Using HotSpotter Animal Individual Recognition Software in the Detection of Ontogenetic Pattern Changes in Panamanian Golden Frogs (Atelopus zeteki)

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Running head: HotSpotter Detection of Pattern Changes

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**Abstract**

Panamanian golden frogs (*Atelopus zeteki*) are a species of bufonid toad native to Panama that are suspected to be extinct in the wild due to infection from an invasive foreign fungus. Adult frogs are bright yellow in color, with numerous individually unique black markings that tend to disappear over time. In this study, a captive population of 44 Panamanian golden frogs were photographed once per month over a period of several months to track the changes in black patterning. These images were compared visually and through the program HotSpotter to develop a method by which HotSpotter may be used to consistently identify individual frogs over time, despite the changes in patterning. By querying against each other in HotSpotter all photos of a given individual frog over time, it was determined that instructing HotSpotter to look at only the dorsum of the frogs, excluding head and legs, returned the most accurate matches. Once this method of matching photos (one at a time, using only the back regions of the frogs) was established as the most accurate, it was used to test how well HotSpotter could match photos over time as the frogs’ patterning changed. The preliminary conclusion was that HotSpotter is less able to match photos of younger frogs whose patterning often changed appreciably in the time between photographs. Further testing is needed to confirm or refute this conclusion.

**Key words:** HotSpotter, mark-recapture, photo identification, *Atelopus zeteki*

**Introduction**

Panamanian golden frogs, *Atelopus zeteki*, are neotropical toads that have recently gone extinct in the wild due to the emergent fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). The prospect of eventual reintroduction of these frogs, now being maintained in captivity until circumstances improve, warrants as much further study on the species as is possible to help facilitate successful reintroduction. The eventual reintroduction effort will likely benefit from a noninvasive method of reliably identifying individual specimens. The identification of individuals by natural coloration and patterning (see Fig. 1)
alone would be preferable to any means that requires physically tampering with the frogs. The aim of this study is to document how the patterning of individual Panamanian golden frogs changes over time and to assess the potential of the program HotSpotter as an aide to identification despite the changes in patterning.

The disease that has extirpated Panamanian golden frogs from their historic habitats, *Bd*, is currently the most dire threat to *Atelopus* frogs. The enzootic has apparently already driven golden frogs to extinction in the wild. Preserved amphibian specimens collected before and during the decades of greatest population decline were tested for *Bd* via PCR, and the results of these tests implicate that *Bd* first appeared in Mexico around 1970 and swept southward into central America over the next 30 years (Cheng et al., 2011) (See Fig. 2). This fungus uniquely affects a wide range of amphibian hosts, causing chytridiomycosis that in many cases is fatal. Chytridiomycosis occurs when *Bd* colonizes a host’s skin. Because the surface of the skin is important to the maintenance of homeostasis in amphibians, the disruption in osmoregulation caused by fungal colonization is a likely cause of death (Voyles et al., 2009). *A. zeteki* is particularly susceptible to this condition (Ellison et al., 2014).

*A. zeteki* is now considered extinct or nearly extinct in the wild, as no wild sightings of the frog have been reported since 2009 (Gratwicke et al., 2016). Scientists fortunately noticed the rapid decline of many amphibian species before Panamanian golden frogs had completely vanished and formed Project Golden Frog in 1999 to make efforts to preserve the iconic amphibians. This organization began collecting healthy specimens for the foundation of an *ex situ* management program (Gagliardo et al., 2008). Husbandry of the frogs has been successful enough that several captive populations persist despite the disappearance of their wild counterparts. The hope is that Panamanian golden frogs may one day be reintroduced to their native habitat.

Researchers project a high probability of success in sparing *A. zeteki* from total extinction through the use of captive breeding programs (Gratwicke et al., 2016), but that probability alone is not enough to
save the frogs or other amphibians imperiled by **Bd**. Any captive breeding program risks loss of genetic material due to genetic drift or inbreeding depression, and if the frogs are kept too long *ex situ*, they may suffer from a loss of genetic diversity and other adaptations which may be too severe to be overcome even after reintroduction to the wild. One study found that golden frogs kept in captivity had significantly different microbial communities on skin surfaces from the microbial communities found on wild frogs (Becker et al., 2014), and this change alone could eventually make reintroduction difficult.

Even if the frogs themselves do not change significantly during their captivity, it is possible that the native habitats to which the frogs are adapted may be altered or destroyed in the frogs’ absence. Due to the drastic lifestyle differences between the amphibians’ larval and adult forms, the disappearance of this species from an ecosystem is doubly detrimental (Whiles et al., 2006). Tadpoles, for instance, have a significant role in the circulation of basal resources in the streams in which they develop (Ranvestel et al., 2004). Additionally, frogs and other amphibians provide an important link between streams and riparian habitats on account of their ability to move nutrients, so their absence from an ecosystem is likely to have significant lasting ecological effects on their former ranges (Whiles et al., 2006).

The sooner Panamanian golden frogs can be reintroduced to their native habitats, the more likely it is that reintroduction will be successful, but researchers have encountered significant barriers to this effort. First, **Bd** is known to infect a great many amphibian species, so it can persist in an environment even after driving one species to extinction. The fungus has also been discovered to use nonamphibian hosts such as crayfish and may by such hosts persist in an environment even after all amphibian species have been extirpated (McMahon et al., 2012). Since it is unlikely, then, that **Bd** can be eliminated from an ecosystem, scientists are exploring the possibility of using probiotics to protect *A. zeteki* from infection with **Bd**. However, studies in which golden frogs have had their skin colonized by bacteria known to act against **Bd** have generally been unsuccessful so far in preventing infection (Becker et al., 2011; Becker et al., 2015). It is becoming evident, though, through the uncommon instances in which frogs
experimentally infected with *Bd* clear the infection and survive, that the community of symbiotic bacteria on individual frogs plays a significant role on the ability of individual frogs to overcome chytridiomycosis (Becker et al., 2014; Becker et al., 2015).

Any knowledge gained toward a better understanding of *A. zeteki* could potentially contribute to successful reintroduction of this species to its natural habitat. The establishment of a noninvasive method of identifying individual frogs could be especially useful to present and future research endeavors. Once frogs begin to be reintroduced to their native range, it may be useful to be able to track the movement, health, and survival of individuals via a method that will not interfere in any way with the frogs’ interactions with their environment. Mark-recapture is the standard technique for population studies, but many methods of marking caught individuals can be potentially harmful. “Marking” individuals by photographing them upon capture is less risky as long as the collected photos can be used effectively to re-identify individuals upon recapture.

Matching individuals to images, especially when those individuals’ distinctive patterning has degraded since the last capture, can be very time-consuming. The use of an image-matching software such as HotSpotter may be useful in speeding the identification process. It will be necessary to determine the most effective method of implementing this program so results will be consistent. Ideally, HotSpotter will be able to successfully identify every individual. If not, or to manually affirm the identifications if so, it will also be helpful to understand the general trends by which Panamanian golden frogs’ patterning changes over time.

To develop a noninvasive identification method by which photos of Panamanian golden frogs are matched in HotSpotter and manually affirmed, individual frogs from a captive population were photographed regularly over six months. The photosets collected were manually identified and then uploaded into HotSpotter to determine whether the program could arrive at the correct matches. A method of using HotSpotter effectively in a standardized manner is yet to be established. Once it is,
however, researchers could use HotSpotter as a tool to observe population dynamics of recently reintroduced golden frogs in nuanced manner, possibly making it easier to discover which populations thrive and which do not. Equipped with such knowledge, researchers could construct and implement more effective reintroduction plans to successfully reestablish Panamanian golden frogs in the wild.

**Materials and Methods**

*Materials*

- 44 Panamanian golden frogs (19 male, 25 female)
- Canon Powershot G15
- Computer with Adobe Photoshop and HotSpotter
- Petri dish, white copy paper
- Calipers, Scale with deli cup
- Isopropyl alcohol and water rinses, Kimwipes

*Methods*

The 44 frogs photographed in this study were housed in two different locations in the Maryland Zoo. First, the males on exhibit were photographed before the zoo opened to the public. All frogs were moved into holding tanks. Then, they were taken out one at a time at a time and identified using permanent ID cards. Each frog was weighed on the scale, using a tared deli cup to prevent them from jumping away, and then snout-urostyle length was measured with the calipers. Next, frogs would be misted to remove debri as necessary and dried with a Kimwipe to prevent glare, and finally, they were placed in a petri dish on a sheet of white printer paper to capture photographs. Both dorsal and ventral photos were taken. When this process was complete, the frog was placed in a “completed” tank, and the process would repeat until all 19 males were photographed. The 25 females were in quarantine, but they were processed in a
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similar manner. They were divided into subsets, each with their own tank, so all equipment was sanitized after each subset was photographed to prevent cross-contamination.

All six photosets, after they were collected, were edited in Adobe Photoshop to correct for frog orientation and zoom. When the photos were edited, they were then uploaded to HotSpotter, where “chips” were made from them (See Fig. 3). A chip is a selected area in an image that the program uses to assess matches. The program automatically creates a high-contrast version of the selected chip, which when used facilitates comparison by contrast rather than hue. Because of variable lighting on the frogs in the photos, the HotSpotter was set to use the high-contrast chips and not the hue chips.

Once chips had been made from all of the photos, the chips were queried one at a time against every other chip in the database, and the program suggested matches. Sets of queries were performed as different tests, and the content of the HotSpotter database as well as the method of chip selection was varied between tests to determine the most effective technique for getting correct matches. The problem of chip-selection methods in particular was investigated. Five potential methods were identified -- full-image chips, in which the entire photo was selected as a chip; spacious chips, in which most of the background was cropped out; restricted chips, in which more of the background and the frogs’ legs were cropped out; head-only chips; and dorsum-only chips.

To determine which of the five chip-selection methods was most effective at getting correct matches from the program, several HotSpotter databases were compiled consisting of all photos collected throughout the study of single individuals. For example, all six images of frog 8112 collected throughout the study period were put into their own HotSpotter database. Chips of all six images were made using one of the chip-selection methods, and these were each queried against each other. It was predicted that the program would match highest the most visually similar images -- those that were taken closer together in time and would have shown less pattern fading (Table 1). Five trials each of the five different chip selection methods were tested in this way. Chip-selection methods in which results matched this
prediction more often were thought to be more accurate than those that did not.

Three of the five chip-selection methods were assessed to have a similar success rate in HotSpotter, so further testing was devised. A subset of 20 images from the February 2018 photoset were queried one at a time into a HotSpotter database of the entire January 2018 photoset. This process occurred in three different tests, using a different chip-selection method in each and a different random subset of 20 images from the February 2018 photoset (to control for differences in image quality). The method that successfully matched the highest proportion of frogs in 20 queries was deemed most effective overall.

Once the most effective chip-selection method was identified, that method was used to test HotSpotter’s ability to match frogs as they age. New databases were created consisting of all images from two adjacent photosets. For example, one database contained all photos from February and March 2018. Chips were made of all February images to serve as a standard of comparison for the program. Then, chips were made, queried, and deleted of one photo at a time from the March photoset. The same process was repeated in databases consisting of all January and February 2018 photos, and all December 2017 and January 2018 photos, and so on. After every query, it was recorded whether HotSpotter correctly matched the photo at all and whether the program ranked the correct match as the best match. Once all six of these month-to-month databases were tested, the proportion of successful matches was determined and graphed as a function of time.

Finally, to determine the relative efficiency of HotSpotter-aided identification compared to manual identification, two blind tests were set up. Both involved a reference set of all February 2018 photos tagged with their correct identification numbers, and a set of 20 photos from the March 2018 photoset labelled A through T. In the first blind test, a subject familiar with Panamanian golden frog identification was asked to use HotSpotter in a standardized way to assign identification numbers to the 20 unknown frogs. In the second test, a different subject also familiar with Panamanian golden frog
identification was asked to perform the same task using only the reference set and not HotSpotter to identify the 20 frogs. Both tests were timed for comparison.

**Results**

Among the five chip-selection methods proposed, those with the highest combined percentage (percentage of queries that either matched or approximated the prediction) were the restricted chip and the dorsum-only chip, both at 48.3%. The chip selection methods that matched prediction most often were the restricted chip at 20.7% and the full-image chip at 17.9%. After these three chip-selection methods -- full-image, restricted, and dorsum-only chips -- were tested further, they were found to identify the correct match as the highest potential match 35.0%, 73.7%, and 85.0% of the time, respectively (See Table 2).

The results from testing HotSpotter’s match accuracy with frogs of different ages are illustrated in Fig. 4. In comparing all October 2017 photos to all December 2016 photos, HotSpotter found the correct match at any rank 1-6 (total match) 30.6% of the time and found the correct match at the highest rank (first match) 12.2% of the time. Comparing November to October 2017 found the total match 51.0% and the first match 40.8% of the time; December to November 2017 found the total match 58.1% and the first match 37.2% of the time; January 2018 to December 2017 found the total match 89.7% and the first match 71.8% of the time; February to January 2018 found the total match 88.6% and the first match 79.5% of the time; and March to February 2018 found the total match 79.5% and the first match 61.4% of the time.

In Blind Test 1, where the subject used HotSpotter to match 20 unknown photos, match accuracy was 100%. The test took 29 minutes and 47 seconds to complete. Blind Test 2, where the subject matched the same 20 unknown photos manually, without the use of HotSpotter, was also 100% accurate. However, Blind Test 2 took only 16 minutes and 8 seconds to complete.
Discussion

Initially, three chip-selection methods -- full-image, restricted, and dorsum-only -- appeared to rival each other in effectiveness at facilitating accurate matching in HotSpotter. After further testing, however, it was determined that the dorsum-only method was clearly the most effective. Once this determination was made, dorsum-only chips were used exclusively for all subsequent testing.

The next set of tests was aimed at exploring how well HotSpotter could match photos of frogs as they aged and their patterns continued to fade. Querying photosets from adjacent months against each other revealed that HotSpotter’s match accuracy generally increases as the frogs grow older, presumably because their ontogenetic patterning begins to look more similar from month to month. This implies that when the frogs are younger and their patterning changes rapidly, HotSpotter is relatively ineffective at matching individuals. This set of tests also revealed a point in time when HotSpotter’s match accuracy suddenly increased significantly -- from a “first match” rate of 37.2% with the December 2017 photos to a rate of 71.8% with the January 2018 photos. By January 2018, the population of frogs used in this study would have been about 22 months old. The leap in HotSpotter’s match accuracy coupled with visual data gathered in observing the frogs over six months implies that it is at that age that the ontogenetic patterning on *Atelopus zeteki* ceases to change appreciably. Thus, it can be surmised that HotSpotter is most effective when used to identify frogs that are at least 22 months of age.

The month-to-month tests performed in HotSpotter in this study were designed to approximate mark-recapture population studies. The earlier set of photos in a given database represented the first capture of a particular population, and the later set of photos in the same database represented their recapture. In these tests, the frogs were “recaptured” each time after only a month, but in a study on a wild population, it is unlikely that individual frogs will be recaptured so frequently. To get a better idea of HotSpotter’s ability to match frogs in a genuine mark-recapture study, it would be necessary to extend
this study to compare photosets that were collected further apart in time in the program. Because the population of frogs used in this study had only recently reached an age where their ontogenetic patterning had ceased to change, this extension was not yet possible.

The blind tests suggested that HotSpotter was as accurate as manual identification of individuals of *Atelopus zeteki*, but far less efficient. However, these tests were operated on a small scale, with only 20 photos in a total set of 44 to identify. Because of the amount of time it takes to make chips of photos for their use in HotSpotter, the HotSpotter identification method took significantly longer than manual identification, which required little more than a visual scan through the 44 reference images to complete. It is possible, however, that in larger data pools, comparing 50 photos to a reference set of 100 or more, for example, may prove to be more efficiently done in HotSpotter. Further testing is necessary to confirm or refute such a prediction.

**Conclusions**

It was determined that among five different chip-selection methods (full-image, spacious, restricted, head-only, and dorsum-only), the dorsum-only method tended to produce the most accurate matches in HotSpotter. Using this chip-selection method, it was then found that the program’s ability to make correct matches generally increases over time. This implies that as the frogs age and the rate that their ontogenetic patterning fades slows or stops, Hotspotter is better able to match them. Conversely, when the frogs are young and their patterning may change dramatically in a relatively short time, HotSpotter is less able to match them correctly. The point when the rate of ontogenetic pattern change slows or stops -- and HotSpotter becomes more effective -- is when the frogs reach about 22 months of age.

Blind tests revealed that manual and Hotspotter-assisted identification is equally accurate (100%), but that with small populations, manual identification is faster, for it lacks the time-consuming necessity of creating chips.
Future directions for this study include collecting photos of a new population of Panamanian golden frogs as they age and testing HotSpotter’s ability to match them as was done in this study, testing HotSpotter’s ability to match photos taken more than one month apart, and conducting further blind tests to determine if HotSpotter is more efficient than manual identification with larger population sizes. The ultimate goal of much of the research being done on Panamanian golden frogs is to re-establish a stable population in their native habitat. Identifying individual frogs by photographs of their dorsal patterning is an effective and harmless method of population study, and the use of HotSpotter to assist in this process may render it quicker and more efficient. Using this system of software-assisted photo identification rather than traditional marking methods like toe-clipping could give recently-introduced populations of Panamanian golden frogs an important survival advantage.

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References


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Table 1. Sample of results from Test 11d. The columns represent each photo of frog 8104 that was queried with a dorsum-only chip in HotSpotter, and the rows list each photo’s match score for the one queried. Scores are index numbers calculated by HotSpotter. Gold cells indicate the highest-matching photo for each query, and light gold cells indicate the second-highest matches.
Table 2. Results from chip-selection method testing. % Successful Matches refers to the proportion of times in a sample size of 20 queries that HotSpotter found the correct match and ranked it at any rank from 1-6. % Highest Matches refers to the proportion of times that HotSpotter found the correct match and ranked it in position 1, the highest match for that query.
Figure Legends

Figure 1. Variable appearance of Panamanian golden frogs, *Atelopus zeteki*. Photos by Brian Gratwicke.
Figure 2. Map of the spread of *Batrachochytrium dendrobatidis* through central America. From Cheng et al., 2011.
**Figure 3.** A progression of a raw photo of an individual frog to hue chip to contrast chip for use in the HotSpotter program. The individual shown is frog 8122 from March 2018.
Figure 4. Results from Tests 15 and 18-22. In each test, one entire photoset from a given month was matched one image at a time to the entire photoset collected the month previously. The percentage of matches from each test in which HotSpotter ranked the correct match as the highest match is depicted by the gold line. The green line depicts the percentage of matches from each test in which HotSpotter recognized the correct match at any rank.