Comparative Survivorship Analysis of Headstarted, Incubated, and Wild Wood Turtle Hatchlings (*Glyptemys insculpta*)

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Introduction

The wood turtle (*Glyptemys insculpta*) is native to North America, with about 30% of the population residing in Canada in patchy populations throughout Nova Scotia, New Brunswick, south-central Quebec, and south-central Ontario (Committee on the Status of Endangered Wildlife in Canada [COSEWIC]; 2007). The three subpopulations in Ontario are particularly isolated, having been extirpated from ten municipalities in southern Ontario, which may have historically connected their range (Committee on the Status of Species at Risk in Ontario [COSSARO], 2017). With the wood turtle being globally endangered, federally threatened, and provincially endangered in Ontario; and limited funding for conservation, much debate has arisen as to which conservation strategies should be prioritized (van Dijk & Harding, 2010; COSEWIC, 2007; COSSARO, 2017; Mullin et al., 2023). One of the discussed strategies is headstarting, an approach in which eggs are incubated and hatchlings captively reared until they have surpassed the small body sizes associated with low survival rates at early life stages, and then released back into the wild, in theory, with improved chances of survival (Mullin et al., 2023). This study will address important knowledge gaps associated with headstarting by comparing the survivorship of headstarted wood turtles to their non-headstarted and wild counterparts from the same population.

Background

Different species and populations have inherently different risks of extinction depending on their life history strategies and population demographics. Iteroparous species (i.e. reproduce many times in their life) such as turtles are particularly vulnerable to increased adult mortality (Johnsson & Ebenman, 2001). Most freshwater turtle species are well-adapted to variation in survivorship at early life stages, their extreme sensitivity to increased adult mortality a result of each individual's reliance on multiple reproductive events per lifetime in order to replace themselves in the population (Heppell, 1998; Enneson & Litzgus, 2008).

Given the heavy impact of adult survivorship on freshwater turtle populations, several researchers have suggested that conservation efforts should be targeted towards reducing adult mortality (Heppell, 1998; Mitrus, 2008; Bougie et al., 2022). Much criticism has been attributed to headstarting programs for not addressing the conditions that are most likely to lead to a population decline (i.e. increased adult mortality), and for not taking the taxon's life history strategy into account (Mitrus, 2008; Heppellb, 1998; Crouse et al. 1998; Frazer 1992).

Conversely, in populations where adult survivorship is already high, increases in juvenile survivorship have been shown to have significant impacts on population growth, largely due to the large initial difference in the survivorship of these two age classes (it is easier to increase 10% juvenile survivorship by 10-20% than it is to

increase 96% adult survival by 1-2%; Enneson & Litzgus, 2008; Cunnington & Brooks, 1996). One study recommended headstarting as a "primary tool for managing freshwater turtles…" (Spencer, 2006, pp. 1340), and a recent growing body of evidence has shown early indicators of success in headstarting programs in reversing population declines (Carstairs, 2019; Wijewardena, 2023; Mullin et al., 2023). However, while most of these studies observed high survivorship among headstarted individuals, most of them lacked wild hatchling control groups, limiting the validity of their findings (Mullin et al., 2023). There is a general lack of research on the fate of wild hatchling and juvenile freshwater turtles, and survivorship estimates for known-age juvenile wood turtles do not exist (Mullin et al., 2023; Enneson & Litzgus, 2008).

In a provincial landscape wherein every freshwater turtle species is threatened with extinction, it is vital to understand which conservation measures are the most effective (Endangered Species Act, 2007). In order to answer this question, studies assessing the validity of headstarting programs must include control groups of non-headstarted wild turtles from the same population (Mullin et al., 2023). It is important to acknowledge that headstarting is not the entire solution to saving declining turtle populations, and protection of all life stages is required (Condon et. al., 1994; Enneson & Litzgus, 2008; Mullin et al., 2023, Keevil et al., 2022). This project aims to assess efficacy of headstarting as one potential piece of the conservation puzzle, using within-study comparisons of the survivorship of headstarted turtles to their wild counterparts.

Project Goal

1. To help fill the knowledge gap of the fate of wild wood turtle hatchlings, including survivorship, growth, and habitat use.
2. To contribute empirical evidence for assessing the efficacy of headstarting as a conservation tool.

Objectives

- Compare one-year survivorship between three groups of wood turtles from the same subpopulation and released in the same year:
    - Wood turtles headstarted for one year (1yHS).
    - Non-headstarted wood turtles, including those:
        - Incubated and directly released (0yDR).
        - Wild-hatched with no intervention (0yWH).
- Monitor survival, growth, and habitat use of wild wood turtles in their first year of life from Clinton subpopulation.

Methodology

Capture and Collection Sites

The study site in southwestern Ontario is habitat to a wood turtle population that has been part of a long-term mark-and-recapture study since 1988 and has used headstarting as a conservation tool since 2003 (Figure 1). The study site contains two artificial nesting sites on private farmlands that are maintained and monitored by a local stewardship council. During nesting season, these artificial nests and surrounding nesting habitat will be monitored using existing HSC nesting survey methodology. Nests will be identified for 1yHS cohort and eggs collected in May-June 2025. The 0yDR and 0yWH cohorts will be identified (and 0YDR eggs collected) in May-June 2026.

Incubation and Captive Rearing

All incubation and captive rearing will take place at the Toronto Zoo under the care of zoo staff using existing methodology.

Release and Monitoring

A total of 45 wood turtles will be outfitted with very high frequency (VHF) radio-transmitters and monitored for one year after release, including 15 wood turtles headstarted for one year, 15 wood turtles released directly after incubation, and 15 wild-hatched wood turtles with no human intervention. All turtles will be affixed with radio-transmitters weighing no more than 5-10% of each individual's body mass, as mandated by Ontario's Wildlife Animal Care Committee (WACC; unpublished guidelines for use of radio-transmitters on turtles in Ontario Parks). Headstarted juveniles and incubated hatchlings will be released at [protected site] approximately 10m apart to minimize group predation (Carstairs et al., 2019).

After release, turtles will be tracked twice per week during the remainder of the active season (April-October; Mullin et al., 2023). Survivorship and growth will be documented twice per week for headstarted turtles and every two weeks for incubated and wild hatchlings to minimize disturbance. Standard morphometric measurements including body mass and straight midline carapace length will be recorded to measure growth, along with location, temperature, and behaviour for analysis of habitat use. Once turtles have reached their overwintering sites, they will be tracked once monthly to check survival and habitat use, but morphometric data collection will be paused during the inactive season to minimize disturbance during this sensitive time (November-March; Mullin et al., 2023). Once turtles have emerged from overwintering the following spring, they will continue to be tracked twice a week using the same parameters as above, however, non-headstarted incubated and wild juveniles will now be measured weekly instead of biweekly. Any turtle that perishes during this study will be investigated to identify the cause of death, using indicators described by Mullin et al. (2023) including "carcass position, limb loss, and state of decomposition" (pp. 5). All radio transmitters will be removed by the end of the 2028 active season.

Data Analysis

Growth rates of each group will be compared using ANOVA. Survival rates of each group will be analyzed using Kaplan-Meier survival curves. I hypothesize that wild hatchlings with no intervention will experience the highest mortality rates immediately post emergence, as they will have the additional life challenge of surviving from emergence throughout dispersal to their wetland. If this hypothesis is supported, I will account for this additional variable using a Cox proportional hazard model.

Project Schedule

Table 1. Project Schedule.*

TASK	2026								2027												2028			
	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR
Captive facility management:																								
Incubation and captive rearing of 1yHS (began May 2025)																								
Incubation of 0yrDR eggs																								
Field Work:																								
Identification, collection and transport of 0yDR eggs to Toronto Zoo																								
Identification of 0yWH eggs																								
Monitoring of protected nest(s)																								
Release 1yHS, 0yDR and 0yWH																								
Monitoring and data collection																								
Remove all radio transmitters																								
Administrative:																								
Data compilation and adjustment																								
Data analysis																								
Preparation of scientific results																								
Publication of scientific results																								
Report to donours																								

**Note that nests for 1yHS cohort will be identified and eggs collected by HSC in May-June 2025; and incubated and captively reared by the Toronto Zoo between spring 2025 and fall 2026. This schedule begins when tasks that fall directly under the supervision of this project begin.*

Expected Outputs

- Publication of scientific paper with results of the project.
- One-year survivorship report for wood turtle hatchlings.
- Comparative observed survivorship data determining whether there are significant differences in survivorship among the three cohorts.
- Replicable methods for incorporating wild control groups into headstart studies.

Expected Outcomes

- Improved understanding of survivorship, growth, and habitat use of wild juvenile wood turtles.
- Expanded habitat protection for additional juvenile habitat identified beyond known adult habitat.
- Standardization of incorporating wild control groups into headstart studies

Resources Required

Table 2. Expenditure Chart.

Item	Quantity	Unit Price ($ CAD)	Total Cost ($ CAD)
PESOLA 10010 Light-line Metric Spring Scale, 10g	2	48.45	96.90
PESOLA 20030 Micro-Line Metric Spring Scale, 30g	2	64.40	128.80
Imperial/Metric Vernier Caliper, 6-pack 15cm	1	12.50	12.50
Garmin eTrex 22x	1	275.00	275.00
J-B Weld New 8277 Waterweld Adhesive Compound Specially Formulated Epoxy Putty	2	13.66	27.32
Elmer's Instant Crazy Glue All Purpose Adhesive	2	4.29	8.58
Isopropyl Rubbing Alcohol 70%, 473mL - 2 bottles	1	17.95	17.95
Prima Mark Spray Paint, Black 1.75 oz (vet approved)	2	8.00	16.00
Celestron Nature DX ED 8x42mm Roof Binoculars	1	242.00	242.00
SRX1200-M1 Receiver	1	3,195	3195.00
F150-3FB, 3 Element Folding Yagi 150Mhz C/W 5' Cable Pouch Grip	1	215.00	215.00
MFT Tag Tag Beeper, MFTB-3A	15	167.00	2505.00
MCFT2 Tag Beeper, NTF-3-2	30	185.00	5550.00
MEC Fulcrum PFD - Unisex	2	127.46	254.92
MEC Nano X-Hail Dry Bag	1	14.97	14.97
Canoe (used)	1	600.00	600.00
Paddle (used)	2	40.00	80.00
Contingency	N/A	200.00	200
Gas	N/A	400.00	400
GRAND TOTAL			**13839.94**

Risks/Assumptions

Table 3. Risk/Mitigation Chart.

Risk	Level (Low, Medium, High)	Mitigation
Depredation of 0yWH eggs during incubation	High	0yWH cohort will only be chosen from clutches laid in artificial nesting sites, which are protected from predators by electric fencing.
Depredation of 0yWH during post-emergence dispersal	Medium	This risk will not be mitigated as it is necessary to understand survivorship of wild control group.
Transmitters could impede turtles' movement, growth, or life quality if they are not removed after completion of study.	Low	If any transmitters fail during the study and the turtles are not able to be captured before the end of the study, intensive mark-and-recapture surveys will be conducted until all transmitters have been removed.

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