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Nesting Biology and Conservation of a Northern Population of Spiny Softshell Turtles (*Apalone spinifera*)

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Abstract.—**The Lake Champlain population of Spiny Softshell Turtle (*Apalone spinifera*) is isolated at the northeastern end of the range of the species in North America. Due to a lack of suitable habitat, nesting is confined to a limited number of sites, including one along a Canadian tributary river: Rivière aux Brochets, Québec. High egg mortality is a conservation concern for this population; however, little is known about its nesting ecology. We observed nesting behavior from 2003–2016 and recorded environmental variables from 2009–2016 at this site. We monitored hatching success of 75 clutches, *in situ* and *ex situ*, and measured morphology of 836 laboratory-incubated hatchlings. Nesting activity occurred mostly in June between 1100–2000. We observed a second clutch within a season four times, laid by two females. Egg laying was more likely on days when the difference between air and water temperatures was smaller. Air temperature, water temperature, cloud cover, and precipitation were not correlated with the probability of egg laying, while high river discharge inhibited egg-laying activities. Nesting behavior and clutch and hatchling characteristics were similar to what has been reported by previous studies. Artificial incubation more than doubled hatching success. Based on our results, we believe that *ex situ* egg incubation is a useful conservation tool for freshwater turtles in critical situations, in locations where hatching success is naturally low, and threats cannot be easily and rapidly mitigated**.

Key Words.—Canada; environment; hatching success; incubation; nesting; Québec

Résumé.—**La population de tortues molles à épines (*Apalone spinifera*) du lac Champlain est isolée à l'extrémité nord-est de l'aire de distribution de l'espèce. En raison d'un manque d'habitat convenable, la ponte est limitée à un nombre restreint de sites, dont un sur le bord d'un tributaire canadien: la Rivière aux Brochets, Québec. La mortalité élevée des œufs est une préoccupation pour la conservation de cette population dont l'écologie de la nidification est peu connue. Nous avons observé le comportement de nidification des femelles en 2003–2016 et mesuré des variables environnementales en 2009–2016 à ce site. Nous avons enregistré le succès d'éclosion de 75 nichées, *in situ* et *ex situ*, et pris des mesures morphologiques sur 836 nouveau-nés incubés en captivité. La ponte avait lieu principalement en juin, entre 1100–2000. Deux femelles sont revenues au site pondre une seconde fois dans la même saison, à quatre occasions au total. Les femelles étaient plus susceptibles de pondre lors des journées où l'écart de température entre l'air et l'eau était plus faible. Les températures de l'air et de l'eau, la couverture nuageuse et les précipitations n'étaient pas corrélées à la probabilité de ponte, alors qu'un débit élevé de la rivière inhibait les activités de ponte. Le comportement de nidification et les caractéristiques des œufs et des nouveau-nés étaient similaires à ce qui est rapporté par des études antérieures. L'incubation artificielle a plus que doublé le taux d'éclosion. En se basant sur nos résultats, nous croyons que l'incubation *ex situ* est un outil de conservation utile pour les tortues d'eau douce en contexte critique, dans les sites où le succès d'éclosion est naturellement bas et où les menaces ne peuvent être atténuées facilement et rapidement.**

Mots clés.—Canada; environnement; incubation; nidification; Québec; succès d'éclosion

Introduction

Freshwater turtles are slow-maturing and long-lived species. Populations with limited recruitment may seem to fare well for a while but will eventually face an unsustainable age structure (Klemens 2000; Browne and Hecnar 2007). Nesting, incubation, and hatching are critical steps in the recruitment process (Kuchling 1999; Moll and Moll 2004) and nesting behavior impacts hatching success (Kuchling 1999). Additionally, environmental factors can modulate the timing of nesting activities and affect hatching success (Iverson et al. 1993; Packard et al. 1993; Kuchling 1999; Moll and Moll 2004). A good understanding of those

Figure 1. Spiny Softshell Turtle (*Apalone spinifera*) male hatchling, released in Rivière aux Brochets, Québec, Canada, after *ex situ* incubation of the nest.

factors is necessary to inform conservation practices, especially when dealing with endangered populations.

The Spiny Softshell Turtle (*Apalone spinifera*; Fig. 1) is the most widely distributed trionychid in North America, but some populations are isolated and endangered in Canada (Galois et al. 2002; Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2016). In Québec, Canada, the Eastern Spiny Softshell Turtle (*A. s. spinifera*) is found only in Lake Champlain and its tributaries (Daigle et al. 2002; Galois et al. 2002). Historical or rare observations exist for two other rivers in southern Québec, from which they are considered extirpated (Équipe de rétablissement des tortues du Québec 2014; COSEWIC 2016). The Lake Champlain population is shared with two U.S. states, Vermont and New York, and is separated by more than 200 km from the nearest population located in Lake Ontario. Due to increasing concern for the long-term survival of this population, the species has been recognized as threatened at both provincial and federal levels (Gouvernement du Québec. 2010. Liste des espèces fauniques menacées ou vulnérables du Québec: Tortue-molle à épines. Available from www3.mffp.gouv.qc.ca/faune/especes/menacees/fiche.asp?noEsp=9 [Accessed 5 December 2018]; Environment Canada. 2017. Species at Risk Public Registry: Species Profile Spiny Softshell. Available from https://tinyurl.com/y3ootzql [Accessed 8 December 2018]). The decline of the species in Canada has been attributed to fishing, injuries from boating activities, poaching, excessive nest predation, loss of suitable habitat due to major shoreline modification, and flooding of nesting sites (Galois et al. 2002; Piraino and Gillingwater 2006; Galois and Ouellet 2007; Équipe de rétablissement des tortues du Québec 2014; COSEWIC 2016). Reduced egg and hatchling survival was one of the main threats identified for the Lake Champlain population, which has prompted the need for directed conservation actions (Équipe de rétablissement des tortues du Québec 2014).

Our goals were to document nesting activity at a site on Rivière aux Brochets, a tributary of Lake Champlain, and understand the conditions required for optimal hatching success. Since 2003 we have carefully monitored the river site during the nesting season. We have also used *in situ* and *ex situ* conservation methods to increase hatching success. Our objectives were to: (1) record *A. spinifera* egg-laying activity in terms of timing and behaviors, (2) determine if and how environmental factors (precipitation, air and water temperatures, cloud cover, water flow rate) affect egg-laying activity, (3) determine clutch characteristics, and (4) evaluate natural hatching success and the impact of nest manipulations. For the latter, our prediction was that hatching success would be higher after nest protection, relocation, and artificial incubation.

Material and Methods

Study area.—Our study was conducted at a single nesting site located on the Rivière aux Brochets, Québec, Canada. The river lies in a heavily drained agricultural basin, which runs through Vermont and Québec into the Missisquoi Bay of Lake Champlain. Over the decades, the region has experienced extensive losses of riparian ecosystems due to clearing and backfilling to facilitate water flow. The nesting area consists of an islet and the adjacent shore a few meters away, formed by gravel and sand deposition in a curve of the river. Based on current knowledge, the study area is the most heavily used nesting area on Canadian soil for the Lake Champlain population. Females using this site represent only a subset of the Lake Champlain population because other females nest on the southern side of the border, in Vermont, USA (Graham and Graham 1997; Galois et al. 2002; Steve Parren, pers. comm.). The extent of suitable nesting area at our study site varies both seasonally and daily depending on river discharge conditions, from approximately 170 m2 to 50 m2 at minimum and maximum water level, respectively.

Nest monitoring.—Multiple observers were involved from 2009 until 2016, all going through the same training and using the same data entry form every season. Dates and hours of monitoring varied over the years (Table 1), based on the accumulated knowledge of timing of turtle activity at the site and resource availability. A single observer at a time sat on the south shore of the river, approximately 60 m from the nesting site. We documented turtle activity by category (e.g., digging, basking, etc.) and recorded any distinctive physical sign of the focal individual. We recorded environmental data upon arrival to the site: water and air temperatures (precision of 0.1° C, mercury thermometer), cloud cover (0–10%, 11–50%, 51–90%, 91–100%), and

Table 1. Observation and camera schedule (beginning and end dates and times) at a nesting site of Spiny Softshell Turtles (*Apalone spinifera*) at Rivière aux Brochets, Québec, Canada, from 2009 to 2016.

	Human presence		Camera	
Year	Date	Time	Date	Time
2009	4 June to 30 June	0530–2030	–	–
2010	1 June to 30 June	1030–1830	–	–
2011	31 May to 8 July	1030–1800	–	–
2012	1 June to 11 July	1030–1800	31 May to 30 June	0000–0000
2013	29 May to 28 June	1030–1800	29 May to 20 June	1400–2030
2014	29 May to 3 July	1100–1900	8 June to 7 July	1730–2100
2015	3 June to 3 July	1100–1900	5 June to 4 September	1100–2100
2016	3 June to 23 June	1100–1900	2 June to 6 July	0900–2000

precipitation (yes/no). We also obtained water flow data from the Centre d'expertise hydrique du Québec (www.cehq.gouv.qc.ca/suivihydro/). In addition, beginning in 2012, we installed one wildlife camera (IR-10 or Live models, SpyPoint, Victoriaville, Québec, Canada) 15 m away from the nesting area, from the end of May to early July (Table 1). We set it up to take time-lapse pictures every five or 10 min. We analyzed the photographs for nesting activities outside the on-site observer presence time. We were able to confirm turtle nesting activities with the photographs, but not egg laying.

Incubation.—Starting in 2003 and until 2013, we monitored 21 natural nests to assess *in situ* hatching success. After recording poor success, we used mitigation measures on some nests: relocation (e.g., Wyneken et al. 1988) and/or protection with wire mesh (e.g., Yerli et al. 1997). We relocated seven nests to higher elevations to reduce the risk of flooding, selecting sites with similar substrate and orientation. We covered three nests (including two that were relocated) with a 1 × 1 m wire screen (2.54 × 5.08 cm mesh) anchored in the sand.

Beginning in 2009, we conducted artificial incubation at two zoological institutions: Zoo de Granby (Granby, Québec, Canada) and Zoo Ecomuseum (Sainte-Anne-de-Bellevue, Québec, Canada). We used sand as the substrate in 2009 and vermiculite or sphagnum moss afterward, at a ratio of 1:1 of substrate and water (Greenbaum and Carr 2001). We used incubators (Nature Spirit LLC, Vicksburg, Michigan, USA) and set them at 28° C with 70–80% relative humidity, based on conditions recorded at the nesting site and information from other sources (Janzen 1993; Scott Gillingwater, pers. comm.; Julie Tougas, pers. comm.). Note that *A. spinifera* is a species with genetic sex determination (Greenbaum and Carr 2001).

We measured egg size and hatchling plastron length to the nearest 0.1 mm with an electronic caliper (Absolute 500-196-20, Mitutoyo, Kawasaki, Japan). We also measured the entire nest mass and individual hatchling mass to the nearest 1 g using an electronic scale (Scout Pro SP2001, Ohaus, Parsippany, New Jersey, USA) and calculated egg mass as an average for each nest. We measured plastron length of hatchlings with a metric caliper and weighed them to 0.1 g (Scout Pro SP401, Ohaus, Parsippany, New Jersey, USA). We used the methods described in Graham and Cobb (1998) to determine sex and then measured average hatchling mass and plastron length. Beginning in 2013, we opened unhatched eggs to document the stage of fetal development (Greenbaum and Carr 2002).

Data analysis.—For date and time of egg laying, we tested for normality with a Shapiro-Wilk test. A linear regression was performed on egg-laying dates versus years to detect a trend toward earlier or later nesting activities over time. We used a multiple binomial logistic regression to test the effect of the environment on egg laying. We chose egg laying on a given day (yes/no) as the response variable, whether there was only one egg-laying event or more on that day. The five predictors initially were: air temperature, water temperature, absolute difference between air and water temperatures, cloud cover, water flow, and precipitation. Although they are biologically relevant for different reasons, the three temperature variables were too highly correlated to be included in a single model. We therefore computed a principal component analysis (PCA) on the temperature variables, resulting in two components. We looked for the most parsimonious model through backward elimination using the Akaike Information Criterion (AIC) as an indicator of model fit. Two models were within three AIC points, so we performed a multimodel inference and model averaging, using R package MuMIn (Barton 2017). For clutch characteristics (clutch size, mean egg mass and diameter, mean hatchling mass and plastron length), we tested linear and quadratic regressions, selecting the best fitted model (adjusted $r2$) to check for an effect of Julian day on each variable.

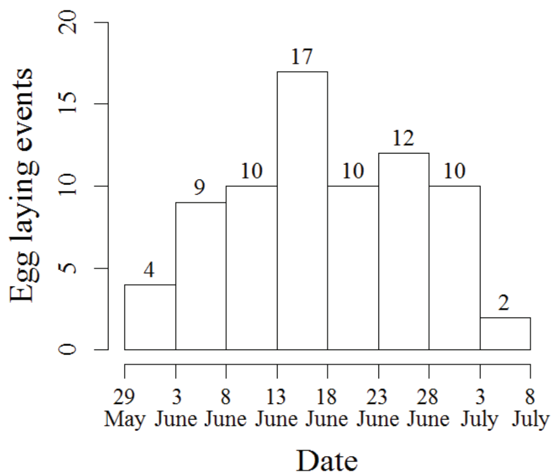

Figure 2. Julian date for 74 egg-laying events for Spiny Softshell Turtles (*Apalone spinifera*) on Rivière aux Brochets, Québec, Canada, between 2009 and 2016. Numbers on top of bars are sample sizes.

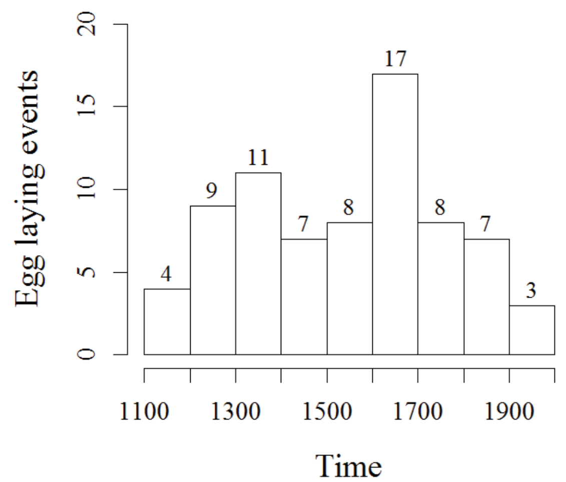

Figure 3. Time of the day for 74 egg-laying events for Spiny Softshell Turtles (*Apalone spinifera*) on Rivière aux Brochets, Québec, Canada, between 2009 and 2016. Numbers on top of bars are sample sizes.

We combined our data with published records of *A. spinifera* clutch size (Gehlbach and Collette 1959; Breckenridge 1960; Webb 1962; Doody, 1995; Graham and Graham 1997; Piraino and Gillingwater 2006) to test for a linear relationship between latitude and clutch size. We performed *t*-tests to compare mass and plastron length of hatchlings between the sexes. We performed all statistical analyses in R 3.5.1 (R Development Core 2018), with the significance level set to 0.05. All means are given with standard deviation (mean ± SD).

Results

Timing of nesting activities.—From 2009–2016, 74 recorded *A. spinifera* nesting events took place between 2 June and 5 July. Start of nesting season varied from 2 to 14 June, the mean date was 18 June, and the latest confirmed date was 30 June. Although the frequency distribution of dates of egg laying appeared to be normal (Fig. 2), it did not follow a normal distribution (W = 0.96, P = 0.017). There was no significant trend for egg-laying dates over the years (t = -0.736, df = 72, P = 0.464). Turtle activity on site, without confirmed egg laying, was observed between 1 June and 9 July. The earliest and latest times of day for egg laying were 1110 and 1947, respectively (EDT; Fig. 3). The distribution of egg-laying events in the day was normal (W = 0.97, P = 0.058) and the mean egg-laying time was 1528 ± 0211. Based on camera recordings, minimum and maximum confirmed hours of turtle activity were 1040 and 2048, respectively. Average number of emergences on land before egg laying was 4.7 ± 3.70, which lasted a mean of 9 m 54 s ± 13 m 08 s. Duration of nesting events (calculated as the time out of water when egg laying occurred) was 48 m 00 s ± 23 m 24 s. One event might include one or more test digs before egg laying.

Four females using the site were recognizable based on their shell characteristics (scars, injuries, and shape). These females were seen multiple times between 2009–2016, ranging from 2–7 y. Two were confirmed to nest twice within a single season, for a total of four females laying two clutches per year over 8 y of monitoring (one female laid twice in 2012, 2013, and 2014, another one laid twice in 2016). Time between egg laying for the same individual ranged between 14 and 23 d. The average number of eggs decreased from 21.3 ± 2.22 for the first clutch to 14.3 ± 5.12 for the second.

Environmental variables and egg laying.—Two principal components explained 91% of the variation in temperature-related variables: PC1 is a combination of air and water temperatures and PC2 is closely related to temperature difference. The two best models (n = 198) included either all variables, or PC1 was removed (Table 2). PC2 was the only significant predictor of egg-laying activity (z = 2.535, P = 0.011), showing that the probability of egg laying decreased as the difference between air and water temperatures increased (Fig. 4). All egg-laying events happened within a more limited range of temperature difference (between −5° and + 4° C) compared to what was recorded during all monitoring days (−9° to + 8° C). Although we observed a positive relationship between egg laying and air and water temperatures, these parameters were not significant in our models.

Clutch characteristics.—We measured clutch characteristics from the 74 nests laid in the field, plus one more nest we found opportunistically at the same

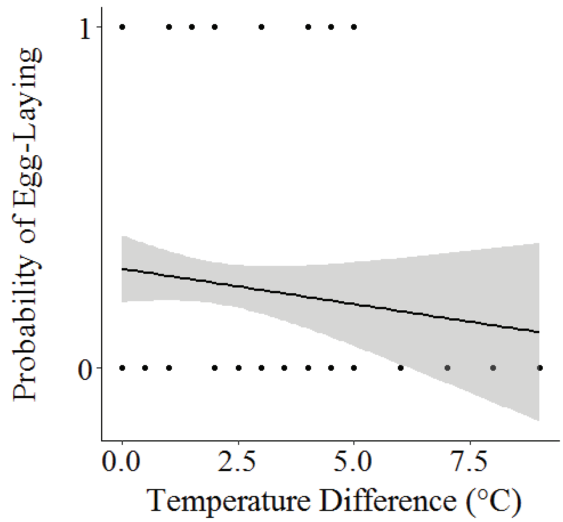

Figure 4. Relationship between the absolute difference between the temperatures of air and water and the probability of egg laying for Spiny Softshell Turtles (*Apalone spinifera*) on Rivière aux Brochets, Québec, Canada. Gray area represents 95% confidence interval.

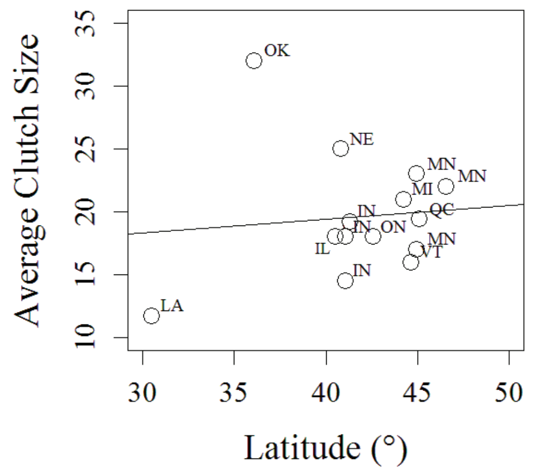

Figure 5. Relationship of clutch size to latitude for 14 populations of Spiny Softshell Turtles (*Apalone spinifera*). State or province codes are given next to each data point (IL = Illinois, IN = Indiana, LA = Louisiana, MN = Minnesota, MI = Michigan, NE = Nebraska, OK = Oklahoma, ON = Ontario, QC = Québec, VT = Vermont).

site. Mean number of eggs per clutch was 19.4 ± 4.78 (n = 75), with a minimum of 11 and a maximum of 31. Clutch size was best predicted by a quadratic relationship with date of laying ($F_{2,46}$ = 17.42, P < 0.001, r^2 = 0.040). There was no significant relationship between clutch size and latitude ($F_{1,12}$ = 0.1, P = 0.748, r^2 = 0.010; Fig. 5). Mean egg mass was 10.1 ± 1.71 g (n = 32). One clutch was much smaller than all other clutches, with an average egg mass of 6.6 g. We removed this clutch, which was more than 1 SD smaller than the average, from the regression calculations. We did not observe a significant quadratic relationship between Julian date and egg mass ($F_{2,29}$ = 2.659, P = 0.087, r^2 = 0.097). Mean egg diameter was 25.3 ± 1.47 mm (n = 621), and 20.4 mm for the small clutch. The quadratic model was better than the linear (r^2 = 0.13 and 0.023, respectively), and the relationship with Julian date was significant ($F_{2,612}$ = 45.60, P < 0.001).

The sex ratio of hatchlings was 1.05:1 (421 males and 416 females). Mean hatchling mass was 6.9 ± 1.28 g (n = 836), with no difference between the sexes (t = 0.40, df = 832, P = 0.687). Mean hatchling mass for the smallest clutch was more than 2 SD smaller than the overall mean (3.5 g). Hatchling mass was associated with date of laying (best model: quadratic; $F_{2,825}$ = 77.96, P < 0.001, r^2 = 0.157). Mean plastron length was 27.6 ± 1.82 mm (n = 785). The smallest clutch had a mean length of 21.8 mm, again more than 2 SD smaller than the overall mean. Mean plastron length was 27.7 ± 1.77 mm for males and 27.6 ± 1.87 mm for females, a difference that was not significant (t = -0.73, df = 779, P = 0.466). Plastron length was also significantly positively related to date of laying (quadratic model; $F_{2,776}$ = 56.7, P < 0.001, r^2 = 0.125).

Hatching success.—*In situ* hatching success of 21 nests, not including those we protected or relocated, was 38% over 10 y (149/390 eggs). The nests we either relocated or protected (n = 6) had a hatching success of 0% (0/114 eggs). Of the two nests that were both relocated and protected, one partially hatched for a total rate of 44% (13/32 eggs). Our first attempt at artificial incubation in 2009 resulted in no hatching success (0/33 eggs). In the seven subsequent years, the hatching success was 84% (1035/1234 eggs) and incubation duration under artificial conditions was 62.7 ± 3.13 d. We observed fetal development in 34% of unhatched

Table 2. Results from multiple binomial logistic regression models of egg laying Spiny Softshell Turtles (*Apalone spinifera*) from Rivière aux Brochets, Québec, Canada, as predicted by environmental variables, showing models with delta Akaike Information Criterion (AIC) < 3 selected through backward selection. The second model with five variables is the global model. PC1 is correlated with air and water temperatures, and PC2 is highly correlated with absolute difference between air and water temperatures. Log L denotes the log likelihood of each model. We selected models based on AIC and number of units from the top model is denoted by ΔAIC. Weight of support for each model is given by w_i in a total of 1.

Model	df	Log L	AIC	ΔAIC	w_i
Rain + Cloud + Flow + PC2	5	-87.55	185.45	0.00	0.74
Rain + Cloud + Flow + PC1 + PC2	6	-87.54	187.57	2.12	0.26

eggs (47/140 eggs), commonly with a fully formed embryo at stage 22 or higher (based on Greenbaum and Carr 2002).

Discussion

We documented nesting activity of *A. spinifera* at the study site for 13 y, indicative of long-term use of a single nesting site (Tornabene et al. 2017). At this site, egg-laying activity was highly predictable with respect to date and time, with egg laying taking place only in June and early July. A similar nesting period was apparent in northern Vermont for the same population (Steve Parren, pers. comm.), with some later dates reported by Freeman (unpubl. report) in a single-year study. In Ontario, at more southern latitudes, *A. spinifera* has been observed nesting at roughly the same time, between the beginning of June and the second week of July (Gillingwater 2004). Similar dates have also been reported from Tennessee (Robinson and Murphy 1978) and Montana (Tornabene et al. 2018) in the U.S. Early literature from the U.S. reports that egg laying occurred from the beginning of June to early July (Webb 1962), with reported exceptions in May in Pennsylvania (Surface 1908) and the end of July in Indiana (Evermann and Clark 1920). Small differences in egg-laying dates among years could be explained by weather during pre-nesting months, as far back as the previous fall, when follicle development starts (Schwanz and Janzen 2008). There was no indication of a broader temporal trend during our intensive seven years of monitoring, but other studies on freshwater turtles (Schwanz and Janzen 2008) and sea turtles (Weishampel et al. 2004) have detected earlier start of nesting activity over the years, possibly linked to climate change.

Time of the day when egg laying occurred in our study was slightly later than the period reported by Tornabene et al. (2018) but was within the timeframe from other studies of egg laying in *A. spinifera* (Newman 1906; Cahn 1937; Breckenridge 1960). We observed that the nesting process followed a simple sequence of behaviors, varying in the number and duration of emergences and test digs prior to egg laying. Nesting behavior was similar to observations of *A. spinifera* in the U.S. (Newman 1906; Cahn 1937; Breckenridge 1960; Tornabene et al. 2018; Steve Parren, pers. comm.). We did not observe the post-nesting burrowing or trenching as reported by Plummer and Doody (2010) in the southern U.S.

Only two of the environmental factors we studied had an influence on the probability of egg laying. We observed that when air and water temperatures were more similar, egg laying probability increased. Fine physiological processes might be at play as it is unclear how this affects egg laying and why this is a cue for the females. Extreme water discharge also hindered nesting activity for more obvious reasons. First, reaching this riverine nesting site always involved swimming upstream for the females (Daigle et al. 2002; Galois et al. 2002). When the water flow is high, this becomes a very strenuous activity for the turtles. Second, at such water levels, the riverbanks were flooded, which reduced the available nesting ground. Flood pulses influenced *A. spinifera* movement and habitat use inhabiting a river system in Montana, USA (Tornabene et al. 2017).

Clutch sizes were similar to those reported in studies from throughout the range of the species (Gehlbach and Collette 1959; Breckenridge 1960; Webb 1962; Graham and Graham 1997; Piraino and Gillingwater 2006) with a notable exception from a study from Louisiana, USA (average clutch size = 11.7 ± 4.14; Doody 1995). Early records indicated an apparent trend toward larger clutch size at northern latitudes in *Apalone* spp. (Webb 1962). A study by Iverson et al. (1993) reported a positive but non-significant relationship specifically looking at *A. spinifera* and we came to the same conclusion integrating more recent data, including our own. Means of other variables (diameter and mass of eggs, and plastron length of hatchlings) were mostly similar to previous studies on *A. spinifera* (Packard et al. 1979; Graham and Graham 1997; Plummer and Mills 2015); however, we observed wider ranges, probably resulting from our larger sample sizes. Egg and hatchling characteristics were highly correlated; smaller eggs (diameter and mass) yielded smaller hatchlings (Ewert 1979). Because we did not catch any adult females in the course of the study, we were not able to test the relationship of clutch or egg sizes with female size, known in other freshwater turtle species (Gibbons 1982, Congdon and Gibbons 1985). Interestingly, Graham and Graham (1997) reported a clutch of eggs they termed under-sized from the Lake Champlain population, with egg characteristics similar to one of the clutches we observed, but ours was even smaller.

We confirmed that multiple clutches can be laid by the same female within the same season in our population, even though this was considered exceptional in Québec freshwater turtle populations due to the prevailing climatic conditions (Équipe de rétablissement des tortues du Québec 2014). Multiple clutches within a season are well known in freshwater turtles (Congdon and Tinkle 1982; Gibbons 1982; Iverson and Moler 1997; Kuchling 1999), including *A. spinifera* (Robinson and Murphy 1978), and this strategy can be a way to reduce the percentage of reproductive failure (Wilkinson and Gibbons 2005). These females also exhibited site fidelity to some extent. We suspect that the shortage of suitable nesting habitat along the river is one of the principal drivers of nest site fidelity, but other reasons might be responsible, such as natal homing or the selection of optimal nest sites based on environmental characteristics (Rowe et al. 2005; Walde et al. 2007).

At 38%, the *in situ* hatching success is low, but not unheard of in other studies that vary greatly among years and sites (0–61%, de Solla et al. 2003; 60–95%, Tornabene et al. 2018). The comparison of management methods among different *in situ* nests was not experimentally tested, but our data indicated that adding a mesh over a nest or relocating it did not increase hatching success. Predation pressure remains high at the site as seen from the abundant presence of eggshell fragments of Snapping Turtles (*Chelydra serpentina*). In any case, runoff from surrounding agricultural fields make nest flooding unavoidable at the site and fencing alone would not improve hatching success. A study of a population of Blanding's Turtles (*Emydoidea blandingii*) in Nova Scotia, Canada, also highlighted the inadequacy of nest protection in the face of adverse environmental factors (Standing et al. 1999). Concerning the failure of relocated nests, we suspect that the higher ground positioned away from potential flooding had low moisture retention, which led to desiccation of the eggs. Hatching rate was more than doubled when nests were artificially incubated, indicating a clear advantage to this method. The ultimate goal of any conservation program, however, is to attain a self-sustainable population, so artificial incubation should be considered a temporary measure. In our case, we are simultaneously working on educating the public, restoring nesting habitats, improving shoreline and water quality, and reducing causes of mortality.

Female behavior and clutch characteristics observed in the Lake Champlain population were similar to information obtained from other locations. Therefore, our proposed conservation actions might be relevant to other *A. spinifera* populations at the northern edge of the distribution of the species, or other riverine turtle species exposed to similar threats. For conservation purposes, knowing the precise dates and timing of nesting activity will allow targeted protective measures such as restricted human access and trapping of predators. We can also estimate the probability of egg laying based on air-water temperature difference and water flow data, which will help in the collection of nests for artificial incubation. As a next step, monitoring behavior and survivorship of *A. spinifera* adult females and juveniles will be imperative to pursue the recovery of this species. To obtain such information, a telemetry program of juvenile turtles was implemented in 2016 and genetic analyses are underway to assess the number and genetic diversity of females using the nesting site and its possible use by recruits.

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