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Original Research

Can You Tell the Turtle by its Eggshell?

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Abstract

Species at risk in Canada receive different levels of habitat protection depending on their status under the federal Species at Risk Act (e.g., Endangered or Threatened, vs. Special Concern). Accurate detection of species presence can facilitate appropriate habitat protection. For example, the Blanding's turtle (*Emydoidea blandingii*; Threatened, COSEWIC 2016) receives legislated habitat protection for its nesting sites on federal lands throughout its Canadian range, and on Crown and private lands in Ontario under the provincial Endangered Species Act. Nesting sites of the sympatric northern map turtle (*Graptemys geographica*; Special Concern, COSEWIC 2012) do not receive the same legislative protection. The eggs of these 2 species look very similar, making nest identification challenging. In this study we tested whether external egg morphology can reliably identify these species' eggs, using measurements from 13 Blanding's turtle clutches (143 eggs) and 72 northern map turtle clutches (873 eggs). Although the mean length, width and volume of eggs differed significantly between the species, strong overlap in the ranges of these measurements prevent reliable identification to species. Alternative methods for egg identification include incubation and identification of the hatchlings, scanning electron microscopy of eggshell fragments, or eDNA analysis of swabs taken from the surface of recently laid eggs.

Key Words: Blanding's turtle; Egg Morphology; *Emydoidea blandingii*; Habitat Protection; *Graptemys geographica*; Maternal Investment; Northern Map Turtle; Species at Risk.

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INTRODUCTION

In southern Canada, high concentrations of species at risk occur on landscapes dominated by urbanization and intensive agriculture (Walters and Shrubsole 2003; Kharouba *et al.* 2008). The federal Species at Risk Act (SARA 2002) provides legal protection for Threatened and Endangered species and their habitats, with similar protection provided by the province of Ontario under its Endangered Species Act (ESA 2007). The SARA protects critical habitat of species at risk on federal land, while the ESA provides habitat protection on Crown (provincial) and private land (OMNRF, undated). Habitat protection may be triggered by the observation of an at-risk species at a site, followed by identification of critical habitat, i.e., features that are required for that species to complete its life processes.

Long-lived species such as turtles that exhibit late maturity and low recruitment may be particularly vulnerable to additive mortality from threats such as road mortality, increased predation, poaching, or interactions with fishing gear (Brooks *et al.* 1991; Congdon *et al.* 1993; Garber and Burger 1995; Klemens 2000; Millar and Blouin-Demers 2012). Habitat protection is an essential component in protecting and recovering turtle populations. Protection can be applied to core habitats such as large wetlands, and to nesting sites, which may be located outside of a turtle's core home range (e.g., Obbard and Brooks 1980; Standing *et al.* 1999). Female turtles are vulnerable during movements to and from nesting sites, and during the nesting process (Haxton 2000). Protection of nest sites and their surrounding habitats allows females to nest successfully, and hatchlings to survive emergence from the nest and dispersal to suitable aquatic habitat (Environment Canada 2016).

Turtle nests may be encountered incidentally during activities such as road construction projects, where anthropogenic substrates such as gravel road shoulders inadvertently provide nesting opportunities. In these cases, correctly identifying the eggs to species can clarify the required habitat protection for the site. However, egg identification is challenging in some cases. Some sympatric species produce similar-looking eggs but receive different habitat protection based on their status under SARA and the ESA.

The Blanding's turtle (*Emydoidea blandingii*; Threatened) and northern map turtle (*Graptemys geographica*; Special Concern) both lay large, ovoid eggs (Ernst and Lovich 2009). The eggshells can be differentiated by microscopic examination of shell fragments, which allows identification of predated nests. However, this approach requires destruction of an egg that may contain a viable embryo if no predated eggs are available (Saumure and Bonin 1998). Differentiating between the eggs of these sympatric species is relevant to their management and protection because Blanding's turtle nests qualify for habitat protection under SARA and the ESA, while northern map turtle nests do not. Under the Ontario ESA, an area of 30 m around Blanding's turtle nesting sites is identified as protected nesting habitat, while the proposed Critical Habitat in the draft federal recovery strategy for Blanding's includes a buffer of 150 m around a Blanding's turtle nest (Environment Canada 2016).

In this study, we tested whether the eggs of Blanding's turtles and northern map turtles could be reliably distinguished based on external morphology, without harming developing embryos. If so, eggs could be used to rapidly confirm the presence of Blanding's and northern map turtles, thereby increasing detection probabilities, informing range estimates, and facilitating the appropriate level of habitat protection for each species. We quantified and compared the size and shape of Blanding's turtle and northern map turtle eggs to explore what external morphological characteristics could best distinguish the 2 groups.

MATERIALS AND METHODS

Egg collection

We collected nests of Blanding's turtles and northern map turtles from 5 June to 14 July, 2017, during an ongoing turtle conservation project on the north shore of Lake Erie (Rondeau Provincial Park, Chatham-Kent, Ontario). This work was approved by Trent University's Animal Care Committee (protocol #24900) and the OMNRF's Wildlife Animal Care Committee (protocol #17-291), and was conducted under a Research Authorization from Ontario Parks. We also measured a subset of eggs incubated at the Ontario Turtle Conservation Centre (OTCC) in Selwyn, Ontario. These eggs were harvested from female Blanding's turtles and northern map turtles that were hit by vehicles and brought to the OTCC between 19 June and 20 July, 2017. These females laid their eggs naturally at the OTCC, if possible, or the eggs were removed during surgery if their injuries were too severe. Eggs were incubated at Rondeau Provincial Park and at the OTCC in plastic tubs with a substrate of damp vermiculite, mixed 1:1 by weight with dechlorinated water. Water was added to tubs weekly to maintain hydric conditions, maintaining the eggs' initial sizes and shapes.

During the second half of the incubation period, we measured the length and width of each egg to the nearest 0.1 mm using an electronic calliper. Volume of every egg was estimated using the equation $V = \pi r^2 l$, where r is egg radius and l is the length. We also examined differences in egg shape by comparing the ratio of egg length to width among eggs.

Statistical analyses

We compared each measurement of egg size and shape between species using generalized linear mixed effects models with a Gaussian distribution (*lme* within the "nlme" package; Pinheiro *et al.* 2016) in R statistical software (version 3.4.3; R Core Team 2017). Maternal effects on egg size and shape have been well documented in freshwater turtles (Beck and Beck 2005; Janzen and Warner 2009). To control for different egg size or shape among clutches related to maternal effects, clutch ID (n = 85) was included as a random effect. Clutch size (the number of eggs laid within each clutch) was included as a numerical covariate to control for potential influence of clutch size on egg size. Finally, we produced kernel density histograms to evaluate whether the variables we measured could discriminate between the 2 species.

RESULTS

On average, Blanding's turtle eggs were wider ($F_{1,83}$=6.01, P=0.016) and longer ($F_{1,83}$=21.08, P<0.0001) than northern map turtle eggs (Table 1). These differences resulted in greater average volume for Blanding's turtle eggs compared to northern map turtle eggs ($F_{1,83}$=13.12, P<0.001; Table 1). There was no difference in egg shape (ratio of egg length to width) between the species ($F_{1,83}$=2.74, P=0.101). Despite the statistically significant differences in the average egg length, width, and volume, the distribution of the measurements overlapped strongly and none could reliably discriminate between the 2 species (Figure 1).

DISCUSSION

Our results demonstrate that eggs of Blanding's turtles and northern map turtles cannot be reliably distinguished based on size or shape. Thus, when intact turtle nests containing large, ovoid eggs are found in the field, they cannot be immediately, non-invasively identified as belonging to 1 species or the other. In parts of Canada where Blanding's and northern map turtles are sympatric, a precautionary approach to encountering turtle nests with large, ovoid eggs in the field should assume that the nest might be that of a Blanding's turtle, pending confirmation by other means.

Given that eggs of these 2 species cannot be identified based on external morphology, what other options are available to ecological and wildlife management professionals? Below, we summarize alternate methods for egg identification that could be used where Blanding's and northern map turtles are sympatric.

MANAGEMENT CONSIDERATIONS

The simplest approach to identify eggs to species is to monitor the nest through incubation and identify the hatchlings. Predator exclusion cages, nest translocation or artificial incubation (Stancyk *et al.* 1980; Congdon *et al.* 1993; Glowacki and Kuhns 2010; Hernandez *et al.* 2010; Paterson *et al.* 2013; Riley and Litzgus 2013; Buzuleciu *et al.* 2015) could be used until hatchlings emerge and species can be confirmed. The hatchlings can then be released into suitable habitat near the original nest site. However, Blanding's and northern map turtle eggs typically require at least 60 d to incubate, so this method risks delaying the appropriate level of habitat protection.

Table 1. Size and shape (mean ± standard deviation for each variable) of eggs laid by Blanding's turtles (*Emydoidea blandingii*; 13 clutches, 143 eggs) and northern map turtles (*Graptemys geographica*; 72 clutches, 873 eggs).

Variable	Blanding's turtle	Northern map turtle
Width (mm)	24.75 ± 1.85	23.53 ± 1.44
Length (mm)	37.0 ± 1.89	34.44 ± 2.06
Volume (mL)	23.95 ± 4.13	20.10 ± 3.08
Shape (Length/Width)	1.50 ± 0.10	1.47 ± 0.10

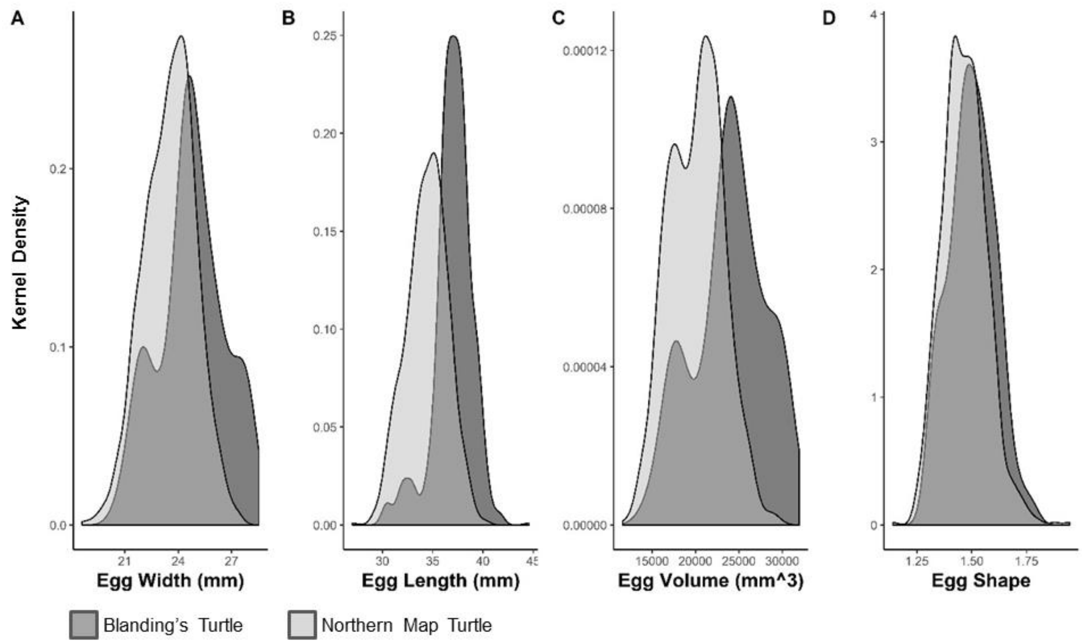

Figure 1. Kernel density estimate plot illustrating substantial overlap between the size and shape of Blanding's turtle eggs (13 clutches, 143 eggs) and northern map turtle eggs (72 clutches, 873 eggs), based on (A) egg width, (B) egg length, (C) egg volume, and (D) egg shape (egg length/egg width).

If time is limited or incubation facilities are not available, scanning electron microscopy of eggshells could be used to rapidly confirm species (Saumure and Bonin 1998). Blanding's turtle eggshells are heavily calcified and rigid, whereas northern map turtle eggs are typically leathery in texture and more flexible. The difference is not apparent when handling intact eggs, but it can be detected by microscopic examination of eggshells. This method is best suited to predated nests where shell fragments are observed, because application to an intact nest would require destruction of an egg. In some cases, it may be appropriate to sacrifice a single egg for identification, if the result will have wider implications (for example, if a construction project is in progress and might need to rapidly implement mitigation measures during work on critical Blanding's turtle habitat).

Environmental DNA (eDNA) could provide another tool for rapid identification of eggs. Blanding's turtle and northern map turtle eDNA have been detected from water samples (Jerde *et al.* 2011; Davy *et al.* 2015), and appropriate primers exist for both species. The egg surface is likely to contain intact DNA from the mother's oviduct, although this DNA likely degrades during incubation. Recently laid eggs could be swabbed in situ for identification, reducing the impact on the developing eggs. Before applying this method in the field, it would be wise to test the temporal variation in detection success during incubation (i.e., test whether eDNA assays targeting maternal DNA on the egg surface become less successful as the egg ages and the maternal DNA degrades).

In summary, it is disappointing that nests of Blanding's and northern map turtles cannot be reliably identified without destroying or hatching the eggs, but the overlap in egg size is important to understand so that nests can be protected as effectively as possible. When a Blanding's or northern map turtle nest is found in a region where both species occur, we recommend a conservative, precautionary approach that assumes potential presence of the Threatened Blanding's turtle until confirmation is possible. If possible, maintaining the integrity of the site until the nest has been positively identified will allow implementation of appropriate habitat protection measures for the correct species.

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