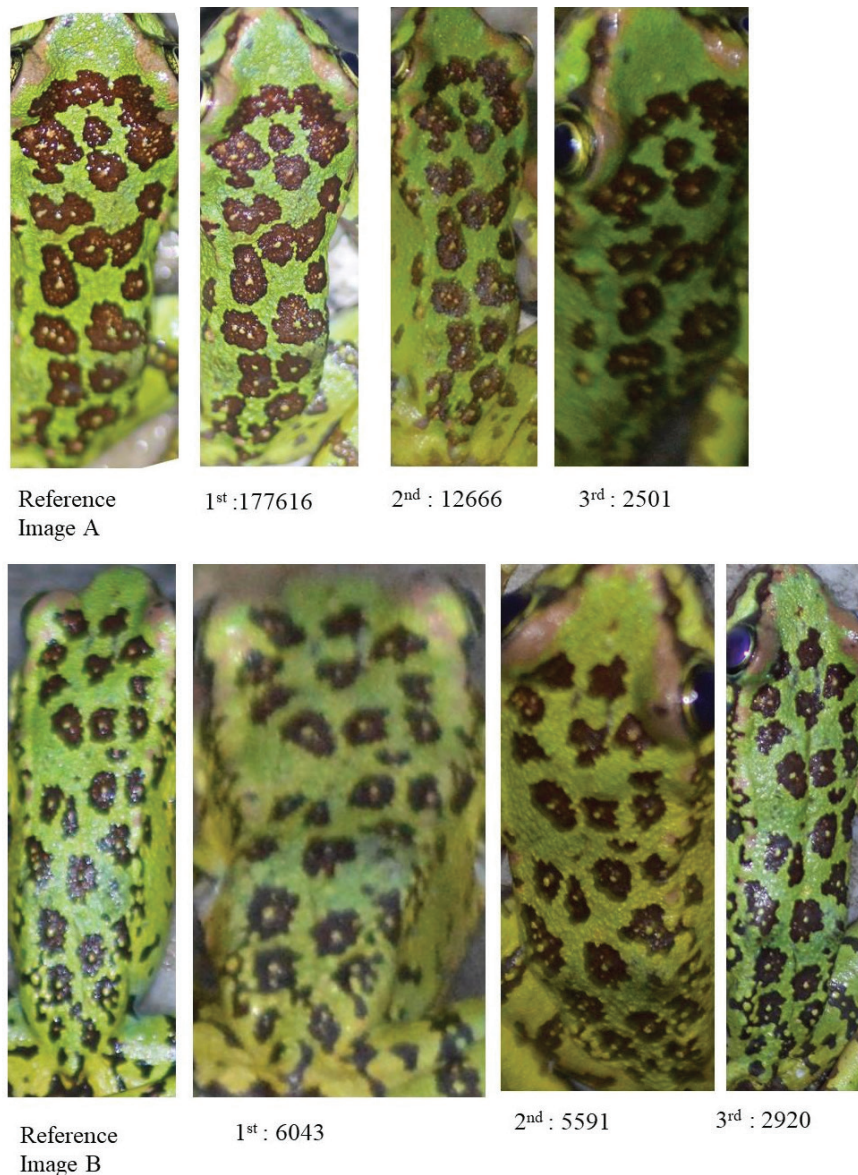
We determined Matching and Non-Matching Image Score for each image for further evaluation. To test the effect of image quality on the identification efficiency, we classified the images into excellent, moderate and poor, based on image clarity, focus and resolution (Fig. 4, Kelly 2001). The scores of the matching and non-matching images were grouped into Matching Score Excellent images (MSE), Matching Score Moderate images (MSM), Matching Score Poor images (MSP), Non-Matching Score Excellent images (NMSE), Non-Matching Score Moderate images (NMSM), and Non-Matching Score Poor images (NMSP). All six groups were analyzed by

the Kruskal-Wallis test with Dunn's post-hoc comparisons (Zar 1999). The differences between the groups were considered significant when  $p < 0.05$ . The statistical analyses were performed using SPSS 16 (SPSS Inc. 2007) and the FSA (Ogle 2010) package in R 3.4.2.

We also investigated the effect of photographic distance on individual identification based on the focal length of the camera as 100 mm  $\approx$  1 meter, 200 mm  $\approx$  2 to 3 meters, and 300 mm  $\approx$  5 to 6 meters. The file size of the cropped dorsal images was also negatively correlated with the photographic distance from the frog (1 meter  $\approx$  1563 kB to 6 meters  $\approx$  57.3 kB). We considered the image size as control for any effect of pixel size or image quality on the identification process. We considered the scores of matching images only since non-matching pairs should always have low scores irrespective of their distance and image quality. We carried out linear regression between the scores of matching images vs. focal length and scores for matching images vs. file size.

### Identification error

We calculated HotSpotter's success rate and error rates on the basis of matching photos considered correct. The matching images were deemed incorrect if non-matching images were scored higher. Success and error rates were calculated as the number of correct and incorrect



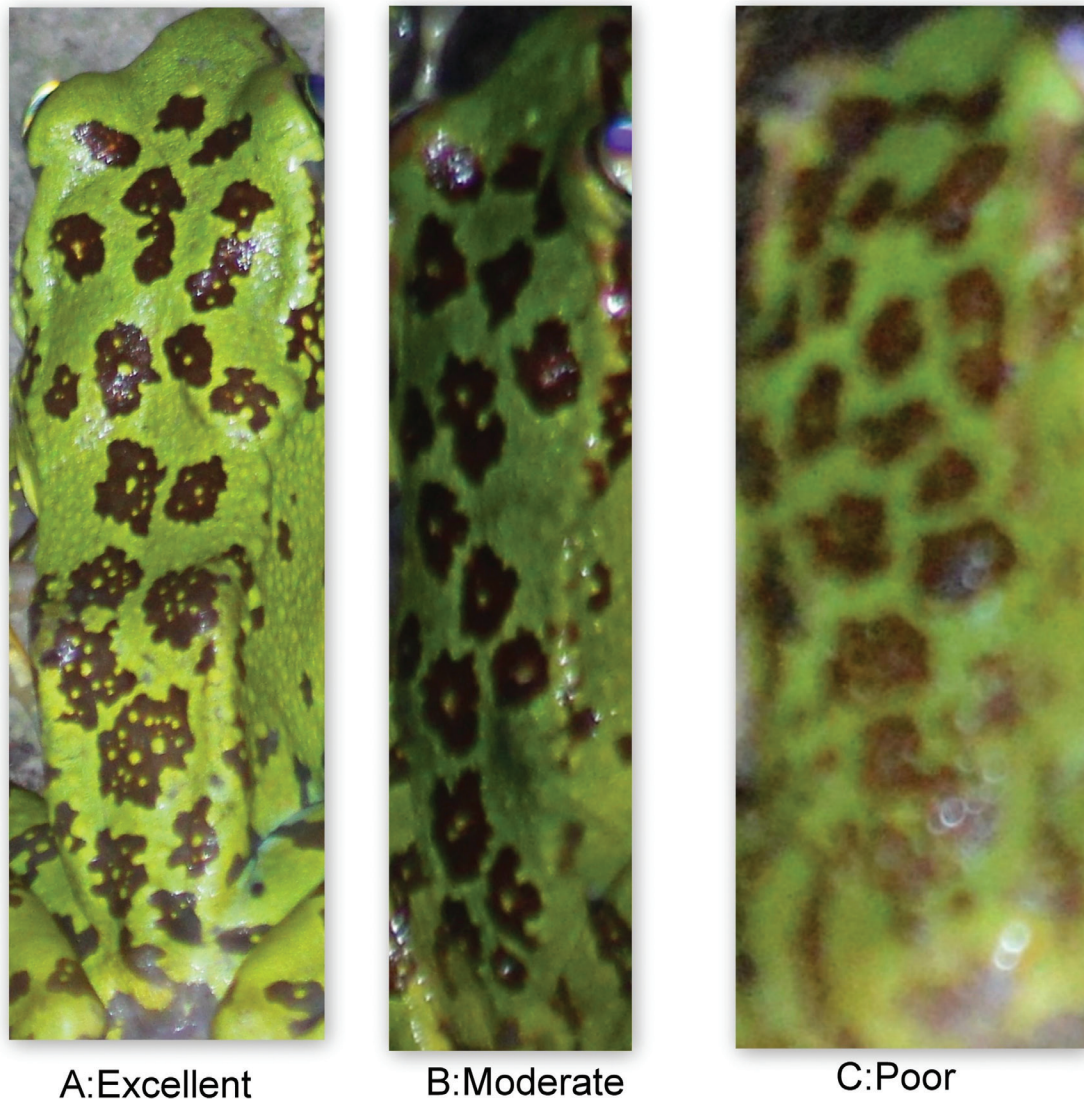
**Figure 3.** Same individuals of *Amolops formosus* recorded on different occasions with the similarity scores.

matches divided respectively by the total number of images. We prepared two capture histories to test the effect of misidentification on the estimation of abundance, one with error and the other with correct identification. For abundance estimate, we prepared individual capture histories for four occasions. Capture-recapture histories from the individual data were analyzed using closed population maximum likelihood estimator (MLE) in Program MARK (White and Burnham 1999). We ran three basic models to estimate abundance (Otis et al. 1978) M(.) – capture probability was constant across individuals and sampling occasions within each night (null model); M(b) – capture probability varied between individuals (behavior effect) but did not vary across sampling occasions; and M(t) – capture probability varied across sampling occasions but did not across individuals. The results were ranked and evaluated using the Akaike's Information Cri-

terion (AIC) (Burnham and Anderson 2002). The total abundance estimate from the model with the lowest AICc was considered to be the best model.

## Results

We recorded 301 photographs taken over 20-man hours of NVES surveys, representing 160 individual *A. formosus*. We found 67 frogs on the first occasion and 81, 89 and 64 frogs on the next three occasions, respectively. Seventy-eight individuals were recorded once and 82 individuals were recorded more than once. Out of 82 recaptured individuals, 42 individuals were recaptured twice, 21 individuals were recaptured three times and 19 individuals were recaptured on all the occasions. *Amolops formosus* was most frequently encountered siting on



**Figure 4.** Photographs were categorized into three categories based on their quality.

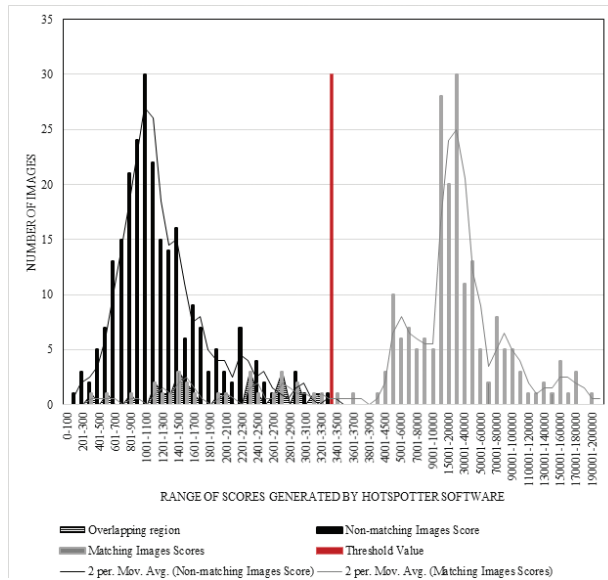
bedrock and boulders followed by branches of the shrub and barren ground. Occasionally, we also found frogs half submerged at the stream edges. Frogs were recorded up to 2 m above the water level and horizontally within 5 m of the stream edge.

### CAPRS efficiency

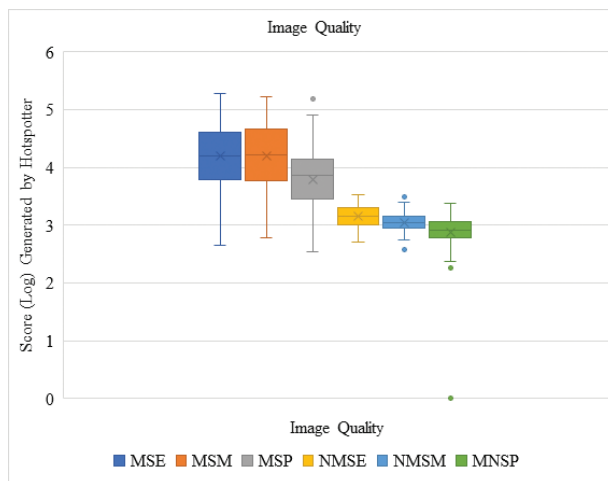
Matching pair scores ranged from 350 to 191644 with a mean value of  $19069 \pm 2800$  and non-matching pair scores ranged from 0 to 3303 with a mean value of  $1089 \pm 36$ , respectively (Fig. 5). Only 14% of the matching scores overlapped with the non-matching scores, and 86% of the matching images scored higher than 3303 (Fig. 5). Log transformation of the score generated by HotSpotter software depicted the difference between matched and non-matched images (Fig. 6). Regardless of the image quality, whether it was a photo of excellent quality or a photo of

poor quality, the matching pair score was much higher than the non-matching one. There was no significant difference between the matching scores of MSE and MSM (Table 1). The matching scores MSP differed compared to the matching scores of MSE and MSP. The matching scores of MSE, MSM and MSP remained significantly higher than all groups of non-matching images (NMSE, NMSM, and NMSP). There was no relationship between matching pair scores with focal length ( $R$ -squared = 0.001,  $p = 0.843$ ) and with file size ( $R$ -squared = 0.001,  $p = 0.648$ ).

The success rate in ranking the same individual's photo based on the similarity score by software was 94.3%, with an error rate of 5.6%. The time dependent model ( $M_t$ ) was the best model to predict *A. formosus* abundance estimates based on lowest AIC scores (Table 2). *Amolops formosus* abundance estimates for two groups “correct identification” and “with identification error” are 179 and 180 respectively (Table 3).



**Figure 5.** Histogram depicting the distribution of scores of matching images and non-matching images of *Amolops formosus* (n= 301) as generated by HotSpotter software. Black bars indicate the distribution of non-matching images scores, and the grey bars indicate the distribution of matching images score. Red bar is threshold value. Grey shaded region on the left side to threshold bar is an overlapping zone between the scores of matching and non-matching images.



**Figure 6.** Distribution of scores (log) generated by HotSpotter. The scores of MSE (Matching Score Excellent), MSM (Matching Score Moderate), and MSP (Matching Score Poor) are higher compared to NMSE (Non-Matching Score Excellent), NMSM (Non-Matching Score Moderate), and NMSP (Non-Matching Score Poor).

## Discussion

This study provides a purely non-invasive and reliable method for individual identification of amphibians with natural marking patterns. The successful recapture of more than 50% of the individuals within four sampling

**Table 1.** Dunn (1964) Kruskal-Wallis multiple comparison. The p-values adjusted with the Benjamini-Hochberg method. MSE (Matching Score Excellent images), MSM (Matching Score Moderate images), MSP (Matching Score Poor images), NMSE (Non-Matching Score Excellent images), NMSM (Non-Matching Score Moderate images), and NMSP (Non-Matching Score Poor images).

Comparison	Z	P.unadj	P.adj
MSE-MSM	0.6495634	5.16E-01	5.16E-01
MSE-MSP	3.1501276	1.63E-03	2.04E-03
MSE-NMSE	10.4214294	1.98E-25	3.71E-25
MSE-NMSM	14.6256754	1.93E-48	7.22E-48
MSE-NMSP	15.5695782	1.17E-54	5.86E-54
MSM-MSP	2.817835	4.83E-03	5.18E-03
MSM-NMSE	10.9538365	6.37E-28	1.59E-27
MSM-NMSM	15.962014	2.35E-57	1.76E-56
MSM-NMSP	16.6177518	5.18E-62	7.78E-61
MSP-NMSE	6.9131649	4.74E-12	7.90E-12
MSP-NMSM	10.7557339	5.57E-27	1.19E-26
MSP-NMSP	12.1053287	9.90E-34	2.97E-33
NMSE-NMSM	3.6733033	2.39E-04	3.27E-04
NMSE-NMSP	5.9042941	3.54E-09	5.31E-09
NMSM-NMSP	2.9470791	3.21E-03	3.70E-03

occasions tends to confirm the validity of this method. The use of zoom lens reduced the flight response of the frogs to the minimum. Only frogs that were encountered too close ( $< 1$  m) had shown flight response.

There is no better approach than noninvasive sampling for population estimation of frogs when physically capturing each frog is not possible. It is feasible to photo-document and to identify individual frogs from within 1 m and thus eliminate the need for capturing and handling (Grafe et al. 2006). In the present study, the frogs were documented from a distance of 1 to 6 m. There was no correlation in the scores of matching images with respect to the file size and the focal length of the images that are substituted for the distance from the animal being documented. Our approach was an effective method in terms of field sampling time. The effort required to catch, handle, and tag or mark the animal was not required in our technique which added an extra buffer time to sample other individuals. Fogarty and Vilella (2001) also showed that in *Eleutherodactylus* frogs, the handling (1.0/survey) required almost double the effort than visual documentation on a transect (1.98/survey).

Pattern recognition gets influenced by animal posture, hormonal status, injury marks, environmental influences, and also dirt (Jørgensen and Larsen 1960; Kindermann et al. 2014). Factors such as glare, focus, camera angle and flash may also influence the pattern recognition (Matthé et al. 2017). Such variations in the quality of the photograph affect the identification of CAPRS (Kelly 2001). We have shown the influence of the angle, focus and glare of the photographs on the recognition efficiency of the HotSpotter (Fig. 3A, B). Excellent quality photographs were identified with ease as their matching scores were much higher than non-matching photos (Fig. 6), but poor quality photographs required careful examination (Table 1;

**Table 2.** Model selection for *Amolops formosus* abundance estimation based on AICc score under closed capture recapture frame work.

Group	Model	Model Name	AIC	Delta AICc	Model likelihood	Number of Parameter
Correct identification	{M(t)}	Time dependent	-452.2	0.000	1.000	5
	{M(.)}	Null Model	-449.10	3.4484	0.1783	2
	{M(b)}	Behavior dependent	-447.08	5.4673	0.065	3
Identification errors	{M(t)}	Time dependent	-446.62	0.000	1.000	5
	{M(.)}	Null Model	-444.81	1.810	0.4045	2
	{M(b)}	Behavior dependent	-443.06	3.560	0.107	3

**Table 3.** Abundance estimation of *Amolops formosus* with identification error and without identification error.

Group	Abundance	Standard Error	Lower Confidence Limit	Upper Confidence Limit
<i>Amolops</i> abundance without error	179.64	6.01	170.92	195.32
<i>Amolops</i> abundance with Misidentification error	180.23	6.56	170.62	197.17

**Table 4.** Frequency of capture histories of *Amolops formosus* for four occasions.

Capture history	With error	Without error
0001	10	10
0010	16	15
0011	11	14
0100	28	26
0101	2	2
0110	13	15
0111	12	11
1000	28	27
1001	1	0
1010	6	7
1011	6	6
1100	3	4
1101	3	2
1110	3	2
1111	16	19

Fig. 5). Such careful examination was only required for 14 % of the photographs, and the remaining 86 % of the photographs were correctly identified without any kind of difficulty (Fig. 5). Therefore, it was possible to identify individuals from poor quality photographs.

It is a fundamental requirement to correctly identify individuals in a mark-recapture population estimate study because misidentification can affect the abundance estimates (Morrison et al. 2011). The identification efficiency and accuracy of the computer-aided matching software varies depending on the species and the program. Here, we only relied on HotSpotter for individual identification. The success rate by HotSpotter in individual identification was 94.3 % and it did not affect the abundance estimate by a large degree. In our study we estimated 179.64 ( $\pm 6.01$ ) individuals of *A. formosus* with correct identification and 180.23 ( $\pm 6.56$ ) individuals with identification error. In this study, the time dependent model performed best as there was a slight increase in the capture rates on the 2<sup>nd</sup> and 3<sup>rd</sup> occasion (Table 2; Table 4). As this study was for a short time, the variation in weather was minimal. This also restricted us to comment on variation in capture rate with

respect to environmental parameter and animal behavior. However, long-term monitoring across the entire growing season will help to better understand this species behavior. In our present study the performance of HotSpotter was satisfactory for *A. formosus* individual identification.

## Conclusions

Pattern recognition is utilized extensively for individual identification, and performs better than other traditional methods. However, the accuracy of software varies depending on the species and their patterns and image quality. Hence, a thorough evaluation of software is recommended. This contribution demonstrates the efficiency of HotSpotter software in estimating the abundance of stream frogs in a non-invasive manner. This technique is quick, easy, cheap and can be utilized in citizen science approach in monitoring amphibian populations. This method can be further improved by collecting parameters such as precise GPS location, time and macrohabitat with each photograph which will help in understanding aspects of species ecology such as home range, site fidelity, activity pattern and macrohabitat use.

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