Using Digital Photographs and Pattern Recognition to Identify Individual Boreal Toads *(Anaxyrus boreas boreas)*

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Individual identification of animals can provide an array of useful capture-mark-recapture information, allowing researchers to estimate survival, movement, abundance, recruitment, and capture probability (Williams et al. 2002). This information can yield valuable insight to field investigators regarding a species' life history (Davis and Ovaska 2001; Phillott et al. 2007). Techniques used to identify individuals of many species have been developed and refined to gather this information. Toe clipping, PIT tagging, polymers and pigments, branding, and pattern mapping are all viable techniques for identifying individuals of many amphibian species (Davis and Ovaska 2001, Donnelly et al. 1994).

In addition to its potential utility in field investigations, individual identification is often important in captive populations. Knowing the identity of individuals allows accurate documentation of animal origin and breeding history, providing essential information when selecting individuals to breed in order to maintain a healthy, genetically diverse population. These goals are the impetus for the keeping of studbooks for a number of imperiled and critically endangered amphibian species. Individual identification capability may also facilitate the separation of brood stocks without the need to apply other marks.

Choosing the technique that best suits the species of interest as well as satisfying the questions at hand is important when determining how to identify individuals (Osbourn et al. 2011). In order to use pattern recognition as a capture-mark-recapture technique, the pattern on the animal should be distinguishable, remain stable, and be unique to the individual (Osbourn et al. 2011). These characteristics must be examined in any species for which pattern recognition is evaluated as a method. When not all of these pattern characteristics are present, false identifications may result (Kenyon et al. 2009). However, patterns that are unique and stable constitute the ideal mark because they cannot be lost, and concerns over deleterious marking effects that could affect results are minimized (Beausoleil et al. 2004).

In the past, pattern mapping has been a time consuming, demanding technique because an investigator had to either sketch the pattern of the specimen or wait to develop film (Donelly et al. 1994; Plăiaşu et al. 2005), accompanied by the uncertainty over the quality of photographs taken. Recently, the affordability and quality of digital cameras have made pattern mapping more feasible. The ability to take, assess the quality, and retake a photograph while the specimen is still available makes pattern mapping and digital photography a suitable option (Bradfield 2004). Furthermore, digital photographs are less intrusive than PIT tagging or toe clipping; there are no wounds or potential infection sites created when taking a digital photo (Carafa and Biondi 2004; Plăiaşu et al. 2005). These advantages make digital photographs for individual identification appealing, especially when working with a state endangered species such as the boreal toad (*Anaxyrus boreas boreas*) in Colorado.

Colorado Parks and Wildlife (CPW) maintains a captive population of ~700 boreal toads at its Native Aquatic Species Restoration Facility (NASRF) for which unique identifications are necessary. Implanting captive toads with PIT tags seemed a logical choice given apparent high retention rates in wild toads (e.g., Scherer et al. 2008, Muths et al. 2010, Pilliod et al. 2010). However, long-term retention rates of PIT tags in our captive population have been much less than 50%, despite being implanted by the same individual who implanted PIT tags into the toads in the Muths et al. (2010) study (personal communication, K. B. Rogers, Colorado Parks and Wildlife). Hatchery toads were smaller on average than wild adult toads, so size at tagging may have had an effect. Whatever the cause of this excessive tag loss, it was clear that CPW would not be able to rely on PIT tags to identify individuals in this captive population. Toe clipping, although a generally reliable, inexpensive method to identify individuals, was deemed unsuitable for identifying the toads housed at NASRF because of the large number of toe removal combinations that would be required.

In order to find a more viable and reliable option, we explored phenotypic characteristics of the boreal toad. The boreal toad's ventral morphology consists of a cream colored belly, creating a contrasting backdrop to the black markings on the belly. The resulting stark contrast makes the boreal toad a good candidate for pattern mapping. However, it was unknown if belly patterns remained unchanged as recent metamorphs or yearling toads mature to adults.

Our goal was to assess the suitability of belly pattern recognition using photography as an alternative method to identify hatchery brood toads, and to ascertain whether boreal toad belly patterns are stable through growth and time.

Methods.—

Cheyenne Mountain Zoo. —

In August 2007, 38 boreal toads originating from a single clutch and aged about 1.5 months post metamorphosis were transferred from NASRF to Cheyenne Mountain Zoo (CMZ) in Colorado Springs, Colorado to assess the uniqueness and stability of the belly patterns as they aged and gained size. There, the toads were randomly assigned to one of two rearing tanks (n = 19 each tank). They were photographed, measured (SV length, mm) and weighed (g) initially on September 8 and each toad was assigned a unique identification number that was annotated onto the original digital photographs. These became the reference photographs. All toads were subsequently photographed once each month from October 2007 through February 2008, then in April and July 2008. Eighteen toads in each tank were implanted with PIT tags at the April 2008 photograph occasion, when average weight was 12.1 gm (SE = 0.51). Upon completion of each set of photographs one author (KZF) matched new photographs with the reference set and labeled each of the new photographs accordingly, but maintained the base photograph number as a component of each photograph name. Matches were later evaluated and confirmed by the remaining authors, hence three investigators had to agree on each photograph match to be considered a successful match. This series of photographs provided a 10-month window to compare the belly patterns using original photographs against the seven follow up photograph sets.

We tested whether investigators unfamiliar with the toads could successfully match photographs by asking ten individuals to match the reference September photos of the toads with one or more of the succeeding sets of photographs. Eight of the ten individuals worked with just one set of succeeding photographs, and two individuals matched reference photographs with two succeeding sets. All seven succeeding sets of photographs were matched against the reference set at least once. These individuals were provided annotated reference photographs, the follow up candidate photographs they were assigned bearing only the file name generated by the camera, and a data sheet upon which to record candidate photograph numbers next to the individually identified reference photograph numbers. Six of the 10 had no previous toad experience. The individuals were informed what tank the toads belonged in, so we achieved twelve blind photo matching opportunities on all toads from each tank. Two authors (AWN, KGT) assessed recording sheets for accuracy in identification by checking the base photograph numbers of candidate photos supplied to the ten individuals against the base photograph numbers of the appropriate follow up set of photographs that included individual toad identification.

Native Aquatic Species Restoration Facility. —

We initiated the use of belly pattern photographs as the primary method to identify individuals at NASRF in 2007. The toads were housed in tanks and separated into "lots", which usually consisted of 10-15 sibling toads. Over 700 toads ranging from age 1 to age 8, as well as some toads brought in as unknown age adults from the wild prior to the year 2000, were photographed in 2007. In subsequent years the additions to the population were photographed at about age 1, or after their first hibernation. Each toad was assigned a unique ID incorporating its lot number at the time its photograph was taken. The belly pattern photographs were copied to a computer and arranged by lots with up to six thumbnail photographs per page along with each toad's unique ID added below its photo. The thumbnail pages were printed and laminated to protect and extend the life of the photographs.

The availability of captive toads at NARSF provided the opportunity to further investigate the stability of belly patterns. In 2009 we photographed five lots of yearling toads twice, in March and November. The lots ranged in number from three to 15 toads, and comprised a total of 29 animals. The photographs were then matched between the March and November occasions within each lot to evaluate stability of pattern over eight months during a period of rapid growth. Consensus on general spot pattern and three or more specific match points (e.g., uniquely shaped spots or placement of spots in relations to others) for March and November photographs among three investigators was considered a successful match.

We also periodically photographed the bellies of two toads brought into captivity from the wild as they matured from age 1 to age 4, when boreal toads generally reach adult size. These animals were housed together, but without other animals, in the display tank at NASRF. At the end of our study the serial photographs of the two display toads were compared both among the photographs taken and with the live toads. Again, consensus on general spot pattern match and three or more specific match points among serial photographs on the part of three investigators was considered a successful match. These trials allowed us to extend observations of belly pattern stability to a longer time period and greater growth difference than we were able to achieve at CMZ.

We also conducted a timed toad identification experiment in 2012 to investigate the efficiency of using belly pattern photographs to ID toads. At NASRF, three separate toad lots were selected for the study; one lot contained 15 animals and the other two lots contained 14 animals. Each toad lot was assigned to four investigators with varying levels of familiarity using photographs to identify individual toads. The investigators were ranked into three categories: novice, experienced, and expert. Novice was defined as having no experience using photographs to ID toads, experienced as having used photographs previously for toad ID but not on a regular basis, and expert as having used photographs frequently to ID toads, either at NASRF or during field studies from 2009 - 2012. An investigator compared a toad in hand to the previously described photographs of the lot, which were placed on a lab counter so that photos of all toads in the lot were visible at once. As toads were presumptively identified, the investigator placed each into a numbered container corresponding to the last two digits of the unique ID. Once all toads from a lot were identified and in containers, two other investigators verified that the identifications were correct by examining each toad's belly and the photograph it was matched with. Consensus on the general spot pattern and three or more specific match points was considered confirmation of the original identification. Timing commenced when the identifying investigator picked up the first toad and ceased when the last toad was placed into a container, and did not include the verification procedure. Time to individually identify toads and the accuracy of identifications was compared among categories of investigators using the single factor ANOVA procedure in the statistical software MINITAB (version 14.1, Minitab Inc., 2003).

Results.—

Cheyenne Mountain Zoo. —

The average size of the toads on September 8, 2007 was 32.7 mm SVL (SE = 0.36) and 3.5 g (SE = 0.12), the average size of the toads at the end of the study on July 17, 2008 was 52.2 mm SVL (SE = 0.82) and 10.9 g (SE = 0.48). One toad died prior to the April and July photographs. The photographs from each of these months were used once in the matching exercise, so a total of 454 matching opportunities were achieved rather than the 456 possible had all toads survived (12 matching exercises x 38 toads).

Investigators correctly matched 449 photograph pairs in 454 opportunities resulting in 98.9% success. Nine out of the 12 blind tests achieved 100% success matching the reference toad photographs to photographs from one to 10 months later (the final photographs taken, Fig. 1). Three errors were encountered during the study. One experienced investigator transposed two toad identification numbers on the recording sheet, but this was a recording error and not a true misidentification resulting from similar patterns. A novice investigator assigned a single follow up photograph to two different toad reference photographs (one was correct) resulting in misidentification of a single toad. The last error occurred when a novice investigator misidentified a pair of toads. Thus there were three true cases of mistaken identity in 454 opportunities (99.3% success). Clearly, belly patterns are distinguishable even to people that are unfamiliar with boreal toads and identification performance may even improve with increasing familiarity with the photography system.

The PIT tags implanted in April were poorly retained. By week 9 post-implantation, retention was just 44% in each tank. At the July photography occasion, 13 weeks post-implantation, only four PIT tags remained implanted, with 16.7% retained in one of the toad tanks and 5.6% retained in the other.

Native Aquatic Species Restoration Facility. —

Among the five lots of yearling toads photographed twice in 2009, all March photographs were matched successfully to November photographs, and both photograph sets were matched successfully to live toads. In no instance was there disagreement among three investigators over matches. These comparisons were limited to within lots, so there were no opportunities to misidentify toads to different lots.

Over the three-year period using serial photographs of two boreal toads held in the display tank at NASRF, the belly patterns of both the subject animals remained stable and identifiable as the toads matured from age 1 to age 4 and increased in mass from 2.5 g average to 59.5 g average (Fig. 2). Even over a three-year period, in no instance was there disagreement between two investigators over the identity of these two toads when assessing general spot pattern as well as multiple specific match points.

Twelve timed ID trials were conducted to identify the toads (three toad lots, four investigators/lot). Only one investigator (from the category "experienced") failed to achieve 100% correct identification, and all errors occurred within a single lot of toads. One hundred sixty-nine of 172 (98.3%) toad identifications were correct. Single factor ANOVA indicated that investigator experience was not a significant predictor of the time required to sort a lot of toads into individual containers ($F = 1.87$, $df = 2, 9$, $P = 0.209$) or of accuracy in doing so ($F = 1.00$, $df = 2, 9$, $P = 0.405$). Two lots were judged as being easy to identify because of the large, distinct blotches characterizing these animals, whereas the third lot was more difficult to identify because the bellies were characterized more by freckling than distinct blotches. The time required to sort all the toads into individual containers averaged 9.5 minutes (SE = 0.7) for the easy lots and 17.5 minutes (SE = 3.6) for the more difficult lot.

Discussion.—

Our results supported the use of belly pattern photographs as a viable method to identify individual boreal toads. The belly patterns exhibited a high degree of stability, fully sufficient to remain effective for identification over considerable growth and time intervals even when slight changes to pigment density were apparent (e.g., Fig. 2). While we did not use a secondary method of identification in these studies, we found that it was generally easy to reach identification consensus among investigators when comparing photographs to other photographs or to live toads. Only in rare instances did we have to resort to discussions of specific match points to confirm identifications or to determine a misidentification. The fact that our toads were housed in discrete tanks containing a known number of toads worked to our advantage. There is a possibility that additional error could occur if unknown toads had been introduced to tanks, or if additional photographs of animals not present in the tanks were available. However, staff at NASRF already encounters the latter situation when replacing breeding animals into their assigned tanks after breeding. In such cases the photographs for at least two lots must be used to discriminate the toads and place them where they belong.

Observations of toads over time at CMZ and NASRF strongly suggest there is little likelihood of false identifications of boreal toads resulting from changing patterns as has been observed for other species (Kenyon et al. 2009, Waye 2013). The chance of error is further reduced if the need for individual identification is limited to one or two lots of toads. If false identifications occur, we think it more likely to occur as a result of similar belly patterns. We are aware of just one instance of such a false identification at NASRF, when an animal sent to another institution was identified as a different animal from the same lot (siblings). The mistake was later discovered and corrected. A precaution against such mishaps is the constant participation of a second observer to confirm putative identifications.

Attempts to identify individuals in other amphibian species via pattern recognition work well with species exhibiting distinct blotching that contrast boldly with the body, such as marbled salamander *Ambystoma opacum* (Gamble et al. 2008), yellow bellied toad *Bombina variegata* (Plăiaşu et al. 2005), and fire salamander *Salamandra salamandra* (Carafa and Biondi 2004). Such species compare favorably with the boreal toad in pattern distinctiveness and contrast. Among these investigators only Carafa and Biondi (2004) observed changes in pattern over time, and those were slight enough that they did not preclude identification. However, in at least one other boldly marked species, tiger salamander *Ambystoma tigrinum,* marking patterns have been observed to undergo significant changes over time (Waye 2013). We observed no such dramatic changes in the spot patterns of boreal toads, despite using many more than the 11 animals observed in the Waye (2013) study. Rather, our results were more like those of Gollmann and Gollmann (2011), who found that although the patterns of early yellow bellied toad metamorphs changed and solidified with growth, they could still identify individual toads.

The stability of the belly pattern in boreal toads over considerable growth is similar to that observed in the yellow bellied toad as well. Plăiaşu et al. (2005) were able to photographically identify individuals that transitioned from sub-adult to adult stages, with associated weight changes. This stability may provide advantages to those conducting long term studies of boreal toad populations by allowing entry of individuals into the marked population at a younger age and smaller size than is presently possible, provided our results could be replicated in field situations when new, previously unknown toads were successfully identified as such. Scherff-Norris et al. (2002) recommended PIT tagging boreal toads that weigh a minimum of 10 g, and other investigators have limited PIT tagging to adult-sized boreal toads (>

55mm SVL, Young et al. 2007). Many investigators have not reported minimum sizes tagged, but their primary tagging periods are during and just after breeding seasons so it is likely that tagged toads are predominantly adults (Scherer et al. 2005, 2008, Muths et al. 2006, 2010, Pilliod et al. 2010). In contrast, photographic identification may allow the entry of toads into the marked population as small as 2 g, as in our trial with the two wild-caught toads. This earlier entry into the marked population could be realized even if investigators ultimately wished to use PIT tags on older animals for the benefit of the quicker in-the-field identification.

Another benefit of photographic identification of boreal toads would be as an ideal second mark to evaluate the loss of other marks or tags. Despite the widespread and apparently successful use of PIT tags in boreal toad field studies, we could find no published evidence of a formal evaluation of PIT tag retention in wild boreal toads. That retention is much higher in wild toads than in our captive toads when PIT tags were implanted by skilled individuals is evident, because studies using PIT tags have produced modeled annual apparent survival rates of 0.75 or more (Scherer et al. 2005, Muths et al. 2010, Pilliod et al. 2010). However, Scherer et al. (2005) investigated models that evaluated tagging effects, and indeed tagging effects had support in their data and were included in all top ranked models. Modeled apparent survival was commonly about 0.20 lower for toads in the first year after tagging than in subsequent years. A follow-up investigation that included a new study area also produced one model ranked among the top three that included a first year tagging effect for the new study area (Muths et al. 2006). Unfortunately, deleterious effects of tagging that may have caused mortality could not be separated from the effects of potential short term tag loss in these studies. A second mark allows the separation of tag loss from tagging effects or other factors affecting survival. We believe a study formally evaluating PIT tag retention in boreal toads could be achieved by the pairing of PIT tagging with photographic identification.

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Figures.—

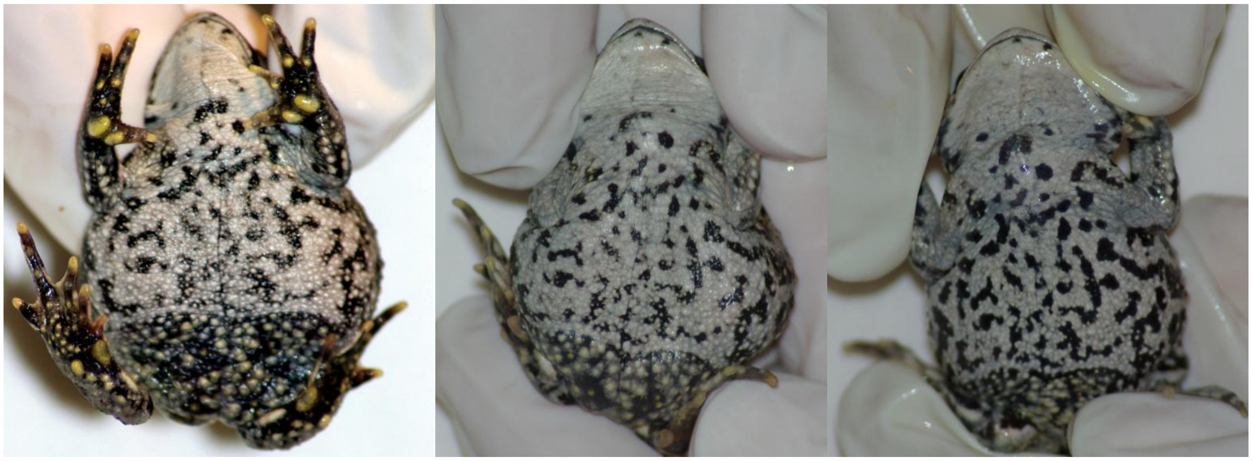

Fig.1. Three photos taken of the same toad at the Cheyenne Mountain Zoo demonstrate pattern stability over 10 months representing the first year of life, in September 2007 (34 mm SVL, 4.2 g, left, age 2 months post-metamorphosis), February 2008 (44 mm SVL, 8.0 g, middle, age 7 months post-metamorphosis), and July 2008 (47 mm SVL, 9.2 g, right, 12 months post-metamorphosis).

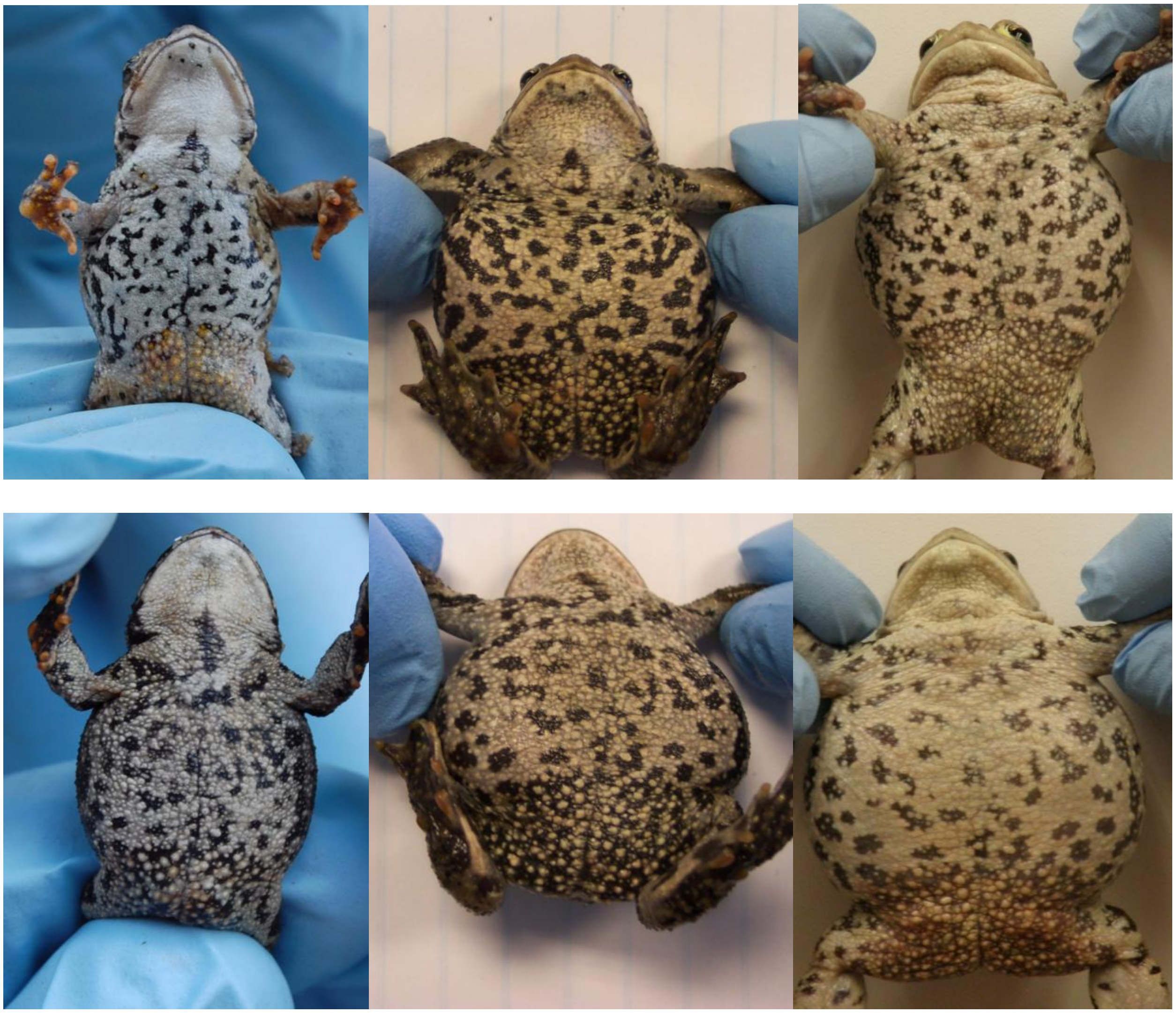

Fig.2. Series of photographs taken at NASRF of two individuals demonstrate pattern stability throughout multiple years of growth. Photographs on the left were taken in September 2009 (average weight 2.5 g, age 13 months post-metamorphosis), middle in March 2010 (average weight 23.8 g, age 19 months post-metamorphosis), and the right in August 2012 (average weight 59.5 g, age 4 years post-metamorphosis).

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