# **CANADIAN WILDLIFE BIOLOGY & MANAGEMENT**



CWBM 2021: Volume 10, Number 2

ISSN: 1929-3100

**Original Research** 

# Using The Blanding's Turtle (*Emydoidea blandingii*) Plastron as A 'Fingerprint': Photo Identification of An Endangered Species

Chantel E. MARKLE<sup>1,2</sup>, Timothy LAW<sup>2</sup>, Hope C. A. FREEMAN<sup>1</sup>, Brennan CAVERHILL<sup>3</sup>, Christina M. DAVY<sup>4,5</sup>, Jeff HATHAWAY<sup>6</sup>, Jeffie McNEIL<sup>3</sup>, Kelsey MOXLEY<sup>6</sup>, Sarah RICHER<sup>7</sup>, and Patricia CHOW-FRASER<sup>2</sup>

<sup>1</sup>McMaster University, School of Earth, 1280 Main Street West, Hamilton, Ontario, L8S 4K1, Canada. Email: <u>marklece@mcmaster.ca</u>

<sup>2</sup>McMaster University, Department of Biology, 1280 Main Street West, Hamilton, Ontario, L8S 4K1, Canada.

<sup>3</sup>Mersey Tobeatic Research Institute, 9 Mount Merritt Rd, Kempt, Nova Scotia, B0T 1B0, Canada.

<sup>4</sup>Wildlife Research and Monitoring Section, 2140 East Bank Drive, Peterborough, Ontario, K9L 1Z8, Canada.

<sup>5</sup>Trent University, Department of Biology, 1600 West Bank Drive, Peterborough, Ontario, K9L 0G2, Canada.

<sup>6</sup>Scales Nature Park, 82 Line 15 South, Oro-Medonte, Ontario, L3V 8H9, Canada.

<sup>7</sup>Royal Botanical Gardens, 680 Plains Road West, Burlington, Ontario, L7T 4H4, Canada.

# Abstract

The ability to uniquely identify individuals is critical to estimating and monitoring trends in population sizes, one of the key metrics used to evaluate a species' conservation status and success of mitigation strategies. For freshwater turtles, shell notching and/or passive integrated transponder (PIT) tags are commonly used to mark individuals. However, because notch codes and PIT tags can be lost over time and require more invasive procedures, we explored if photographs offer an effective method to reliably identify individuals. The Blanding's turtle (*Emydoidea blandingii*) is a globally endangered species with distinct black and yellow markings on its plastron. We used the I<sup>3</sup>S Pattern software with custom parameters to classify patterns on Blanding's turtle plastrons and to identify individuals. We

*Correspondence:* Chantel E. Markle, McMaster University, School of Earth, 1280 Main Street West, Hamilton, Ontario, L8S 4K1, Canada. Email: <u>marklece@mcmaster.ca</u>

analyzed 826 plastron images from 707 individual Blanding's turtles taken between 1998 and 2019 from 12 study areas distributed throughout their Canadian range. When plastron photos were pooled across the sampled range (i.e., all study areas), there was an 84% probability of correctly identifying an individual turtle within the top 3 suggested matches, whereas when identifying Blanding's turtles within a specific study area, identification accuracy was 82% in Central Ontario and 97% in Nova Scotia. Individual identification from plastron markings did not work well in areas where iron staining obscured the plastron pattern or for hatchlings and juveniles whose patterns changed over time. For example, the only misclassification in the Nova Scotia study area was for a turtle with photos through various life stages. In areas without iron staining, plastron photo identification offers a cost-effective, non-invasive method to identify individual adult Blanding's turtles to support population monitoring and community science initiatives, and has the potential to assist with range-wide coordination to counteract illegal wildlife trade.

**Key Words**: Blanding's Turtle; Digital Photographs; *Emydoidea blandingii*; Mark-recapture; Pattern Analysis; Photo Identification; Plastron; Species at Risk.

# Introduction

The ability to uniquely identify individuals is critical to estimating and monitoring long-term trends in population sizes. Monitoring change in population size is one of the key metrics used to evaluate a species' conservation status and to assess the success of rehabilitation and mitigation strategies. Common approaches for estimating survival rates, capture rates, population sizes, and recruitment rely on capturing and recapturing marked individuals (Jolly 1965; Seber 1965). Mark-recapture analyses assume that animals do not lose their marks and that marking does not change the probability of capture (e.g., painting numbers on a turtle's carapace making them more visible), but when these conditions are violated, it can have serious effects on resulting estimations (Arnason and Mills 1981). Since individual animals must be followed through time and re-identified, a variety of methods are employed to mark different species. Marking techniques include but are not limited to shell notching (Cagle 1939), painting (Brown et al. 1984; Koper and Brooks 1997), passive integrated transponder (PIT) tagging (Buhlmann and Tuberville 1998; Gibbons and Andrews 2004), and leg bands (Marion and Shamis 1977). Although marking techniques are meant to be permanent, shell notches can fade, be misread, or become damaged (e.g., shell chips), paint requires reapplication (Koper and Brooks 1997), and PIT tags can be lost or migrate (e.g., Roark and Dorcas 2000; Feldheim et al. 2002; Wyneken et al. 2010). Thus, there has been considerable interest in using natural patterns and markings as a method for identifying individuals (e.g., Pennycuick 1978).

Many species have distinct markings that can act as a "fingerprint" to allow for individual identification (e.g., Jackson *et al.* 2006; Speed *et al.* 2007; Harihar *et al.* 2010), which could reduce the need for invasive marking techniques and provide a cost-effective approach to population monitoring (Morrison *et al.* 2011). Moreover, photographs

of individuals can be stored in a digital database and used to support landscape-level monitoring across a species' range. Identifications can be made visually (e.g., Jackson et al. 2006; Harihar et al. 2010), although manual identification becomes difficult with increasing database size. Therefore, more automated identification processes are required to facilitate landscape-level population studies and provide opportunities for collaborative datasets among researchers, community scientists, law enforcement, and conservation practitioners. For example, photo identification of individuals has been used for a wide range of species such as sharks (Van Tienhoven et al. 2007; Holmberg et al. 2009), rays (González-Ramos et al. 2017), insects (Caci et al. 2013), reptiles (Knox et al. 2012; Moro and MacAulay 2014; Bauwens et al. 2017), amphibians (Hoque et al. 2011) and mammals (Kelly 2001; Hiby et al. 2009; Halloran et al. 2015). Although data from photo identification has been used to estimate population trajectory (Holmberg et al. 2009) and survival estimates (Morrison et al. 2011), accurate identification of individuals is key for monitoring endangered species as misclassifications can result in inflated population estimates (Suriyamongkol and Mali 2018; Johansson et al. 2020).

Reliable marking techniques are especially critical when monitoring long-lived species such as freshwater turtles. Photo identification has been used to identify individuals with fairly good success in freshwater turtle species such as *Chrysemys picta belli* (Cooley *et al.* 2013), *Terrapene carolina carolina* (Cross *et al.* 2014), *Trachemys scripta elegans* (Janzen *et al.* 2000a, b), *Chelydra serpentina* (Kolbe and Janzen 2001), and *Pseudemys gorzgui* (Suriyamongkol and Mali 2018); however, success was limited to adult turtles (e.g., Cross *et al.* 2014) or within a season for species whose pattern changes or fades over time (Janzen *et al.* 2000a, b; Kolbe and Janzen 2001; Suriyamongkol and Mali 2018). In *Clemmys guttata*, the plastron pattern appeared unique to turtles studied in Pennsylvania while changes to the spot pattern on the carapace of some individuals were observed (Gray 2008). Although carapace spot patterns may be unique to adult individuals, they were unable to identify a turtles' origin population (Brown and Davy 2021). Overall, the accuracy of photo identification for many species of freshwater turtle has made photo identification a successful marking method in many studies (e.g., Janzen *et al.* 2000a, b; Kolbe and Janzen 2001; Cross *et al.* 2014; Suriyamongkol and Mali 2018).

Similar to other freshwater turtles, the Blanding's turtle (Emydoidea blandingii) also has seemingly unique markings on its plastron that may permit individual identification. The Blanding's turtle is considered Endangered globally (Rhodin et al. 2018) and in Canada (COSEWIC 2016; ECCC 2018) where it is legally protected in all provinces where it occurs (Nova Scotia Endangered Species Act 2000; Québec Endangered Species Act 2002; Ontario Endangered Species Act 2007). Blanding's turtles are a semi-aquatic species that use a variety of wetland and upland habitats across their range (e.g., Ross and Anderson 1990; McMaster and Herman 2000; Joyal et al. 2001; Beaudry et al. 2009; Ernst and Lovich 2009; Millar and Blouin-Demers 2011; Markle and Chow-Fraser 2014) and are known to make long overland movements (> 6 km, Edge et al. 2010). As a result of anthropogenic activities, many populations of Blanding's turtle across their range are in decline and geographically isolated (COSEWIC 2016). The longevity of Blanding's turtles (confirmed to live beyond 83 years, Congdon et al. 1993, 2001; COSEWIC 2016) combined with their potential for relatively long-distance movements further emphasizes the importance of viable, long-term identification techniques especially given that large-scale population monitoring is a vital component to managing extant populations.

The purpose of our study was to determine if the pattern on the Blanding's turtle plastron could be used to uniquely identify individuals. We combined plastron photos from different study areas across Canada to determine if plastron patterns accurately identify individual turtles (1) within a distinct study area and (2) among all sampled study areas. We systematically varied the default input parameters in the identification software and conducted a sensitivity analysis to identify custom parameters that optimized Blanding's turtle identification accuracy. We also examined plastron photos collected over multiple years to assess plastron pattern stability and retention because patterns may change during maturation from hatching to adult and over time for adult turtles.

Materials & Methods Photographic data

We created a digital database of 826 Blanding's turtle plastron photos from 707 individuals that were photographed between 1998 and 2019, and that represented Blanding's turtles from 12 study areas distributed throughout Ontario and Nova Scotia in their Canadian range. Approximately 50 additional plastron photos were collected but did not get included in the digital database due to extremely poor photo quality (e.g., blurry, dark). Turtles were captured by hand, with dip nets, or in baited hoop traps. Each turtle's plastron was photographed (camera specifications vary widely) before release at their initial capture sites. For all plastron photos, the actual individual identification was known based on a notch code system (e.g., Cagle 1939) which was required for conducting accuracy assessments. Age class of each individual turtles was also provided by the contributing group. We used the entire database (826 photos of 707 individuals) to determine if the plastron pattern of a Blanding's turtle could be used to accurately identify the individual turtle when compared to plastrons across a wide range of study areas. We also had a sufficient number of plastron photos of re-captured Blanding's turtles from a study area in central Ontario (395 images of 342 individuals) and Nova Scotia (108 images from 57 individuals) to evaluate accuracy within each of the 2 study areas.

#### Photo analysis

We used the Pattern software package (Version 4.0.2) in the Interactive Individual Identification System (I<sup>3</sup>S, den Hartog and Reijns 2014) to classify and identify patterns on Blanding's turtle plastrons. I<sup>3</sup>S Pattern is an open-source program developed with Java 1.4.2 and C++ (Van Tienhoven *et al.* 2007, http://www.reijns.com/i3s, accessed November 2019). For each plastron photo, we identified 3 reference points to permit 2-dimensional transformation to allow images to be comparable (Van Tienhoven *et al.* 2007). We defined the 3 reference points as the top of the plastron where the gular scutes meet and the bottom of the left and right anal scutes (Figure 1) because they were reliably identifiable in each photograph and covered most of the plastron (den Hartog and Reijns 2014).

After the user manually identified the reference points and delineated the plastron (approximately 1 min depending on user speed), 35 key points are automatically extracted in I<sup>3</sup>S. Each set of key points were used as a fingerprint to compare individual turtles (< 10 s, duration may vary based on database size and computer specifications). Key points were represented as circles on the plastron (see Figure 1), which were compared between each pair of individuals in the database. A distance metric was calculated in I<sup>3</sup>S, which is the sum of the distances between each key point pair divided by the square of the number of key point pairs (Van Tienhoven *et al.* 2007; den Hartog and Reijns 2014).



Figure 1. A fully annotated image of a Blanding's turtle (*Emydoidea blandingii*) plastron. Reference points A, B, and C are located at the top of the plastron where the gular scutes meet and the bottom of the left and right anal scutes, respectively. The outer edge of the plastron is the delineated region of interest (green circles and line). The key points are automatically extracted by I<sup>3</sup>S Pattern (red circles and numbers) and is the first step to quantifying the plastron pattern.

Potential matches are then scored where the most likely match receives the lowest score. The top 10 most likely matches with the lowest scores are presented in order and a split-screen allows the user to visually compare the unknown turtle with the probable matches. The user either selects a match (i.e., the turtle is a recapture) or enters the turtle as a new individual. Because the user manually confirms a match (or enters a new individual), the more often the correct identification is presented in the top few suggestions, the quicker the identification process is in the field (using a tablet) or in the lab. Although we only relied on plastron photos for identification in this study, the program also allows for additional information to be stored such as sex, deformities, and biometric data to assist with individual identification and monitoring turtle growth. Prior to key point extraction, I<sup>3</sup>S Pattern converts the colour photo to grayscale. The grayscale image is created from a sum of the luminance values from the red, green and blue channels, which are each weighted by a conversion value and summed to 1. Adjusting the weights will change the emphasis on different colours. For example, if the pattern is mostly green, increasing the weight of the green channel can increase the contrast of the grayscale image and improve key point extraction. Since I<sup>3</sup>S Pattern was tested for pattern identification of facial scutes on green sea turtles (*Chelonia mydas*, see den Hartog and Reijns 2014), we altered the red, green, and blue conversion values and the number of key points to customize pattern identification for Blanding's turtle plastrons. Using a subset of plastron photos to

Table 1. The percentage of individual Blanding's turtles ( <i>Emydoidea blandingii</i> ) correctly identified in the testing dataset
(simple evaluation; 625 images of 551 individuals) based on plastron pattern when using the default red, green, and blue
conversion values compared to the custom values calibrated for Blanding's turtles.

	Conversion Values			Percentage of correctly identified turtles				
	Red	Green	Blue	Top 1	Top 2	Top 3		
Default	0.299	0.587	0.114	82.8	88.3	93.0		
Custom	0.466	0.333	0.201	87.5	93.0	94.5		

minimize processing time (395 photos of 335 individuals; the first set of plastron photos compiled at the start of the project), we varied the number of key points in intervals of 5, starting with the default of 35 points and ending when the accumulated score no longer decreased. For conversion values, we varied the weights in intervals of 10%, starting with an even weighting and ending when the accumulated score no longer decreased, and we tested two channels simultaneously while holding the third channel constant. We also conducted a sensitivity analysis to determine if the number of key points extracted or the conversion values (red, green, blue) influenced identification accuracy. Finally, we used a larger testing dataset (625 photos of 551 individuals) to compare the accuracy of the default parameters to the custom parameters selected during the sensitivity analyses.

We used the simple and elaborate evaluation tools within I<sup>3</sup>S Pattern to determine the accuracy of identifying Blanding's turtles based on their plastron pattern. The purpose of simple evaluation is to determine if individuals can be accurately identified using the software. For example, the simple evaluation method compares multiple photos of the same turtle to determine if accuracy of the identification changes over time and how variable an individual's pattern is during the sampling period. We recorded the percentage of time that the correct identification was made within the top 1, 3, 5, and 10 suggested matches. The elaborate evaluation simulates the real-life addition of a plastron photo of a new or recaptured turtle into an existing database. To conduct this assessment, 1 reference photo per individual was randomly selected and compared to the remaining photos (den Hartog and Reijns 2014). The random selection of a reference photo was repeated over 10,000 iterations to negate random effects. Percentage of correct turtle identifications within the top 1, 3, 5 and 10 suggested matches were calculated and recorded.

# Results

Although plastron photos in the compiled database were all pre-labelled with the individual turtle identification to facilitate accuracy assessments, we found that for turtles with multiple recaptures, 17% contained a mis-labelled photo (e.g., duplicate notch code, mis-read notch code, data entry error). Mis-labelled turtles were given new identification codes and accuracy assessments were re-run.

We increased the likelihood of identifying the correct Blanding's turtle by 5% when using the custom red, green, and blue conversion values (Table 1). Compared to the default program parameters, there was a 56% increase in the red value weight, 76% increase in the blue value weight, and 43% decrease in the green value weight (Appendix 1). Altering the number of key points from the default did not increase the accuracy of the software when identifying Blanding's turtles; therefore, we used the default setting to extract 35 key points. Identification of all individual Blanding's turtles and accuracy assessments were conducted using our custom parameters (Appendix 1).

Individual identification accuracy was highest (82.4– 96.9%) in study areas with no iron staining and when there were multiple, high-quality photos per adult turtle (e.g., our sites in central Ontario and Nova Scotia; Table 2). Identification accuracy was lowest (77.3–87.7%) when sites had iron staining (e.g., our sites in southern Ontario; Figure 3) and when hatchlings or juveniles had grown since the reference photos were taken (Figure 4). Across all 12 study areas, including those with iron staining, different photos of the same turtle were correctly identified in the top 3 suggested matches 77.3–87.7% of time according to simple evaluation (Table 2; Figure 2) and 84% of the time with elaborate evaluation (Table 2; Figure 3). When comparing accuracy within study areas without extensive iron staining, Table 2. The percentage of individual Blanding's turtles (*Emydoidea blandingii*) correctly identified by plastron pattern across all study areas (826 images of 707 individuals), in the central Ontario study area (395 images of 342 individuals), and in the Nova Scotia study area (108 images from 57 individuals) using simple and elaborate evaluations (1 reference image for elaborate evaluation). Simple evaluation compares multiple images of the same turtle to determine how accurately individuals can be identified by plastron pattern and provides context regarding pattern consistency through time. Elaborate evaluation simulates the real-life addition of an unknown turtle into an existing database and the likelihood of a correct identification based on plastron pattern.

		Percentage of correctly identified turtles					
		Top I	Top 2	Top 3	Top 5	Top 10	
	Simple	77.3	84.2	87.7	90.6	93.1	
All study areas	Elaborate	73.0	81.0	84.0	86.4	89.9	
C	Simple	72.9	81.3	83.3	90.6	94.8	
Central Ontario	Elaborate	70.0	79.3	82.4	88.6	94.0	
No. Conto	Simple	92.9	97.6	98.8	98.8	100.0	
Nova Scotia	Elaborate	91.3	96.2	96.9	97.9	99.0	



Figure 2. Example of a consistent adult Blanding's turtle (*Emydoidea blandingii*) plastron pattern successfully used for image-based identification. This individual adult Blanding's turtle was photographed 4 years apart in 1998 (a) and 2002 (b).



Figure 3. Comparison of 5 adult Blanding's turtle (*Emydoidea blandingii*) plastron patterns among different individuals from 5 study areas throughout Canada (a–e). Example of 3 different adult Blanding's turtles with significant iron staining on plastron which obscures pattern necessary for identification (f–h).

the program correctly matched different photos of the same turtle with 72.9–83.3% and 92.9–98.8% accuracy in the top 3 suggested matches within central Ontario and Nova Scotia, respectively (Table 2; simple evaluation).

We had an 82.4% and 96.9% probability that the correct Blanding's turtle was identified within the top 3 suggested matches within central Ontario and Nova Scotia, respectively (Table 2; elaborate evaluation). The Nova Scotia study area had the highest number of individual Blanding's turtle with multiple photographs over time (mean  $\pm$  SD = 1.9  $\pm$  1.1, range 1–6) and the highest classification accuracy with a 91% probability of correctly identifying an individual in the first suggested match (Table 2). In comparison, the Central Ontario study area had a smaller number of recaptures (1.0  $\pm$  0.22, range 1–3) and it required 5 suggested matches to exceed a classification accuracy of 88% (Table 2).

#### **Discussion**

When assessing Blanding's turtle plastron pattern uniqueness across all 12 study areas, we found that different photos of the same turtle are within the top 3 suggested matches 88% of the time (Table 2). When we simulated the addition of an unidentified turtle into the pooled database, there was an 84% probability that the correct Blanding's turtle would be identified within the top 3 suggested matches. These findings highlight the uniqueness of many adult Blanding's turtle plastron across a large part of their Canadian range and the potential for photo identification as a method for supporting large-scale monitoring across multiple study areas (Table 2; Figure 3a–e). When combining plastron photos from across a large region, we found that misidentifications primarily occurred when the plastron pattern was obscured by iron staining. In particular,



Figure 4. Example of plastron pattern changes in an individual Blanding's turtle (*Emydoidea blandingii*) across 14 years (2001–2015) as they mature from a juvenile (a, b), subadult (c), then an adult (d). Example of 3 different hatchling Blanding's turtles (e–g) with indistinguishable plastron patterns and patterns which are not retained with age.

there were study areas where a majority of the photographed individuals had almost no discernable plastron pattern (e.g., Figure 3f-h), limiting the application of this method to study areas without iron staining. However, where water chemistry did not obscure the pattern, the uniqueness of a Blanding's turtle plastron also meant that we only needed to use a single reference photo per individual turtle in the database (up to 5 photos can be used) which enables most individuals to be identified when they are first photographed. For example, in Nova Scotia, only a single plastron photo was required to identify an individual Blanding's turtle whose recaptures were 13 years apart. Nevertheless, photographing individuals across multiple years remains important as identification accuracy increases as reference photos are added to the database (Van Tienhoven et al. 2007; Moro and MacAulay 2014).

In study areas where significant iron staining was not present and sample size was sufficiently high, the probability of correctly identifying an adult Blanding's turtle was 82.4% (Central Ontario) and 96.9% (Nova Scotia; Table 2). The higher overall accuracy for Nova Scotia was likely due to the inclusion of multiple clear, high-quality photos of the same individual over many years which provided a large catalogue of reference photos. The uniqueness of the plastron patterns and the large catalogue of photos resulted in a 91% probability of correctly identifying an individual from the Nova Scotia study area in the first suggested match. Despite excluding photos that were extremely poor quality, photos with some blemishes (e.g., shadows, low-light, plastron photographed on an angle) were still retained in the Central Ontario database and did lead to misidentifications. Because identification accuracy typical increases as additional highquality photos are added to the database (Van Tienhoven et al. 2007; Moro and MacAulay 2014), it is important that the plastron of each turtle be photographed on an annual basis or whenever it is encountered. We recommend that photos are taken in bright natural light with the camera parallel to the plastron (i.e., not an angle that might obscure some details or alter the shape of the plastron markings), and ensure the photo is free from glare or shadows. The plastron should take up most of the photo field and be completely unobstructed (e.g., remove dirt, if necessary). Cataloguing high quality photos (e.g., Figure 1) will improve individual identification and is a key step in providing a long-term database to aid in the identification and monitoring of Blanding's turtles.

In addition to pattern uniqueness, pattern stability and retention are necessary assumptions for image-based identification (Vincent et al. 2001). Although we found that instances of lower identification accuracy were due to iron staining and poor photo quality, identification errors also occurred because younger juvenile and adult plastron patterns for the same individual did not match (Figure 4). In fact, the only misidentification in the Nova Scotia study area was for a single Blanding's turtle who was photographed twice as a juvenile (2001, 2002), once as a sub-adult (2007), and once as an adult (2015). Interestingly, there was another sub-adult that was correctly identified but the photos were only taken a year apart and the pattern had remained consistent enough to permit identification. Overall, similar to juvenile Glyptemys insculpta (Cowin and Cebek 2006), pattern recognition is not suitable for identifying Blanding's turtle hatchlings or younger juveniles since patterns change during these life stages (Figure 2; Figure 4). Although we did not have the data required to pinpoint the exact age at which the plastron pattern becomes stable, Blanding's turtles that are near-maturity appear to retain most of its pattern (Figure 2). Moreover, adult Blanding's turtle plastron patterns do not appear to fade or depigment with age as observed in adult Glyptemys insculpta (Jones 2009). Therefore, we found that adult Blanding's turtle plastron patterns are consistent enough over time for image-based identification (Figure 2; Figure 4) and, in the Nova Scotia study area, all adult Blanding's turtles were correctly identified. For populations where plastron patterns are undistinguishable due to iron staining (see Figure 3), future work could investigate the possibility of identifying individuals based on their scute suture lines, lip banding pattern, or carapace pattern. Furthermore, a similar approach to this study could be applied to other species of freshwater turtles such as identifying Chrysemys picta marginata based on their plastron suture lines and Clemmys guttata based on their plastron or carapace patterns (Gray 2008, but see Brown and Davy 2021).

We did not use any image preprocessing so users have the option of photographing a Blanding's turtle plastron in the field and using the I<sup>3</sup>S software to immediately identify if the individual has been previously captured. Including a preprocessing step to automatically delineate the plastron would require additional computational power and time, and

this may not be available on a field tablet or desirable under field conditions. Furthermore, since the source code and our modifications are freely available, an android/iOS app can be developed to make the process more convenient and seamless by enabling immediate individual identification in the field. The final step of the identification process requires the user to select the correct plastron match (or add the new individual to the database) based on the list of top possible matches provided. In our study, the correct match appeared in the top 3 options 82-97% of the time, making this is a relatively quick step. A benefit to this manual step is the ability for the user to see beyond photo blemishes such as leeches, leaves, shadows, glare, etc. which can obscure parts of the plastron pattern. This manual check was also useful when we used older photos of lower quality (e.g., Figure 2a) or when photos were blurry or taken in low-light conditions (e.g., in the evenings during nesting surveys) since photo quality significantly affects identification accuracy (e.g., Speed et al. 2007). However, this manual step could introduce inter- or intra-observer identification errors which were not evaluated in this study. Using the program option to store and refer to additional information such as sex, deformities, and biometric data could be useful when identifying individuals with more obscure plastron patterns thereby reducing identification errors.

Individual identification through pattern recognition provides benefits over passive integrated transponder tagging, notching, and painting since the latter marking options can be lost or fade through time, require additional technician training, are more invasive, and are subject to errors (notch codes can be misread or duplicated). Here, we found that 1 in every 6 turtles with multiple plastron photos had a mis-labelled photograph possibly due to duplicate notch codes, misread notch codes, and photo labelling errors. For populations without significant iron staining and high individual identification accuracy (i.e., 97% accuracy in Nova Scotia study area), plastron photo identification offers a low-cost method to identify and monitor adult Blanding's turtles which is essential for a species that can live longer than 83 years (Congdon et al. 2001). However, identification accuracy is critical to modeling population dynamics, and long-term mark recapture studies likely require identification accuracies higher than found in some study areas (i.e., 82% accuracy in Central Ontario) and the ability to mark juveniles. In these populations, plastron photo identification is likely not sufficient to replace other marking techniques but can be an important complimentary method to verify turtle identification and support population monitoring. Especially for a long-lived species like the Blanding's turtle, plastron photos can be extremely beneficial for identification of turtles caught and photographed in years where marking with

other methods was limited or to fill out capture histories in data sets with imperfect marking. It is also important to note that replacing marking techniques such as scute notching may not be desirable in areas where notches are suspected to make the individual less desirable in the illegal wildlife trade markets. Therefore, in these study areas, pairing pattern recognition with traditional marking methods would ensure the most accurate data for population monitoring.

In addition to supporting population monitoring, identifying adult Blanding's turtles with plastron photos can also support community science initiatives and has the potential to assist with combatting illegal wildlife trade. In particular, the expansion of community science programs has greatly increased our ability to collect valuable data and track biodiversity metrics over large spatial scales (e.g., iNaturalist 2019; Ontario Nature 2019). Image identification can be a useful component to existing outreach programs where community members partner with local research groups to participate in surveys under the appropriate permits (e.g., Scales Nature Park, Ontario, Canada; Kejimkujik Area Stewardship Program, Nova Scotia, Canada). Image identification could also be valuable when individuals are confiscated from the illegal wildlife trade markets. In many cases, the exact origin of the confiscated individual turtle is unknown which makes reintroduction unlikely and, consequently, individuals are placed in zoos or breeding programs (Turtle Conservation Fund 2002). With coordinated image-identification databases, however, a photographed plastron could help identify the turtle to its population as long as its plastron has been previously photographed.

## Acknowledgements

We thank the many students and technicians who assisted in collecting the plastron images and Kevin Chau for helping with image cataloguing. We thank Alanna Smolarz for providing plastron images and feedback on an early draft. We are grateful to Paul Yannuzzi for providing plastron images. Funding was provided in part by the Royal Bank of Canada Blue Water Foundation Project (awarded to Chantel Markle and Patricia Chow-Fraser). All animal handling was conducted under approved institutional and/or provincial animal care (e.g., Animal Utilization Protocols 11-02-05, 13-02-07, 14-09-35), provincial and/or federal species at risk (e.g., ESA M-102-6326447; AY-B-005-13; SARA-OR-2014-0260; WSCA 1076122, 1073523), and research area permits. We are grateful to 2 anonymous reviewers and Damien Mullin for thoroughly reviewing our paper and providing helpful feedback.

CEM and PC-F formulated the study. CEM, KM, BC, CMD, JH, JM, PY, AS, SR collected the data. CEM, HF, and TL analyzed the data. CEM led the writing of the manuscript with assistance from TL, HF, and CMD. All authors contributed critically to the draft manuscripts and gave final approval for publication.

### **Literature Cited**

- Arnason, A. N., and K. H. Mills. 1981. Bias and loss of precision due to tag loss in Jolly–Seber estimates for markrecapture experiments. Canadian Journal of Fisheries and Aquatic Sciences 38: 1077–1095.
- Bauwens, D., K. Claus, and J. Mergeay. 2017. Genotyping validates photo-identification by the head scale pattern in a large population of the European adder (*Vipera berus*). Ecology and Evolution 8: 2985–2992.
- Beaudry, F., P. G. deMaynadier, and M. L. Hunter. 2009. Seasonally dynamic habitat use by spotted (*Clemmys guttata*) and Blanding's turtles (*Emydoidea blandingii*) in Maine. Journal of Herpetology 43: 636–645.
- Brown, L. J., and C. M. Davy. 2021. Evaluation of potential information available from spot pattens and carapace abnormalities of an endangered freshwater turtle (*Clemmys guttata*). Endangered Species Research doi: 10.3354/esr01120.
- **Brown, W. S., V. P. J. Gannon, and D. M. Secoy. 1984.** Paint-marking the rattle of rattlesnakes. Herpetological Review 15: 75–76.
- **Buhlmann, K. A., and T. D. Tuberville. 1998.** Use of passive integrated transponder (PIT) tags for marking small freshwater turtles. Chelonian Conservation and Biology 3: 102–104.
- Caci, G., A. B. Biscaccianti, L. Cistrone, L. Bosso, A. P. Garonna, and D. Russo. 2013. Spotting the right spot: computer-aided individual identification of the threatened cerambycid beetle *Rosalia alpina*. Journal of Insect Conservation 17: 787–795.
- **Cagle, F. R. 1939.** A system of marking turtles for future identification. American Society of Ichthyologists and Herpetologists 1939: 170–173.
- Congdon, J. D., A. E. Dunham, and R. C. van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's Turtles (*Emydoidea blandingii*): Implications for conservation and management of long-lived organisms. Conservation Biology 7: 826–833.
- Congdon, J. D., R. D. Nagle, O. M. Kinney, and R. C. van Loben Sels. 2001. Hypotheses of aging in a long-lived vertebrate, Blanding's turtle (*Emydoidea blandingii*). Experimental Gerontology 36: 813–827.

- **Cooley, C., S. Smith, C. Geier, and T. Puentes. 2013.** The use of photo-identification as a means of identifying western painted turtles (Chrysemys picta bellii) in long-term demographic studies. Herpetological Review 44: 430–432.
- **Committee on the Status of Endangered Wildlife in Canada [COSEWIC]. 2016.** COSEWIC assessment and status report on the Blanding's turtle *Emydoidea blandingii*, Nova Scotia population and Great Lakes/St. Lawrence population in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario, Canada.
- **Cowin, S., and J. Cebek. 2006.** Feasibility of using plastron markings in young wood turtles (*Glyptemys insculpta*) as a technique for identifying individuals. Herpetological Review 37: 305–307.
- Cross, M. D., G. J. Lipps Jr., J. M. Sapak, E. J. Tobin, and K. V. Root. 2014. Pattern-recognition software as a supplemental method of identifying individual eastern box turtles (Terrapene c. Carolina). Herpetological Review 45: 584–586.
- **den Hartog, J., and R. Reijns. 2014.** I<sup>3</sup>S pattern manual: interactive individual identification system. Available at: www.reijns.com/i3. Accessed July 2017.
- Edge, C. B., B. D. Steinberg, R. J. Brooks, and J. D. Litzgus. 2010. Habitat selection by Blanding's turtles (*Emydoidea blandingii*) in a relatively pristine landscape. Ecoscience 17:90–99.
- Environment and Climate Change Canada [ECCC]. 2018. Recovery strategy for the Blanding's turtle (*Emydoidea blandingii*), Great Lakes/St. Lawrence population, in Canada. Species at Risk Act Recovery Strategy Series. Environment and Climate Change Canada, Ottawa, Canada.
- Feldheim, K. A., S. H. Gruber, J. R. C. de Marignac, and M. Z. Ashley. 2002. Genetic tagging to determine passive integrated transponder tag loss in lemon sharks. Journal of Fish Biology 61: 1309–1313.
- Gibbons, W. J., and K. M. Andrews. 2004. PIT tagging: simple technology at its best. BioScience 54: 447–454.
- González-Ramos, M. S., A. Santos-Moreno, E. F. Rosas-Alquicira, and G. Fuentes-Mascorro. 2017. Validation of photo-identification as a mark-recapture method in the spotted eagle ray *Aetobatus narinari*. Journal of Fish Biology 90: 1021–1030.
- **Gray, B. S. 2008.** A study of carapace and plastron patterns in the spotted turtle, *Clemmys guttata*, and their use as a technique for individual recognition. Bulletin of the Chicago Herpetological Society 43: 109–114.
- Halloran, K. M., J. D. Murdoch, and M. S. Becker. 2015. Applying computer-aided photo-identification to messy datasets: a case study of Thornicroft's giraffe (*Giraffa*

*camelopardalis thornicrofti*). African Journal of Ecology 53: 147–155.

- Harihar, A., M. Ghosh, M. Fernandes, B. Pandav, and S.
  P. Goyal. 2010. Use of photographic capture-recapture sampling to estimate density of Striped Hyena (*Hyaena hyaena*): implications for conservation. Mammalia 74: 83–87.
- Hiby, L., P. Lovell, N. Patil, N. S. Kumar, A. M. Gopalaswamy, and K. U. Karanth. 2009. A tiger cannot change its stripes: using a three-dimensional model to match images of living tigers and tiger skins. Biology Letters 5: 383–386.
- Holmberg, J., B. Norman, and Z. Arzoumanian. 2009. Estimating population size, structure, and residency time for whale sharks *Rhincodon typus* through collaborative photo-identification. Endangered Species Research 7: 39– 53.
- **Hoque, S., M. A. H. B. Azhar, and F. Deravi. 2011.** ZOOMETRICS – biometric identification of wildlife using natural body marks. International Journal of Bio-Science and Bio-Technology 3: 45–53.
- iNaturalist. 2019. iNaturalist. http://inaturalist.org. Accessed November 2019.
- Jackson, R. M., J. D. Roe, R. Wangchuk, and D. O. Hunter. 2006. Estimating snow leopard population abundance using photography and capture-recapture techniques. Wildlife Society Bulletin 34: 772–781.
- Janzen, F. J., J. K. Tucker, and G. L. Paukstis. 2000a. Experimental analysis of an early life-history stage: avian predation selects for larger body size of hatchling turtles. Journal of Evolutionary Biology 13: 947–954.
- Janzen, F. J., J. K. Tucker, and G. L. Paukstis. 2000b. Experimental analysis of an early life-history stage: selection on size of hatchling turtles. Ecology 81: 2290– 2304.
- Johansson, Ö., G. Samelius, E. Wikberg, G. Chapron, C. Mishra, and M. Low. 2020. Identification errors in camera-trap studies result in systematic population overestimation. Scientific Reports 10: 1–10.
- **Jolly, G. M. 1965.** Explicit estimates from capture-recapture data with both death and immigration—stochastic model. Biometrika 52: 225–247.
- Jones, M. T. 2009. Spatial ecology, population structure, and conservation of the wood turtle, *Glyptemys insculpta*, in Central New England. PhD dissertation, University of Massachusetts Amherst, Massachusetts, USA.
- **Joyal, L. A., M. McCollough, and M. L. Hunter Jr. 2001.** Landscape ecology approaches to wetland species conservation: a case study of two turtle species in southern Maine. Conservation biology 15: 1755–1762.

- Kelly, M. J. 2001. Computer-aided photograph matching in studies using individual identification: an example from Serengeti cheetahs. Journal of Mammalogy 82: 440–449.
- **Knox, C. D., A. Cree, and P. J. Seddon. 2012.** Accurate identification of individual geckos (*Naultinus gemmeus*) through dorsal pattern differentiation. New Zealand Journal of Ecology 37: 60–66.
- Kolbe, J. J., and F. J. Janzen. 2001. The influence of propagule size and maternal nest-site selection on survival and behavior of neonate turtles. Functional Ecology 15: 772–781.
- **Koper, N., and R. J. Brooks. 1997.** Population-size estimators and unequal catchability in painted turtles. Canadian Journal of Zoology 76: 458–465.
- Marion, W. R., and J. D. Shamis. 1977. An annotated bibliography of bird marking techniques. Bird-Banding 48: 42–61.
- Markle, C. E., and P. Chow-Fraser. 2014. Habitat selection by the Blanding's turtle (*Emydoidea blandingii*) on a protected island in Georgian Bay, Lake Huron. Chelonian Conservation and Biology 13: 216–226.
- McMaster, N., and T. B. Herman. 2000. Occurrence, habitat selection and movement patterns in juvenile Blanding's turtles (*Emydoidea blandingii*) in Kejimkujik National Park, Nova Scotia. Chelonian Conservation and Biology 3: 602–610.
- Millar, C. S., and G. Blouin-Demers. 2011. Spatial ecology and seasonal activity of Blanding's turtles (*Emydoidea blandingii*) in Ontario, Canada. Journal of Herpetology 45: 370–378.
- **Moro, D., and I. MacAulay. 2014.** Computer-aided pattern recognition of large reptiles as a noninvasive application to identify individuals. Journal of Applied Animal Welfare Sciences 17: 125–135.
- Morrison, T. A., J. Yoshizaki, J. D. Nichols, and D. T. Bolger. 2011. Estimating survival in photographic capture-recapture studies: overcoming misidentification error. Methods in Ecology and Evolution 2: 454–463.
- Nova Scotia Endangered Species Act. 2000. Nova Scotia Endangered Species Act, 1998, c. 11, s. 1, Halifax, Canada.
- **Ontario Endangered Species Act. 2007.** Endangered Species Act, 2007, S.O. 2007, c. 6., Toronto, Canada.
- **Ontario Nature. 2019.** Ontario Reptile and Amphibian Atlas. http://ontarionature.org/programs.citizen-science/reptile-amphibian-atlas/. Accessed November 2019.
- **Pennycuick, C. J. 1978.** Identification using natural markings. Pages 147–159 *in* B. Stonehouse, editor. Animal marking. MacMillan, London, United Kingdom.

- **Québec Endangered Species Act. 2002.** Québec Act Respecting the Conservation and Development of Wildlife, 2002, C-61.1, c. 82, s. 1., Québec.
- Rhodin, A. G., C. B. Stanford, P. P. Van Dijk, C. Eisemberg, L. Luiselli, R. A. Mittermeier, R. Hudson, B. D. Horne, E. V. Goode, G. Kuchling, A. Walde, E. H.W. Baard, K. H. Berry, A. Bertolero, T. E.G. Blanck, R. Bour, K. A. Buhlmann, L. J. Cayot, S. Collett, A. Currylow I. Das, T. Diagne, J. R. Ennen, G. Forero-Medina, M. G. Frankel, U. Fritz, G. García, J. W. Gibbons, P. M. Gibbons, G. Shiping, J. Guntoro, M. D. Hofmeyr, J. B. Iverson, A. R. Kiester, M. Lau, D. P. Lawson, J. E. Lovich, E. O. Moll, V. P. Páez, R. Palomo-Ramos, K. Platt, S. G. Platt, P. C. H. Pritchard, H. R. Quinn, S. C. Rahman, S. T. Randrianjafizanaka, J. Schaffer, W. Selman, H. B. Shaffer, D. S. K. Sharma, S. Haitao, S. Singh, R. Spencer, K. Stannard, S. Sutcliffe, S. Thomson, and R. C. Vogt. 2018. Global conservation status of turtles and tortoises (order Testudines). Chelonian Conservation and Biology 17: 135–161.
- **Roark, A. W., and M. E. Dorcas. 2000.** Regional body temperature variation in corn snakes measured using temperature-sensitive passive integrated transponders. Journal of Herpetology 34: 481–485.
- Ross, D. A., and R. K. Anderson. 1990. Habitat use, movements, and nesting of *Emydoidea blandingii* in central Wisconsin. Journal of Herpetology 24: 6–12.
- Seber, G. A. F. 1965. A note on the multiple-recapture census. Biometrika 52: 249–259.
- **Speed, C. W., M. G. Meekan, and C. J. A. Bradshaw. 2007.** Spot the match – wildlife photo-identification using information theory. Frontiers in Zoology 4: 1–11.
- Suriyamongkol, T., and I. Mali. 2018. Feasibility of using computer-assisted software for recognizing individual Rio Grande Cooter (*Pseudemys gorzugi*). Copeia 106: 646–651.
- Turtle Conservation Fund. 2002. A global action plan for conservation of tortoises and freshwater turtles. Strategy and funding prospectus 2002–2007. Conservation International and Chelonian Research Foundation. Washington, DC, USA.
- Van Tienhoven, A. M., J. E. den Hartog, R. A. Reijns, and V. M. Peddemors. 2007. A computer-aided program for pattern-matching of natural marks on the spotted raggedtooth shark *Carcharias taurus*. Journal of Applied Ecology 44: 273–280.
- Vincent, C., L. Meyner, and V. Ridoux. 2001. Photoidentification in grey seals: legibility and stability of natural markings. Mammalia 65: 363–372.

#### MARKLE et al.

Wyneken, J. E., S. P. Epperly, B. Higgins, E. R. McMichael, C. O. Merigo, and J. P. Flanagan. 2010. PIT tag migration in sea turtle flippers. Herpetological Review 41: 448–454.

# **About the Authors**

Dr. Chantel Markle is a postdoctoral research fellow in the



School of Earth at McMaster University in Ontario, Canada. Her research focuses on spatial ecology of freshwater turtles and use of remote sensing and geographic information systems for landscape-level conservation of at-risk reptiles. Current studies focus on mapping habitat suitability and occupancy for at-risk reptiles, ecohydrology of reptile habitat, and examining how changes in climate and land-use

impact the availability and resilience of critical habitats.

Timothy Law is a Master's student in the Department of Biology Concordia at University in Montréal, Canada. His research focuses on understanding the drivers freshwater shaping fish communities across large environmental gradients. Current projects are aimed at



understanding the role of abiotic versus biotic processes in shaping freshwater fish communities.

Hope Freeman is a Master's student in the School of Earth, Society, and Environment at McMaster University in Ontario, Canada. Her research focuses on the ecohydrological controls in freshwater turtle nesting and overwintering habitat along the coast of Georgian Bay to inform habitat restoration strategies.

Brennan Caverhill is

biologist, photographer, and teacher. Born and raised in the Maritimes, he attended Acadia University for his BSc and MSc degrees in Conservation Biology, and the University of Toronto for his Education degree. For over a decade he worked for

a

universities, governments, and nonprofit organizations conducting research and engaging communities in

biodiversity conservation. His past work for Parks Canada, the Mersey Tobeatic Research Institute, the Toronto Zoo, and the Royal Ontario Museum involved a blend of science, art, and education to promote the conservation of Canada's



Species at Risk. Brennan is currently a classroom teacher in a downtown Toronto elementary school, focused on STEAM (Science, Technology, Engineering, the Arts and Mathematics) in education.

Dr. Christina Davy is an Assistant Professor in the

Department of Biology at Carleton University. Her studies research group conservation of small and declining wildlife populations impacted by emerging infectious diseases and anthropogenic environmental change.



**Jeff Hathaway** is the founder of Scales Nature Park, a

conservation centre focused on Canadian reptiles. He leads field projects primarily in central Ontario and on Pelee Island, that have marked thousands of turtles, generated legal protection for over 2700 km<sup>2</sup> of habitat, and released

39,000+ turtle hatchlings. Jeff strives to use research results, new techniques and community engagement at a landscape scale for effective conservation and recovery of species at risk.

**Jeffie McNeil** is a Co-Director and Species at Risk Biologist at the Mersey Tobeatic Research Institute (MTRI). She began working with



Blanding's turtles in the mid-1990s when she did her honor's thesis at Acadia University, followed by her MSc thesis. Upon graduation she continued working with Blanding's turtles, eastern ribbonsnakes and other reptiles at risk as recovery coordinator. She is a co-chair of the NS Reptile and Amphibian Recovery Team and manages the provincial reptiles databases.

#### MARKLE et al.

**Kelsey Moxley** is the Field Projects Manager for Scales Nature Park and the Georgian Bay Turtle Hospital, overseeing all aspects of field work for our projects across Ontario. She directly coordinates the Saving Turtles at Risk

Today (START) project in central Ontario, which works to reduce or mitigate threats, monitor populations and trends, protect habitat, and engage the community in species at risk conservation. Under her guidance, START



has grown into the largest project of its kind in Canada. Kelsey has also been extensively involved in public education and nature interpretation.

Sarah Richer began as Royal Botanical Garden's Species at Risk Biologist in January 2016, and brings 18 years of



experience with stewardship, restoration, conservation, and invasive species management in both pristine and heavily urbanized habitat. Her background spans a diverse range of locations and organizations, including the Ontario Ministry of Natural Resources and Forestry; Canadian Wildlife Service; Ducks Unlimited Canada; Wye Marsh Wildlife Centre; Algonquin

Provincial Park; and various consulting companies; with the

bulk of her experience dealing directly with species at risk birds, reptiles, and plants. She was the Ecologist Team Leader at Pukaskwa National Park and was lead ecologist for baseline pre-development surveys on Canada's largest wind power project on First Nations land at Henvey Inlet First Nations. She currently implements the Site-Specific Turtle Recovery Plan at the Royal Botanical Gardens in Hamilton, Ontario as their Species at Risk Biologist.

**Dr. Patricia Chow-Fraser** is Professor in the Department of Biology, where she teaches ecology, and conducts research on conservation and restoration of aquatic ecosystems,

primarily in the coastal zone of the Laurentian Great Lakes. A primary goal of her research is to develop indicators to monitor impacts of human activities on the long-term health of wetlands, streams and forest ecosystems. Another goal is to increase the capacity for citizen science and to fully engage and empower students in outreach opportunities.



Received 26 March 2021 – Accepted 17 September 2021

APPENDIX 1. The matrix of red (R) and green (G) conversion values tested and the resulting accumulated score. Blue (B) conversion values are calculated with the equation B = 1 - R - G. The lowest accumulated score indicates that plastrons of the same individual were matched more accurately and is denoted with an asterisk. Conversion values were tested with a subset of plastron images (395 images, 335 individuals). Custom conversion values used to identify Blanding's turtle based on their plastron pattern were 0.466 (red), 0.333 (green), and 0.201 (blue).

	i.	0.166	0.199	0.233	0.266	0.299	0.333	0.366	0.399	0.432	0.466	0.499
0. 0. 0.	0.166	2256	2183	1908	1870	1765	1679	1825	1765	1761	1705	1593
	0.199	2304	2200	1837	1847	1673	1722	2072	1752	1688	1689	1622
	0.233	2235	1856	1898	1776	1749	1881	1840	1830	1747	1703	1582
	0.266	2133	1960	1746	1704	1759	1789	1757	1860	1777	1614	1601
	0.299	1928	1969	1762	1814	1722	1709	1842	1839	1835	1610	1585
	0.333	2009	2006	1780	1842	1797	1824	1912	1799	1600	1526*	1605
	0.366	1731	1837	1851	1850	1935	1905	1934	1880	1651	1592	1571
	0.399	1785	1866	1779	1800	1939	1855	1857	1809	1625	1572	1690
,	0.432	1874	1984	1805	1992	1910	1951	1833	1773	1734	1726	1710
	0.466	1949	1939	1861	1952	1912	1911	1755	1720	1744	1722	1710
	0.499	1986	1912	1966	1842	1957	1886	1728	1684	1689	1741	1713