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Pattern-Recognition Software as a Supplemental Method of Identifying Individual Eastern Box Turtles *(Terrapene c. carolina)*

Identifying and monitoring individuals is essential in behavioral and ecological studies of wild animals. Traditional methods for permanently marking turtles include shell notching, tagging with aluminum bands, and insertion of passive integrated transponder (PIT) tags (reviewed in Ferner 2007 and Plummer and Ferner 2012). These methods have been shown to be effective, but applying such marking techniques may result in increased stress, altered behavior, or opportunities for infection (McGregor and Peake 1998; Markowitz et al. 2003; Fisher 2007). Therefore, it is desirable to use methods of long-term identification that minimize the potential impact on the organism.

As technology improves, researchers have taken advantage of photographing naturally occurring or conspicuous marks to identify individuals (reviewed in Reisser et al. 2008 and Bolger et al. 2012). Traditionally, pattern-recognition involved comparing hard copies of photographs against catalogues of photographs to identify recaptures. In the case of large image catalogues, the number of images makes pattern-matching a time-consuming process and increases the probability of visual errors (Hammond et al. 1990; Katonas and Beard 1990; Sears et al. 1990; Gamble et al. 2008).

Shell patterns have been suggested to be a viable means of identifying individual box turtles (Budischak et al. 2006; Wynn and Moody 2006; Weiss 2009; D. E. Hoss, pers. comm. 2013). Photo-recognition techniques offer several advantages such as reduced handling time of the animal, an increase in the efficiency of identifying individuals, and elimination of the problems associated with loss of tags or other artificial marks (Reisser et al. 2008). In addition, pattern recognition is inexpensive, less invasive than most of the traditional methods of permanently marking turtles, and lessens the chance of identity failure due to wear or malfunction. As such, pattern-recognition software has the potential to be a cost-effective alternative method of identifying box turtles.

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At our study site in northwestern Ohio, USA, the required method of marking Eastern Box Turtles (*Terrapene carolina carolina*) is use of PIT tags. The expense of using PIT tags and the readers required to confirm the presence of a PIT tag can quickly eat away at the small grants available to fund local mark-recapture studies. Additionally, this method of marking carries the extra complication of making it impossible to identify recaptures without a PIT tag reader. To aid in future mark-recapture studies and to involve the public in local Eastern Box Turtle conservation efforts, we sought to use digital photography to supplement our use of PIT tags. The goals of our project were to: 1) test pattern recognition as a viable method of identifying individual Eastern Box Turtles; 2) to determining if top-down carapace, off-center carapace, or plastron photos were more diagnostic; and 3) to test the ability of a pattern recognition program to identify individuals from different populations.

Methods.—We collected photographs of Eastern Box Turtles from three areas in two states: the Oak Openings Region (OOR) of northwestern Ohio (41.556°N, 83.854°W) from 2004–2013, Ft. Custer Training Center (FCTC) and the adjacent Ft. Custer Recreation Area (FCRA) in Michigan's southwestern Lower Peninsula (42.324°N, 85.298°W) in 2005 and from 2011–2013, and the Manistee National Forest (MNF; 43.875°N, 85.914°W) from 2009–2013. The impetus for obtaining images from multiple states was to determine whether or not images from different locations would result in false matches. Upon capture, top-down pictures were taken of the turtle's carapace and plastron (N = 610 of each; Ohio = 170; FCTC/FCRA = 307; MNF = 133). Additionally, we used a subset of images (194) taken without standardizing (i.e., pictures taken from any angle; hereafter "off-center") to compare the efficacy of these two methods. A minimum bounding rectangle that reduced the amount of visible background present was used to crop each image. The pictures from all locations were combined into carapace, off-center,

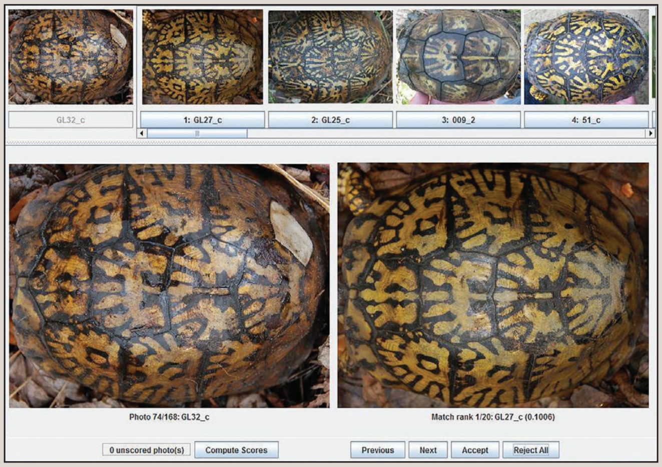

Fig. 1. User interface of WildID showing the focal image (bottom left and first in the row along the top), the active comparison window of the top-ranked marching score (bottom right), and the ranked potential images (top row). Note the loss of pleural scute 4 in the recapture image (active comparison window).

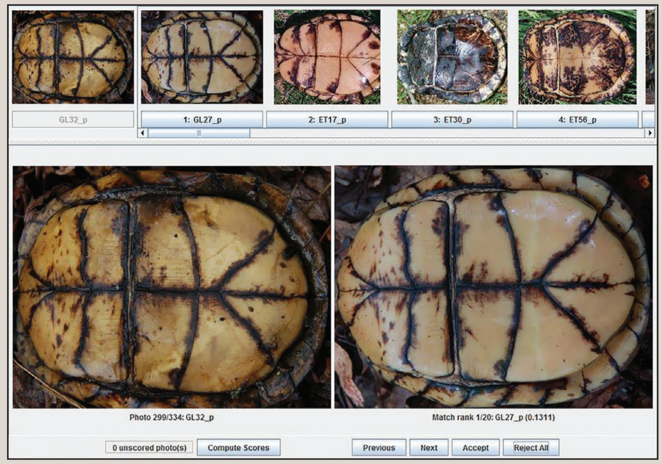

Fig. 2. User interface of WildID showing the focal image (bottom left and first in the row along the top), the active comparison window of the top-ranked marching score (bottom right), and the ranked potential images (top row) for the same turtle in Fig. 1.

and plastron categories within an image database. Turtles with a plastron length less than 7.0 cm were not used because their shell patterns do not appear to be fully developed (D. E. Hoss, pers. comm. 2013).

To identify individual Eastern Box Turtles in our study, we employed the relatively new pattern-recognition software Wild-ID (http://www.dartmouth.edu/~envs/faculty/bolger.html; Bolger et al. 2012). Wild-ID is stand-alone, open-source, multi-platform software that uses Java to implement pattern-recognition. The Wild-ID software uses a Scale Invariant Feature Transform Operator (SIFT; Lowe 2004) to find and extract distinctive features invariant to the scale, rotation, viewpoint, local distortion, and illumination of the image. The geometric arrangements of these SIFT features for each pair of images in the dataset are compared to one another. The program then calculates the goodness-of-fit between the images and assigns a matching score (values range from 0.0000 to 1.0000, where values closer to 1.0000 indicate a stronger match). A full description of these steps can be found in Bolger et al. (2012).

Once Wild-ID has completed the above steps, the user interface displays each focal image along with 20 of the top-ranked images (Figs. 1 and 2). This allows the user to assess the images visually and based on matching scores to conclude whether or not the focal image has a match. Once matching scores were assigned, we used the Kruskal-Wallis procedure and *post hoc* Mann-Whitney U-tests in R (R Development Core Team 2011) to compare carapace, off-center, and plastron matching scores. For this project, a correct response from Wild-ID was recorded if: 1) a successful recapture was identified and 2) if no mismatches between individuals or sites occurred. The individual marking techniques used at the study sites (PIT tags in the OOR; shell notches at FCTC/FCRA and MNF) were used to verify the matches reported by Wild-ID.

Results.—Mean (± SE) recapture matching scores for carapace, off-center, and plastron images were 0.1918 ± 0.0266 for carapace, 0.0396 ± 0.0111 for off-center, and 0.1813 ± 0.0373 for plastron. In no case was the highest-ranked image a different turtle if it was a recapture. Additionally, Wild-ID did not erroneously match images between sampling locations or states and verified recaptures, as confirmed by shell-notching and PIT tagging, at each location. Results of the Kruskal-Wallis tests indicated that there was a significant difference between matching scores of the three image categories ($\chi^2 = 59.91$, df = 2, $P < 0.0001$); *post hoc* tests indicated that there were significant differences in matching scores between carapace and off-center ($P < 0.0001$) and plastron and off-center ($P < 0.0001$), but not between carapace and plastron ($P = 0.6492$).

Discussion.—Overall, Wild-ID performed ideally for identifying Eastern Box Turtle recaptures from carapace and plastron images, but exhibited lower efficacy when off-center images were used. We initially expected plastron images to be less accurate because the plastron occasionally lacks distinguishing patterns (MDC, pers. observ.), but there was no statistical difference between the plastral and carapace images. Plastron images were correctly matched just as often as carapace images and what little pattern may be present was sufficient for the program to identify a match. Because this method has not been tested before with Eastern Box Turtles, we wanted to make sure the program would not mismatch turtles from Ohio with turtles from Michigan; we found no mismatches, suggesting that Wild-ID is in fact suitable for identifying individual adult Eastern Box Turtles from their shell patterns.

Wild-ID represents a cost- and time-effective method of identifying individual eastern box turtles for mark-recapture studies. However, this is not to say that this should be the only means of identifying turtles. For long-lived species like Eastern Box Turtles, whose patterns do not fully develop until later on in life, photo-recognition software alone may not be sufficient for life-long identification and should still be coupled with an additional marking technique until the long-term validity of computer-assisted pattern-recognition has been verified. Additionally, shell damage is a potential source of error, especially in the case of burn scars where a large portion of the shell may be rendered patternless. Photographic mark recapture relies on three conditions: 1) individuals can be photographed, 2) individuals bear some phenotypic pattern variation that easily identifies them from other individuals, 3) an individual's pattern does not vary through time (Bolger et al. 2012). The unique patterns of Eastern Box Turtle shells lend themselves to this method with the exception of the criterion that the pattern remains unchanged.

Many turtles in our study had visible carapace imperfections that appeared to be the result of fires, mower blades, cars, or general wear. Shell damage may influence the results of programs such as Wild-ID, but as we saw with several of our turtles, as long as a majority of the pattern remained, Wild-ID could identify the image as a match. While carapace injuries and subsequent pattern alterations are relatively common, we observed that the plastron pattern remained relatively unchanged in most individuals. Whereas carapace images have been suggested as the minimum requirement to identify a turtle (Wynn and Moody 2006), based on our results, we strongly suggest photographing the plastron as a secondary form of identification, particularly in the case of individuals with damaged carapaces.

Although Wild-ID surpassed our expectations for pattern-recognition with Eastern Box Turtles, we noted that there are a number of ways future studies can improve the probability of the program identifying a positive match. We identified soil and glare on the turtle's shell and amount of background as potential factors contributing to lower matching scores. We recommend that turtles be photographed on a uniform background (e.g., a blank piece of paper) in an area that reduces the amount of glare and shadow on the shell and that soil be cleaned from the shell prior to image-capture. Similarly, for studies specifically seeking to accurately record recaptures, we recommend using cameras with the same resolution. Glare, patterns marred with soil, and images with a disparity in resolution can all lead to reduced matching scores. The SIFT operator, however, will still work with whatever pattern is present in the image (D. Bolger, pers. comm. 2013). Standardizing the method of image-capture in the manner we suggest should lead to increased matching scores, but is not necessary and will depend on the goals of the study. For instance, if the goal of the project is to reduce the amount of handling, off-center photos could be used as long as photos are taken from approximately the same angle. Again, as there is currently no way to confirm a correct identification prior to the development of a pattern on the shell, this method should be considered a supplement to traditional marking techniques.

We encourage researchers and naturalists who have taken photographs of Eastern Box Turtles in the past to utilize this software as a means of rapidly analyzing historical photographs to identify recaptured individuals. This sort of recognition software, combined with citizen-science programs (e.g., Davidson College Herpetology Laboratory's Box Turtle Mark-Recapture Program; Hester et al. 2008), could provide the means for mark-recapture studies of Eastern Box Turtles over a large geographic range.

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Literature Cited

Bolger, D. T., T. A. Morrison, B. Vance, D. Lee, and H. Farid. 2012. A computer-assisted system for photographic mark-recapture analysis. Methods Ecol. Evol. 3:813–822.

Budischak, S. A., J. M. Hester, S. J. Price, and M. E. Dorcas. 2006. Natural history of *Terrapene carolina* (box turtles) in an urbanized landscape. Southeast. Nat. 5:191–204.

Ferner, J. W. 2007. A review of marking and individual recognition techniques for amphibians and reptiles. Herpetol. Circ. No. 35, Society for the Study of Amphibians and Reptiles. 72 pp.

Fisher, A. R. 2007. Turtle assemblages in the eastern panhandle of West Virginia with an emphasis on *Pseudemys rubriventris* (LeConte). M.S. Thesis, Marshall University, Huntington, West Virginia. 75 pp.

Gamble, L., S. Ravela, and K. McGargial. 2008. Multi-scale features for identifying individuals in large biological databases: an application of pattern recognition technology to the marbled salamander, *Ambystoma opacum*. J. Appl. Ecol. 45:170–180.

Hammond, P. S., S. A. Mizroch, and G. P. Donvan. 1990. Individual recognition of cetaceans: use of photo-identification and other techniques to estimate population parameters. Rep. Int. Whal. Commn., Special Issue 12:3–17.

Hester, J. M., S. A. Budischak, and M. E. Dorcas. 2008. The Davidson College box turtle mark-recapture program: urban herpetological research made possible by citizen scientists. *In* J. C. Mitchell, R. E. Jung, and B. Bartholomew (eds.), Urban Herpetology: Conservation and Management of Amphibians and Reptiles in Urban and Suburban Environments. Volume 3, pp. 549–555. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah.

Katonas, K., and J. A. Beard. 1990. Population size, migrations and feeding aggregations of the humpback whale (*Megaptera novaeangliae*) in the western North Atlantic Ocean. Rep. Int. Whal. Commn., Special Issue 12:295–305.

Lowe, D. 2004. Distinctive image features from scale-invariant keypoints. Int. J. Comput. Vis. 60:91–110.

Markowitz, T. M., A. D. Harlin, and B. Würsig. 2003. Digital photography improves efficiency of individual dolphin identification. Mar. Mamm. Sci. 19:217–223.

McGregor, P., and T. Peake. 1998. The role of individual identification in conservation biology. *In* T. M. Caro, (ed.), Behavioral Ecology and Conservation Biology, pp. 31–55. Oxford University Press, New York.

Plummer, M. V., and J. W. Ferner. 2012. Marking Reptiles. *In* R.W. McDiarmid, M.S. Foster, C. Guyer, J. W. Gibbons, and N. Chernoff (eds.), Reptile Biodiversity: Standard Methods for Inventorying and Monitoring, pp. 143–150. University of California Press, Berkeley, California.

R Development Core Team. 2011. R: A Language Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Reisser, J., M. Proietti, P. Kinas, and I. Sazima. 2008. Photographic identification of sea turtles: method description and validation, with an estimation of tag loss. Endangered Species Research 5:73–82.

Sears, R. J., J. M. Williamson, F. W. Wenzel, M. Berube, D. Gendron, and P. Jones. 1990. Photographic identification of the blue whale (*Balaenoptera musculus*) in the Gulf of St. Lawrence, Canada. Rep. Int. Whal. Commn., Special Issue 12:335–342.

Weiss, J. A. 2009. Demographics, activity, and habitat selection of the eastern box turtle (*Terrapene c. carolina*) in West Virginia. M.S. Thesis, Marshall University, Huntington, West Virginia. 96 pp.

Wynn, D. E., and S. M. Moody. 2006. Ohio Turtle, Lizard, and Snake Atlas. Ohio Biological Survey, Columbus, Ohio. 81 pp.