Eastern Georgian Bay Initiative
2021 Funding
Final Report

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Table of Contents

1. Summary and Milestones

In general, field work aspects of this year's projects went very well, while community engagement efforts continued to be hampered by the Covid-19 pandemic and the resulting restrictions. The project was about 50% funded relative to the original proposal. As a result, some activities were cancelled or postponed, in favour of supporting others. Other sources of funding were also leveraged. Table 1 shows the proposed milestones, modifications, and results for 2021.

In general, field work focused primarily on Eastern Massasauga, particularly identifying and monitoring hibernation (17) and gestation (70) sites, as well as new targeted walking surveys (128) for snake road mortality. Similar targeted walking surveys (149) were continued for turtles. Road mortality mitigation for turtles through direct patrols continues to show promise for mitigating this threat along two lane, paved roads. Encouraging preliminary results are shown in section 10.

Community volunteer training continued virtually, with 33 volunteers completing training this year. Only 62 educational programs, both virtual and in person, were delivered due to pandemic-related complications, especially the spring lockdowns.

Habitat protection under the Endangered Species Act from our observations of threatened and endangered species increased by 669 km^2, from 3547 km^2 in 2020 to 4827 km^2. Over 4000 species at risk reptile observations were recorded. Especially noteworthy observations included:

-a female massasauga that gestated successfully in 2020 and again in 2021; this species normally gives birth every other year in this population

-a female Blanding's turtle that moved over 32 km (straight line distance) between 2017 and 2021

-a female Blanding's turtle that laid eggs on our own property, a first (since 2002)

Table 1. 2021 proposed project milestones, modifications, and results.

Proposed Milestone	Modifications	Results	Notes
4 First Nations of Georgian Bay youth enrolled in Herpetology Internship Program	Retained pending applications, however no candidates applied so postponed.	Not applicable	We hope to recruit suitable candidates in the future.
5 Community Volunteer training workshops, 30 volunteers trained	Reduced number of virtual workshops due to pandemic and reduced funding	3 workshops, 33 volunteers trained	Milestone exceeded.
1 Educational Video created	Eliminated due to reduced funding	Not applicable	Proposed for reinstatement due to reduced

			education output
100 Educational Programs delivered	2020 Programs also held over (120)	61 programs delivered	Incomplete due to Covid-19. Alternatives proposed
4 snake walking surveys, twice weekly	Increased to 6 sites due to increased focus on snake road mortality mitigation	6 sites, 128 surveys conducted	Completed
16 turtle walking surveys, every other week	Reduced to 15 sites due to access issues on one road	15 sites, 149 surveys conducted	Completed
900 hotline calls/texts	None	828 hotline reports received	Almost met despite lockdown during the most important season
2000 species at risk observations recorded	None	4065 species at risk observations recorded	Milestone greatly exceeded.
10 hotline signs installed	Postponed due to reduced funding	Not applicable	Although not done in 2021, we are still pursuing this.

2. Introduction

Funding from the Eastern Georgian Bay Initiative (EGBI) supported both field conservation activities and community engagement efforts for both the Georgian Bay Conservation, Action, Research, and Education about Snakes (CARES) Project and the Saving Turtles At Risk Today (START) Project. This report discusses the activities conducted directly or indirectly under this funding, and their results for the conservation of our target species- primarily Eastern Massasauga, Eastern Hog-nosed Snake, Blanding's Turtle, and Spotted Turtle. Eastern Foxsnake and Eastern Musk Turtle have so far been tangential to our efforts, though we may expand our work with these species in 2022 depending on available resources.

The CARES Project was launched last year with funding support from the EGBI. The CARES Project focuses on the conservation of species at risk snakes in the eastern Georgian Bay region and is focused primarily on addressing road mortality, habitat loss, filling knowledge gaps, and decreasing persecution of the Eastern Massasauga Rattlesnake (*Sistrurus catenatus),* Eastern Hog-nosed Snake (*Heterodon platyrhinos),* and to a lesser extent, the Eastern Foxsnake, (*Pantherophis gloydi).* This project works in tandem with the START Project and leverages its

large amount of funding and resources for turtles to create more conservation effort for snakes in the region. Both CARES and START teams are trained to work with local turtles and snakes. This was the second year of the CARES Project and despite the difficulties presented by the COVID-19 pandemic, it was a massive success as far as the number of observations reported and other goals, including assessing habitat connectivity, road mortality, identification of hibernacula/gestation sites, etc.

The START Project and the CARES Project are conducted in collaboration with the Georgian Bay Turtle Hospital, the Canadian Wildlife Federation, Laurentian University and The Land Between. Additional partners include McMaster University, Johnston's Cranberry Marsh, the Gravenhurst Rifle and Revolver Club, Lake Simcoe Region Conservation Authority, the Nottawasaga Valley Conservation Authority, the Couchiching Conservancy, the Nature Conservancy of Canada and the Georgian Bay Biosphere Reserve. As usual, the project was well received within the community and we expect additional academic and community partners to join in the future.

The START Project commenced in 2013 to investigate turtle nest predation across a gradient of human influence, to estimate the population of Blanding's turtles in Muskoka and to protect habitat through new observations of this threatened species. Due to the need for turtle conservation work in central Ontario, the scope of the project rapidly expanded. Results from this project are especially relevant to the management and conservation of turtles across the southern edge of the Canadian shield, sometimes known as "The Land Between", which is estimated to contain a large proportion of Ontario's remaining turtle populations. Other long term turtle work in central Ontario occurs along the Georgian Bay coastline and in Algonquin Park, however, these locations don't share the typical habitat conditions, anthropogenic influences and climate of the region, so their results are unlikely to be representative.

Five of six turtle species (Blanding's, spotted, snapping, map, musk and midland painted) found in the region are at risk, with a seventh (wood) already eliminated. Blanding's and spotted turtles have suffered catastrophic declines south of the Canadian shield. Anthropogenic threats, such as road mortality, habitat loss, subsidized predators, collection and persecution are the primary causes of decline. Some objectives of this project are to prevent, reduce and offset road mortality and subsidized predation as threats to populations of these species, to report observation for species at risk to facilitate habitat protection, to estimate population sizes of turtle species found in the area, to identify nesting, overwintering, aestivation and road crossing hotspots, to reduce collection for the food and pet trades, and to increase public knowledge and awareness regarding related conservation issues in the region.

The education goals related to EGBI funding pertain to both turtles and snakes, and details are presented in section 8.2 Educational Programming. However, in general, our education efforts continue to be greatly constrained by the Covid-19 pandemic. Some progress was made this year, with 61 programs being delivered virtually or in person, however we were hoping for more progress.

The START project received core funding ($173,000) from the Rogers Foundation through the Canadian Wildlife Federation (as it has for the last 8 years). With funding from the EGBI being more limited this year, it was not directly focused on turtle work, however since the project goals and activities are so closely entwined, we will present some results about it. The EGBI remains our only core funder for snake work in central Ontario.

Our work also received some funds through a partnership with The Land Between through their federally funded Community Nominated Priority Places project; this was primarily for turtle conservation. Several federal wage subsidy programs allowed for hiring additional field technicians, such as Young Canada Works, Canada Summer Jobs, and Youth Employment Strategy internships through Eco Canada and the Clean Foundation. Funding totalled over $689,000 this year.

3. Project Area and Permitting

The overall 2021 project area extended from the Georgian Bay coastline to the eastern edge of Haliburton County, and from Bradford to Parry Sound and Huntsville, which can be seen in Figure 1. Expansion this year was limited to western Simcoe County, to include all of the Minesing Wetlands which became an area of focus thanks to a new partnership with the Nottawasaga Valley Conservation Authority and the Nature Conservancy of Canada, as well as Blanding's turtle funding from Environment Canada which was specific to that area. All of Simcoe County is now included in our permitting. Some project activities may occur across the entire landscape (i.e. hotline call response) while others happen only within specific areas (i.e. radio-telemetry, turtle notching) or for specific species (i.e. blood sampling). Snake-focused fieldwork occurs only in northern Simcoe County, Muskoka, and Parry Sound within the range of the Eastern Massasauga. Additional geographic expansion may occur in the future but is not currently planned.

Multiple permits and authorizations are required to conduct many of these project activities. Endangered Species Act (ESA) B Permits were registered for and obtained from the Ministry of Conservation, Environment and Parks, for activities related to species at risk, including capture, processing, blood sampling, radio-telemetry, etc. One B permit was specific to radio-telemetry and a second was for all other project activities. Wildlife Scientific Collector Authorizations (WSCA) were obtained from the Ministry of Natural Resources and Forestry (OMNRF). One from the Parry Sound district was specific to radio-telemetry activities with Blanding's and Spotted turtles. The second was from the Southern Region for all other project activities. An additional permit was obtained from Ontario Parks regarding work within parks and protected areas. This year we were extremely grateful to receive all permits in a timely manner, so we could start our activities close to our projected start date.

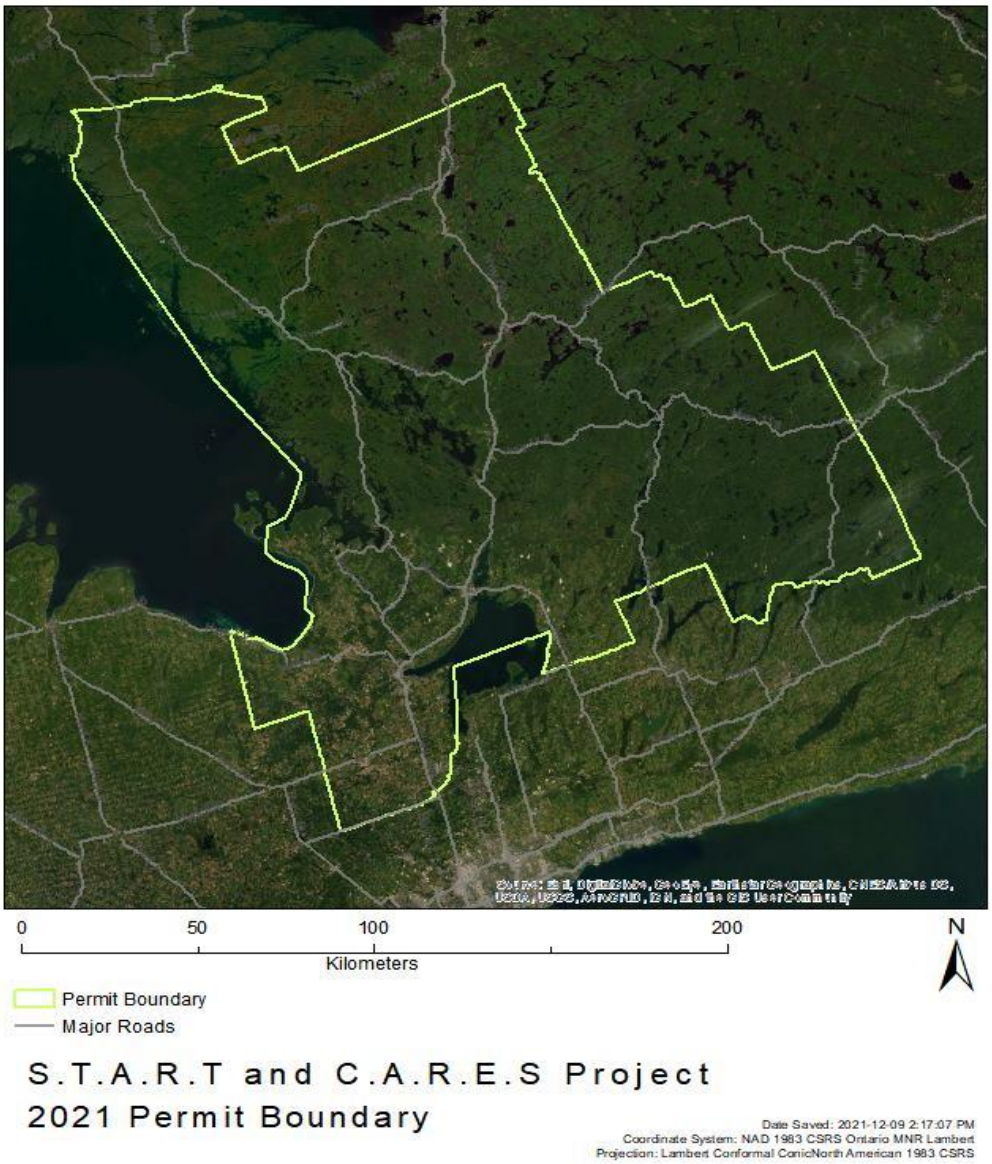


Figure 1. The current permitted study boundary of our central Ontario conservation efforts is shown in yellow.

4. Combined START and CARES Activities

4.1 Walking Surveys (May-October)

In the 2021 field season, we completed our third year of walking surveys for the START Project. The results of these are in Table 2. Walking surveys consisted of teams of 2 or 4 people surveying for herptiles along a designated 1km stretch of road. Within the project area, there were 16 roads designated for walking surveys for turtles, and each is surveyed bi-weekly from April to September. The goal of these surveys is to identify how different road types (speed, traffic, substrate, etc.) impact herptiles. One of these roads was dropped due to a locked gate

installed in 2020 that blocked access. In total, 149 surveys were conducted across the 15 sites.

This year we also added 6 CARES walking surveys for snakes, which focused on areas where higher snake mortality rates were found in 2020. These were completed weekly. In total, 128 surveys were conducted across the 6 sites. Please see Table 3 for the results of CARES walking surveys. We hope to continue these surveys for an additional year before quantifying our results. In total, 1823 road-associated observations were recorded, of which 417 were reptiles. Thirty of these were confirmed as species at risk and 10 were primary target species under the EGBI funding.

Table 2. 2021 START walking survey observation results for turtles, snakes and amphibians.

Species	**Alive**	**Injured**	**Dead**	**Total**
Turtles	14	0	117	131
Blanding's Turtle	1	0	1	2
Midland Painted Turtle	13	0	87	100
Snapping Turtle	0	0	8	8
Unknown Turtle	0	0	21	21
Snakes	16	2	71	89
Massasauga Rattlesnake	0	0	1	1
E. Hog-nosed Snake	0	0	0	0
Dekay's Brownsnake	0	0	11	11
E. Gartersnake	5	0	18	23
E. Milksnake	0	0	4	4
N. Watersnake	7	1	15	23
N. Red-bellied Snake	0	1	14	15
N. Ribbonsnake	2	0	1	3

N. Ring-necked Snake	0	0	3	3
Smooth Greensnake	1	0	3	4
Unknown Snake	1	0	2	3
Five-lined Skinks	0	0	0	0
Anurans	283	0	625	863
Salamanders	2	0	16	18
Totals	268	2	813	1083

Table 3. 2021 CARES walking survey observation results for turtles, snakes and amphibians.

Species	**Alive**	**Injured**	**Dead**	**Total**
Snakes	7	1	174	182
Massasauga Rattlesnake	0	0	5	5
E. Hog-nosed Snakes	0	0	2	2
Eastern Foxsnake	0	0	0	0
Dekay's Brownsnake	0	0	40	40
E. Gartersnake	6	0	50	56
E. Milksnake	0	0	4	4
N. Watersnake	0	0	21	21
N. Red-bellied Snake	0	0	15	15
N. Ribbonsnake	1	1	3	5
N. Ring-necked Snake	0	0	8	8

Smooth Greensnake	0	0	12	12
Unknown Snake	0	0	14	14
Turtles	2	0	11	13
Blanding's Turtle	0	0	0	0
Midland Painted Turtle	1	0	9	10
Snapping Turtle	1	0	1	2
Unknown Turtle	0	0	1	1
Five-lined Skinks	0	0	2	2
Anurans	10	0	489	499
Salamanders	1	0	43	44
Totals	20	1	719	740

*None of our walking surveys target Eastern Foxsnake habitat.

5. Saving Turtles at Risk Today Project

5.1 START Project Field Activities

Wetsuits, binoculars, and canoes all continue to be used extensively by project staff and volunteers to enable wetland surveys. In general, we have found that wetsuits are more durable and more comfortable to wear over long periods of time in the field than waders. As some of our wetlands are disjunct from others, it is often necessary to hike between areas, which is much easier with the use of a wetsuit instead of waders. In many instances, binoculars were used by teams to survey for basking turtles in wetlands where we didn't have access to the private land. We found these particularly effective when surveying in the Lake Simcoe watershed, where most of the land is private. Canoes were used extensively this year in open water areas, particularly for Map Turtle surveys, and for remote survey trips.

5.2 Preseason Surveys (April)

Preseason turtle fieldwork began in early April, with limited staff and some volunteer

field technicians under the guidance of Field Projects Coordinator Kelsey Moxley. This is a critical time for Spotted Turtle activity, due to their early emergence from hibernation.

Limited preliminary snake surveys began in April to assess the amount of ice still present at confirmed and suspected Massasauga hibernacula as well as to determine the potential suitability of newly selected sites. Sites were selected using satellite imagery as well as prior knowledge of the area due to past surveys from the last season and previous START Project surveys.

Early in the season, previously identified populations of Spotted Turtles were targeted for surveys. We have begun to analyze data and generate population estimates for our three largest populations within the project area. Even though we didn't discover a new population of Spotted Turtles this year, they continue to be a target species for the project and field technicians were able to capture and process 72 individuals and observed an additional 20 this season. We were able to collect data from 19 new individuals to the project that were captured at 6 of our known populations, expanding our demographic datasets for them. One individual was observed traveling on a road not close to any of our current populations and we have yet to determine the location of its origin. Surrounding habitat for this observation is private property, therefore we are working on contacting associated landowners with the intention to obtain permission and survey suspected wetlands for Spotted Turtles and any other species at risk. Another notable find for this year includes a juvenile Spotted Turtle found within a population surveyed regularly over the season and over the years. In this population we have never come across a juvenile during the previous 7 years of surveying the habitat. We will continue to monitor this population by surveying it during the optimal season to gain more insight on the population's dynamics.

5.3 Boot Camp (early May)

Due to COVID-19, this year's boot camp once again included a requirement for quarantining upon arrival and throughout the season. Project staff candidates and volunteers quarantined into the Scales Nature Park 'bubble' in order to participate in training. Training included classroom presentations, and hands-on practice with captive turtles using the live collection at Scales Nature Park, in addition to training days in the field. Specific topics included identification of species at risk reptiles, safe handling of live turtles and snakes, sex determination of turtles and snakes, measurement methods, weighing turtles and snakes using Pesola spring scales, blood sampling, data collection and entry, mapping, satellite image interpretation, field site selection and survey planning, field safety procedures, first aid, canoe paddling and canoe-over-canoe rescue, and cold water safety.

Field training days included introduction to a wide variety of sites, such as bogs, swamps, beaver ponds, fens and rock barrens. This provides opportunities to learn which survey methods are appropriate for the terrain, and what equipment is necessary to make the field day a success.

Training was led by Kelsey Moxley, our Field Projects Manager with 8 years of experience directly with the START Project. This year we were safely able to have 44 staff and

volunteers participate in the training, while operating within strict COVID-19 quarantine protocols. Staff candidates who successfully completed training were hired for the summer. Some volunteers who completed training were also hired and others continued to volunteer for a minimum of one month following the training period. This ensured they were confident and competent with all field procedures, provided an opportunity to learn nesting season protocols, which aren't taught during Boot Camp, and were available to help with field work during turtle nesting season, our busiest survey time. Volunteers completing this process learn many transferable skills and gain critical hands-on experience.

Training for snake methods was provided by Taylor Kennedy and Maria Ning, CARES Project Coordinators, and Kelsey Moxley, Field Projects Manager. Venomous snake training during Boot Camp was mainly theoretical. Selected staff were trained later to tube and process Massasauga Rattlesnakes and other species-at-risk (SAR) snakes.

5.4 START Field Surveys (May-September)

Following Boot Camp, teams were quickly deployed to their field stations across the study area. Due to the generous support of Johnston's Cranberry Marsh, we were able to base a remote field team to live in the north-west end of the active survey area on their property near Bala. Due to the renewed support by the Gravenhurst Rifle and Revolver Club and the Gravenhurst Conservation Club, we were also able to base a team in the east side of our active survey area on their property. These bases helped to keep mileage costs down, allowed us to have a larger reach geographically, and allowed for faster response times to hotline calls. We are thankful to have the continued support of organizations like the Johnston's Cranberry Marsh and the Gravenhurst Rifle and Revolver Club in 2021.

Blanding's Turtles continued to be a priority target species for the project. Field technicians were able to capture and notch 157 new Blanding's Turtles, recapture 92 previously notched individuals, and observe an additional 235 this season (such as basking turtles that evaded capture, or were observed on private property where we don't have access to enter wetlands). The total number of notched Blanding's Turtles is now 1569 individuals across all years of the project. We are unaware of any other project with such a large collection of data on this species in Ontario and we hope to continue collecting this data to aid with future conservation efforts, such as road mitigation and contributing to the scientific community through analysis of individual morphometrics and genetic studies.

Unfortunately, the one juvenile Blanding's Turtle affixed with a radio-transmitter in 2020 within Parry Sound District was presumably lost due to another malfunctioning transmitter. It may have traveled too far away to pick up the signal, though this seems unlikely as we covered a wide area. We went out in January 2021 to try to locate this turtle but were unsuccessful. We also tried to track this turtle again at the beginning of May, to no avail. Field technicians performed a line survey of the wetland the turtle was originally captured in, in an attempt to find and capture the individual once again to remove the defunct transmitter; unfortunately this was unsuccessful.

It is our hope that we will be able to find this turtle and replace or remove this transmitter next season, since we survey this area frequently.

During nesting season, a Blanding's Turtle was found on the property of Scales Nature Park- the first time in our 19 year history! Permission was sought and granted to radio-track this turtle, since there are so few in the area and we hope to find more. Due to our previous experiences with transmitter failure, we opted to attach two different transmitters to this turtle. Since these transmitters are of a suitable mass for juveniles, putting two on a large adult female posed no problem. Currently she has been tracked from June 20 to November 18. She was tracked a total of 12 times, and captured twice. Her transmitter will turn back on for three weeks in January, for us to confirm her hibernation site. We are hopeful to find her nesting again to be able to headstart her hatchlings, as we did this year.

5.5 START Nesting Surveys (June-July)

EGBI funding was not used directly for activities pertaining to turtle nests. However, since our snake teams find turtles and our turtle teams find snakes, these efforts are synergistic. For this reason, some information on nesting season activities has been included here. More details are available if required. Please note that turtle nesting surveys are only performed along roads, so they also double as road mortality surveys during this peak time. However, our intent with these surveys goes well beyond counting dead animals and excavating turtle eggs. We actively work to mitigate road mortality of turtles, especially Blanding's turtles, through these intensive surveys and the resulting monitoring of females at roadsides. Details regarding this effort, and a preliminary analysis of data, may be found in section 10.

Eggs are the most vulnerable life stage of turtles. Most turtle eggs are eaten by predators, primarily mammalian mesopredators such as raccoons, foxes, and opossums. Although natural nest predation rates are high, artificially increased predation rates ("subsidized predation") in areas of human influence have been identified as a significant threat to turtles. Based on past nesting surveys and analyses done by our previous M.Sc. student Hannah McCurdy-Adams while at Laurentian University, the predation rate of turtle nests along roadsides is approximately 80% in Muskoka. It is unclear whether this rate is natural or not, as it is difficult to determine a natural rate in a modified landscape. Increasing hatching rates and recruitment into the population has been documented as an effective strategy to offset threats and slow or reverse turtle population declines. Since road mortality is a significant threat in our region, and subsidized predation may also be significant, at least in some areas, a goal of the project is to increase hatching rates by reducing predation of turtle eggs. However, it takes many turtle hatchlings to offset a single adult killed on a road, so directly reducing road mortality is more important than simply hatching more turtle eggs. Both strategies are used with this conservation project, since neither is likely to be sufficient in isolation in the foreseeable future.

Nesting season started in early June. Field staff and volunteers received additional training regarding nesting protocols. During nesting season, staff and volunteers conducted

evening (and sometimes morning) surveys along roads in addition to daytime surveys in wetlands. Road surveys were conducted by car, bicycle, or occasionally on foot. Hotline calls also led to nest locations along roads and on private land as well as females crossing the road before or after laying their eggs. All nest locations were recorded. Nests were classified as intact, predated, partially predated (some eggs remaining), or other (i.e. dug up by another nesting turtle, etc.). Where feasible, intact turtle nests may be protected with a nest cage, constructed of wire mesh, or wood and wire mesh, with exit openings for hatchlings. These cages can be effective at reducing the predation of nests by mammalian mesopredators. Due the concerns expressed by local municipalities in 2015 about nest caging on road shoulders, and our research results from 2014-15 regarding cages being run over, stolen, picked up and moved by the public, nests being destroyed by grading as well as results from other projects with stolen eggs, etc., we continue to avoid nest caging on road shoulders. Since 2015, most of our nest caging has been done on private lands, and roadsides outside of the right-of-way or where guardrails would protect the cages. Also, on private or public lands, there are places where it is unsuitable to cage nests, such as active construction sites, sporting facilities, and private driveways. All of these locations are deemed "precarious", in that caging is not feasible to protect the eggs, leaving them vulnerable to predators with a high probability of predation. Nests that cannot be caged are typically excavated and the eggs extracted for captive incubation. Eggs from partially predated or other nests are also recovered for captive incubation. Since the vast majority of our nesting survey effort is focused on roads and road sides, partially to help reduce road mortality of adult females, most of the nests that we encounter are excavated. We do not typically actively survey for nests in locations where we could cage them. Some eggs are also recovered from dead or injured turtles found on roads, though these aren't 'nests' in the strictest sense. Table 4 summarizes nest survey results. In total, 21,103 turtle eggs were excavated for incubation, and 16,726 hatchlings were released.

Another noteworthy find during nesting season was a female Blanding's Turtle captured in Severn Township, that we had previously captured and notched in Muskoka in 2017. This turtle crossed Highway 11, and travelled through multiple watersheds, for a total of 32 km straight line distance between these observations. This is an extremely large movement for this species. Observations like this are a benefit of our landscape scale approach to conservation.

Table 4. Turtle nest survey results for the 2021 season. These numbers partly reflect that Snapping Turtle nests are easier to find following the female leaving the nesting site. We also don't specifically target non-anthropogenic nest sites, which would be used by Spotted Turtle.

Species	Predated Nests	Caged Nests	Excavated Nests	Extracted Eggs
Blanding's Turtle	11	0	45	4
Map Turtle	0	0	29	0
Musk Turtle	0	0	0	0

Midland Painted Turtle	175	3	89	7
Spotted Turtle	0	0	0	0
Snapping Turtle	389	16	455	1
Total	575	19	618	12

6. Conservation Action, Research and Education about Snakes Project

6.1 CARES Project Activities

This year the majority of intentional snake surveys were focused on Massasauga Rattlesnakes. Due to their cryptic nature, broad habitat preferences, and wide distribution, Eastern Hog-nosed snakes were located incidentally during general surveys. Targeted surveys for Massasaugas are more useful due to their predictable habitat choices, basking behaviour, and limited home range size. Mid-season snake surveys are terrestrial in nature, often focusing on edge habitat and rock barrens that are adjacent to confirmed or suspected Massasauga hibernacula; however, during ingress (fall) and egress (spring) periods we survey suitable wetlands. Overall, this year's field season was focused on evaluating road mortality threats in the eastern Georgian Bay region south of Parry Sound, identifying and confirming hibernacula and gestation sites, monitoring gravid females at gestation sites, and filling data gaps.

Technicians were equipped with closed-toe footwear that is at least ankle high, such as rubber boots, neoprene moque boots, or hiking boots. Snake hooks, triangles and modified pillow cases were used to safely capture rattlesnakes. Clear plastic tubes were used to safely hold the snakes in order to obtain morphometric data, as well as allow the painting of rattles for capture-mark-recapture purposes.

Every team is amphibious in nature in order to allow for incidental turtle captures. CARES technicians carry wetsuits and binoculars in case of incidental turtle sightings. Technicians also carry morphometric tools needed to process both turtles and snakes, and any other sampling equipment required.

Both START and CARES Project data was recorded in the field, on water resistant Android tablets with daylight readable screens (Samsung SM-T360/T380) or on waterproof Android smartphones (Sonim XP8). We continued to restructure our custom database and unfortunately it was still not ready for use again this season . Instead, we continued to use customized cloud-based Google Sheets for this field season. This approach worked but once again did have some issues and a small amount of data was lost as a result and significant staff time was required to error check and correct any errors found. It is our hope that we will be able to use different software to collect data next year to decrease lost data and staff time. Getting our

custom database up and running has proven difficult with our programmer returning to school; we have been unable to recruit a replacement with the necessary skills.

6.2 CARES Egress Hibernacula Surveys (April-May)

There was typically one team per day focused on snakes at the beginning of the field season, as previous CARES Coordinator Josh Porter left our employment just prior to the start of the field season, and senior field technician (now Assistant Coordinator) Taylor Kennedy was already committed to an intensive spring survey on Pelee Island for Blue Racer and Eastern Foxsnake. This daily snake team consisted of Assistant CARES Coordinator Maria Ning and any available senior field technicians, who were already trained with rattlesnakes. Volunteers and junior technicians were also recruited in order to increase search effort of field sites, but their main role was to observe the presence of snakes in a site.

Following the acquisition of permits, the snake team focused on monitoring and confirming hibernacula, and surveying viable habitat to confirm new sites for Massasauga Rattlesnakes. Hibernacula are typically fen habitats with a moderate inundation of moss hummocks, and an appropriate saturation of water. Appropriate fens cannot be too saturated for risk of anoxia or freezing during the winter, or too dry as the snakes need access to the water table to survive the winter. Hibernacula were confirmed by observing Massasaugas that had not yet left their hibernation sites for the spring. Our first observation in a hibernation site was on April 24, 2021 in one of last year's confirmed hibernation sites. The first new hibernation site was found on May 13, 2021. The CARES Project observed rattlesnakes in 17 hibernation sites in 2021; of these sites 7 were new.

6.3 Additional CARES Training (June-July)

Training was provided by Assistant Project Coordinator, Taylor Kennedy, to select field technicians and volunteers on how to safely catch and process Massasauga Rattlesnakes, Eastern Hog-nosed Snakes, and Eastern Foxsnakes. Lead field technicians were trained to hook, bag and tube rattlesnakes for processing. Their partners were taught "secondary duties", which consisted of measuring, counting subcaudal scales and painting rattles to determine year and site of capture. Each year a unique colour is assigned to paint the proximal rattle segment, this year's colour was neon red, whereas neon green on the proximal rattle indicates the snake was caught in 2020. In following years, when a snake is caught, a new colour will be assigned. To identify the hibernaculum a snake uses, an additional segment was painted with a unique colour designated to that site. Painting the rattle allows technicians to visually identify if an individual snake has previously been captured, which prevents unnecessary recaptures and reduces the stress for the snake.

6.4 CARES Gestation Surveys (June-Aug)

We focused more effort on Massasauga gestation surveys, and CARES Project field

technicians were split into two separate teams to cover more ground. Taylor Kennedy led one team, and a second team was typically led by senior field technicians Meg Britt or Isabelle Summers. A trained pool of technicians cycled between the teams. These teams focused mainly on Severn township and southwestern Muskoka District. A third team led by Matt Rudland, stationed at our Bala field base (Johnston's Cranberry Marsh) surveyed for Massasauga gestation locations in that area, while a remote team, led by Jose Rodriguez surveyed in less accessible areas throughout Muskoka and Parry Sound Districts.

Gestation sites were considered confirmed if a female was present for 5 consecutive days. Sites were also confirmed if a female with a larger than average girth was observed late in the season. Gestating females were only processed if lead technicians felt that minimal stress would be incurred by the snake. Very few gestating females were processed from mid-July to mid-August to minimize stress during this crucial stage in fetal development. After site confirmation, females were monitored weekly or bi-weekly when possible until neonates were born. The CARES Project confirmed 70 Massasauga gestation sites for the 2021 field season with 92 gestating females. Of these 70 sites 56 of them were confirmed to be birthing sites. Through careful monitoring of a large number of females, we have noticed that some of them seem to leave their gestation site just prior to giving birth. They move to another suitable rock or crevice in the vicinity, where they give birth. We hope to study this behaviour in more detail in the future, as it could have an impact on the success of gestation site construction projects which are often created for mitigation purposes. .

Of note, a female ("Marta") who was observed gestating at a site in 2020 returned to the same location to give birth again this year. This is thought to be rare, as Massasauga Rattlesnakes are well-known to give birth biennially.

Switching the focus of some field technicians from turtles to snakes surveys during this critical period proved invaluable. Monitoring snakes on a landscape scale over our large permit area is a tall task, so a high level of search effort is required.

6.5 CARES Neonate Surveys (July- Aug)

Neonate snake surveys consisted of returning to confirmed gestation sites to capture and process neonate (young of year) rattlesnakes. The first neonates were found on August 1, 2021. As more neonates were born over the following weeks we added additional teams to help keep up with capturing and processing of these babies. When a clutch of neonates was found they were caught using small snake hooks, a triangle, and a modified pillow case, just as adults are captured. Neonates were weighed, measured, photographed, and released. We tried to mark neonates by painting their natal segment (button), but if this is done before their first shed, the paint is lost with the rest of the shed skin. This makes capture-mark-recapture with neonates challenging, as they typically disperse from the gestation site soon after their first shed. Dorsal patterns will be used for identification as a contingency to rattle painting, although it does require more staff time for analysis. In total, 555 Massasauga neonates were observed during the 2021

field season, a ~400% increase from last season's 110 neonate observations.

6.6 CARES Ingress (Sept- Oct)

Once neonate rattlesnakes were fully dispersed from their gestation sites, technicians began focusing on wetland and terrestrial edge habitat surveys. This time was used to resume habitat viability surveys of newly suspected hibernation sites, and to continue the monitoring of previously confirmed hibernation sites. Temperatures in the fall were above average, and this allowed technicians to survey confirmed hibernacula until the first week of November. The last rattlesnake of the season was found in a confirmed hibernaculum on October 6, 2021.

7. Habitat Protection

Another goal related to targeting Blanding's Turtles and Eastern Massasaugas is the generation of new observations in new locations to extend habitat protection under the Endangered Species Act (ESA). In 2021 we were able to continue extending our survey efforts to sites that had not previously been surveyed by the project, or had been unsuccessfully surveyed in the past. This led to an increased number of species at risk reptile observations in new areas. Other threatened and endangered species observations also trigger protection, including Spotted Turtle and Eastern Hog-nosed Snake. With these observations, we were able to build upon habitat protection from the last seven years and the project has almost completely covered southern Muskoka with habitat protection due to our species at risk observations. There were many new Blanding's turtle locations identified, resulting in significant wetland area protection under the Endangered Species Act (ESA). Geographic Information Science (GIS) mapping of all threatened and endangered herpetofauna encounters for the 2021 season was completed this fall and protected habitat resulting from threatened and endangered species observations was mapped as shown in Figure 2. The annual amount for 2021 was 4003.2 km^2, of which 669.2 km^2 does not overlap with that of previous years. Figure 3, shows the cumulative habitat protection triggered by all threatened and endangered species encountered from 2013-2021, which is now 4827.2 km^2. Table 4 shows a breakdown of habitat protection by taxa group and project year. More significantly, 4253.6 km^2 of this area was not previously protected through conservation reserves or provincial parks. This is equivalent to about two thirds of the land area of Algonquin Park, and is greater than the entire District of Muskoka. While this protection is not absolute, at a minimum it triggers requirements for mitigation or other measures to protect species at risk. We believe this to be a significant achievement for the conservation of these target species, and all other species that share their habitats across the Georgian Bay watershed.

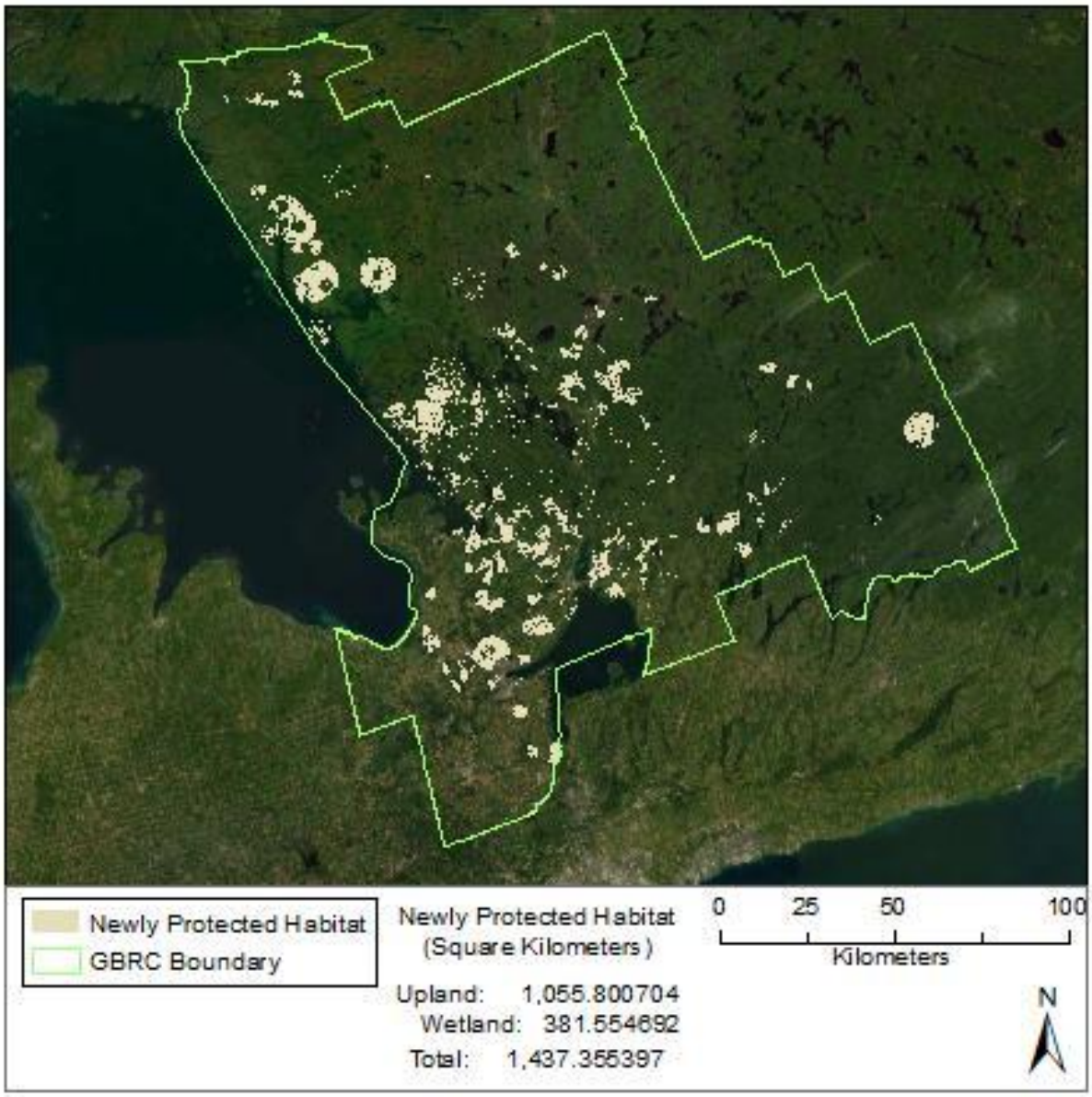


Figure 2. Map showing newly protected habitat resulting from 2021 observations of threatened and endangered species.

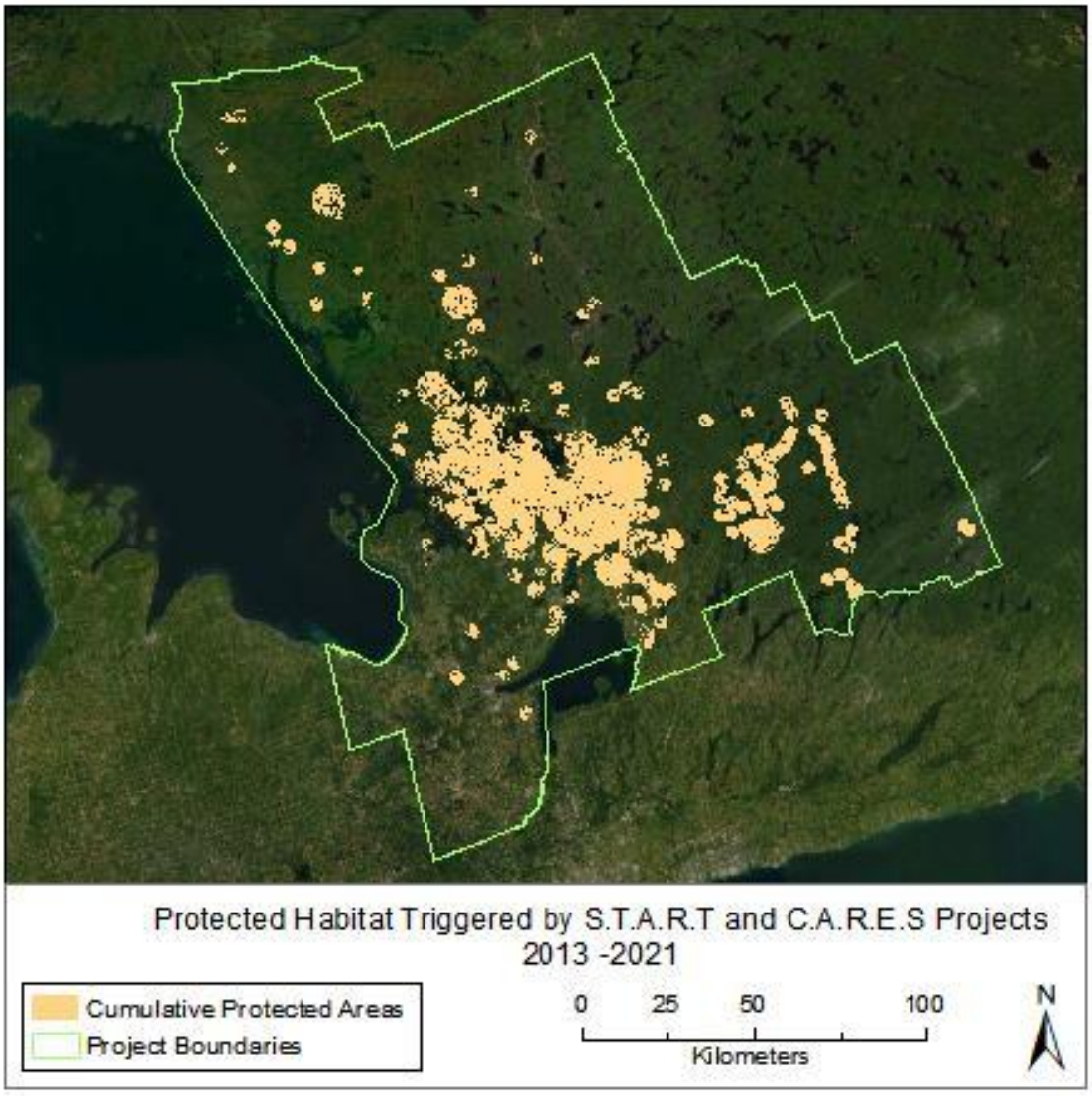


Figure 3. Map showing cumulative protected habitat resulting from 2013- 2021 observations of threatened and endangered species.

Table 5. A breakdown showing the designated habitat protection (in km^2) for threatened and endangered species due to eight years of START Project data collection, by taxa group (with overlap). Critical habitat was calculated from a species observation within 2 km for Blanding's Turtles, 1 km for Spotted Turtles, 1.2 km for Massasauga Rattlesnakes, and 5 km for Eastern Hog-nosed Snakes. The bottom row of totals represent the cumulative amount of protected area within the START study area, excluding overlap.

	2013	2014	2015	2016	2017	2018	2019	2020	2021
Cumulative Protected area by Turtle Observations	636.4	782.8	785.3	1060.5	1390.3	1634.0	1924.1	2746.8	3682.1
Cumulative Protected area by Snake Observations	0.3	352.7	624.4	755.2	906.4	982.7	1133.1	1616.9	1913.5
Cumulative Total (excluding overlap)	636.7	932.6	1067.6	1407.2	1732.7	1732.7	2126.7	3527.4	4827.2
Annual Increase	636.7	295.9	135.0	339.6	325.5	0	394.0	1420.1	669.1
Cumulative Total (outside of existing parks, etc.)	486.2	739.6	868.8	1165.8	1463.0	1463.0	1827.2	2759.5	4253.6

To give a better understanding of how protected habitat is designated, we will break down Blanding's Turtle habitat protection into the descriptive habitat categories. There are three categories under which Blanding's habitat can be designated in the OMNRF General Habitat Description for the Blanding's Turtle. Category 1 includes protection of nesting and overwintering habitat and the area within a 30 m radius of the occurrence. Category 2 includes the wetland complex (all suitable wetlands and water bodies within 500m of each other) up to 2 km away from an occurrence, and the terrestrial area within 30m around the suitable habitat. Category 3 encompassses a buffer zone from 30m to 250m around the suitable habitat areas identified in category 2, within 2km of the occurrence.

This can be done for Massasauga Rattlesnakes as well. Category 1 includes 30m around gestation sites and 100m around hibernacula. Category 2 protects open and semi-open habitats (include sparse forests, forest clearings, forest edges, fields, meadows, alvars, shorelines, rock barrens, and wetlands (such as fens, bogs, marshes and swamps),) within 1.2km of an observation. Category 3 includes all forests up to 1.2 km surrounding any rattlesnake observation.

8. Community Engagement

The reptile hotline continues to be a useful tool for getting local insight about places to survey, for recruiting volunteers, and for meeting landowners who give us permission to access their land. This continues to increase in effectiveness, with over 828 reports (calls/texts) in 2021, as discussed further below in section 8.3. This is a slight increase in contacts despite pandemic lockdowns and stay at home orders during the peak spring season.

New landowners continue to show interest in allowing us access to excellent habitat on private lands, due in part to our community involvement, and the turtle hotline. However, due to the pandemic restrictions, we had far fewer interactions with people during the course of our work and this limited our ability to explore many new properties this year.

The project generates a lot of interest in volunteering with our conservation efforts. Due to COVID-19, we were unable to accept many new volunteers that couldn't go through our rigorous quarantine protocol. The volunteers we did have included our trained community members, and biology students. Volunteers would accompany staff into wetlands to catch and process turtles, assist with data entry, etc, in addition to going out nightly with staff completing road nesting surveys.

Educational programming continued to be hampered by the pandemic, however 61 programs, most of which were virtual, were conducted over the course of the year. Most of these were prior to the spring lockdowns. More details are in section 8.2.

8.1 Community Volunteer Training (April - June)

This year we continued our training program for keen members of the public who were interested in becoming more involved in our turtle conservation efforts but were unable to participate in Boot Camp due to physical limits, full time jobs, etc. This two stage training process allowed community volunteers to become more actively involved in our work.

For stage one we educated 33 local community members in how to identify, handle and help conserve turtle species identified along roadsides. Training included the collection of accurate location data, road safety, usable digital images, and communications with the project turtle hotline dispatch. This training was specifically to provide better data for the START Project and to enable community members to be more involved in the conservation of reptiles. Additionally, community members were also trained in how to identify species at risk snakes and to report their sightings to the hotline. They were also trained to take tissue samples of dead specimens, as appropriate, for our partnership with Dr. Steve Lougheed at Queen's University. These volunteers were added to the list of names on our various permits.

A select group of 10 stage one community members were invited to participate in the second stage of training. This stage of training consisted of nest caging, egg excavation, and a better understanding of relevant permitting. The addition of these community volunteers allowed project activities to expand northwards, in addition to being able to respond to more hotline calls

quickly and to excavate more at risk turtle eggs.

8.2 Educational Programming

The Covid-19 pandemic continued to wreak havoc on our plans for public education and community engagement in 2021. Thanks to the support of the Eastern Georgian Bay Initiative in 2020, we were able to develop an education broadcast studio within our facilities to enable virtual programming for schools and other groups. This studio is equipped with a powerful computer, software, cameras, lights, and related equipment. It gives us considerable capability to conduct high quality virtual programming, though this requires four staff to conduct a program and considerable extra training compared to delivering in person presentations with two staff.

The studio became fully operational in January, 2021 and we began to conduct virtual programming for schools in the Georgian Bay watershed. Uptake was good and the program pace accelerated until April. We were on track to complete the previously proposed amount of educational programming. Unfortunately, the third wave of Covid-19 then caused province-wide lockdowns and stay at home orders, causing schools to be closed. Demand for the programs dried up and did not resume until mid-summer. We began some programming again in mid-July, at a slower pace though also adding some in-person programming at outdoor venues. Figure 4 shows the number and timing of programs completed over the season.

Staffing challenges have also contributed to our difficulties. In August, our Education Coordinator, Adam Moxley, left our operations to focus on his teaching career in the public school system. Educator Shawna Shiel was promoted to replace Adam. In addition to losing some wonderful summer staff in August, we also lost senior staff member Nathan Grant who had contributed greatly to program delivery. We tried to hire two new educators during the fall, but so far we have not been able to fill these positions- in part due to the pandemic related quarantine restrictions that our staff live within. Finally, our primary studio operator, Marta Aveta, got married in November and took a leave to visit family in Europe. As of early December, 61 programs have been completed- much less than we had hoped for this year. We hope to overcome some of these challenges, and catch up on program delivery in 2022. We have also proposed some alternative activities related to public education that could be done regardless of the pandemic. These are currently under consideration.

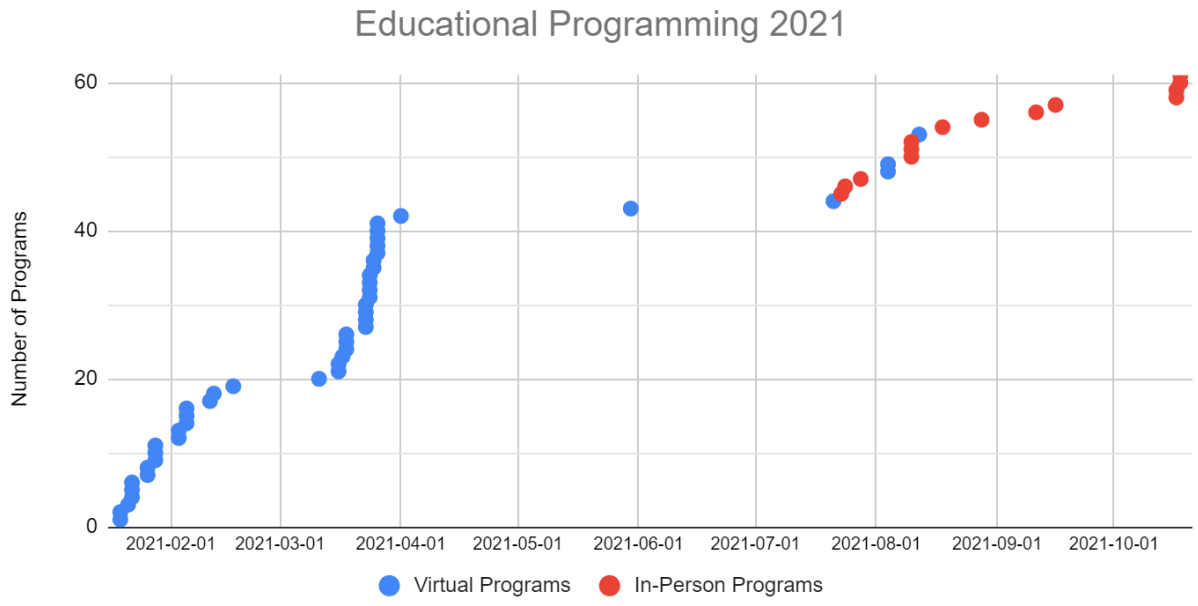


Figure 4. Graph showing number and timing of virtual and in-person educational programs completed over the season.

8.3 Reptile Hotline

The START Project hotline continued to gain traction this year. There were social media posts, and information provided at road-side stops during field work about how and when to call the number. Members of the public were encouraged to call this number whenever they encountered a 'rare, nesting, dead, or injured turtle' within our study area. Depending on the location, species, and circumstances, a research team would be deployed to the reported location to process the turtle and cage or excavate the nest. There were over 828 calls/texts in 2021, despite Covid-19 stay at home messaging and reduced travel due to lockdowns from April to June. Our busiest day occurred on June 7th with 53 calls in one day. Notable calls provided 161 Blanding's Turtles, 5 Eastern Hog-nosed Snakes, along with other species at risk observations.

As shown in Figure 5, hotline calls continue to generate a significant and increasing number of Blanding's Turtle (BLTU) observations, often in new areas where we would not have otherwise encountered them. We hope to continue the promotion and use of this hotline for the upcoming field season, including posting additional signage along roads that are hotspots for reptiles within the project area.

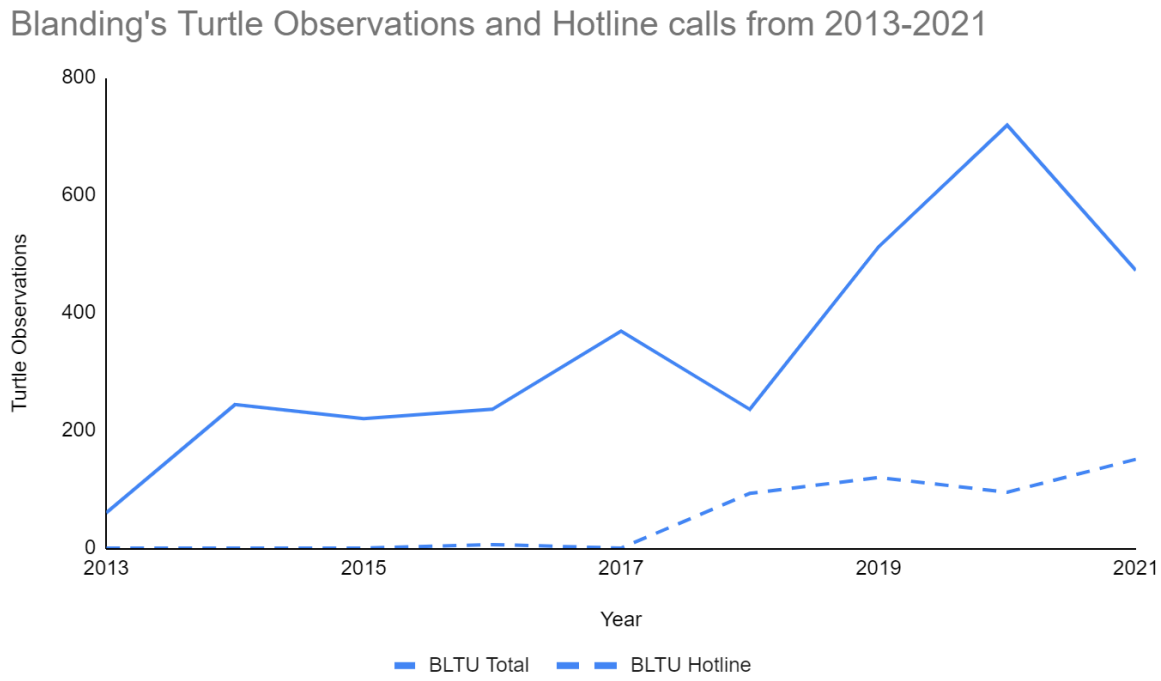


Figure 5. Total Blanding's Turtle (BLTU) observations (solid line) and Blanding's turtles observations from hotline callers (dotted line) in 2013-2020.

9. Observational Data

9.1 Observations

We record observations of all reptile and amphibian species, particularly species at risk (SAR). Typically, every individual observation of a species at risk is recorded. Species that are not at risk may only be recorded to confirm presence in an area, note calling dates, track road mortality, etc. In 2021, a total of 8127 individual observations of 32 reptile and amphibian species were recorded (Table 6). Of these, 4065 observations were of 10 different species at risk, which is similar to last year's number of species at risk observations. Of particular note, we put much more search effort into finding and processing Massasauga Rattlesnakes (due to the CARES Project), and this is clearly reflected in the numbers. This trend is also seen with Map Turtles, as the increased search effort in open water habitat in 2021 allowed us to process as many Map Turtles as we did in the past 8 years (2013-2020).

All of our observation data was submitted to the OMNRF Natural Heritage Information Centre and observations that occurred in Parks and Protected Areas were also submitted to iNaturalist under the Ontario Herps Project. Figure 6 shows the locations of all herptile species that we recorded in 2021.

Over the last 9 years of the project we've processed a total of 4031 SAR turtles within

our study area and observed 4352 more. The ratio of processed to observed individuals testifies to the great efforts of our staff and volunteers to capture and mark turtles, as well as the consistent strong leadership and training we have provided through Boot Camp. For the second year of the CARES Project we processed 659 snakes and observed an additional 1327 SAR snakes. These numbers easily triple the numbers we had processed and observed in the 2020 season.

Table 6. Species at risk totals for processed, and observed (not captured) in 2021 and between 2013-2021. These numbers partly reflect that we were surveying predominantly for turtles and snakes; lizard encounters were generally through incidental observations. This table does not include non-species at risk individuals, nor invasive species.

	2021			Cumulative 2013-2021		
Species	**Processed Individuals**	**Observed (not captured) Individuals**	**Total Individuals**	**Processed Individuals**	**Observed Individuals**	**Total: All Years**
Blanding's Turtle	339	235	574	1751	1279	3030
Spotted Turtle	79	15	94	427	61	488
N. Map Turtle	186	389	575	225	813	1038
Snapping Turtle	285	407	692	1616	2171	3787
Musk Turtle	3	8	11	12	28	40
Massasauga Rattlesnake	628	1185	1814	823	1613	2436
E. Hog-nosed Snake	30	40	70	37	108	145
E. Foxsnake	1	3	4	0	11	11
N. Ribbonsnake	0	99	99	0	455	455
Five Lined Skink	0	132	132	0	627	627
TOTALS	1551	2513	4065	4891	7166	12,057

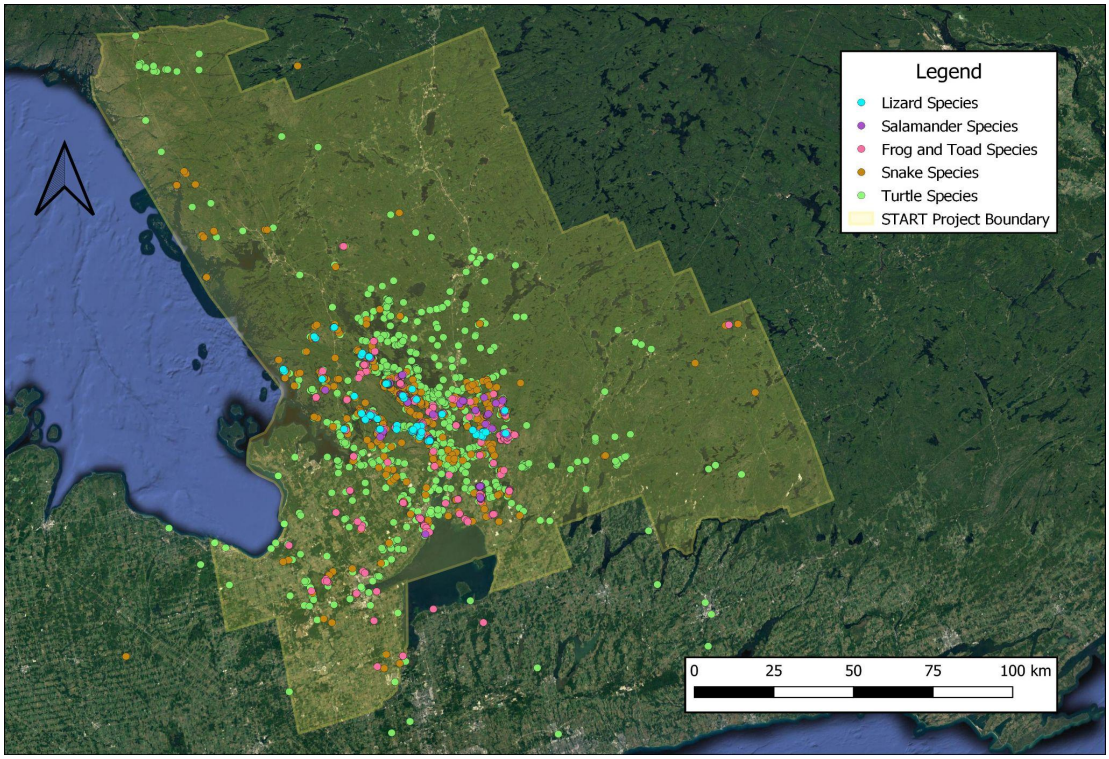


Figure 6. All herptile species (SAR, non-SAR individuals) that were observed and/or processed within the START/CARES project boundary in 2021. Please note, dots outside the project boundaries are only reports of observations.

9.2 Injured and Dead Reptiles

As in years past, this year we functioned as a transportation node for the Ontario Turtle Conservation Centre (OTCC), facilitating the movement of injured and rehabilitated turtles back and forth between the Georgian Bay watershed and Peterborough. Through START and CARES Project field surveys, public hotline calls and calls into the Scales Nature Park office, 64 injured turtles were collected to be transported to OTCC, or their approved drop off veterinarians, to receive treatment. Three turtles (1 Midland Painted Turtle, 1 Snapping Turtle and 1 Blanding's Turtle) were able to receive treatment at the Georgian Bay Turtle Hospital for minor injuries. Five Blanding's Turtles, 32 Midland Painted Turtles, 20 Snapping Turtles, 2 Map Turtles, 1 Musk Turtle and 4 unidentified species of turtles (unmarked on the documentation) came through Scales Nature Park before being transferred to the OTCC. Unfortunately, 13 of these turtles died while waiting for transportation to another facility for treatment. Ten died while in transport (7 while on route to Scales Nature Park and 3 on route to OTCC) and 7 died while in care at the OTCC. So far, 5 of the turtles that have received medical treatment have successfully recovered and were able to be released, 2 of which were rehabilitated by GBTH.

Injured snakes that survive to reach treatment, or beyond any initial treatment, are rare

compared to turtles due to their lack of a protective shell and the positioning of vital organs along most of their body length. This year, our field teams brought in 2 injured Eastern Massasaugas for treatment. One had severe head injuries and died. The other case was a suspected predator attack. While the injuries were significant, this snake has survived and will likely be released in the spring. Our Bala team also recovered some Eastern Hog-nosed Snake eggs that were exposed on a gravel driveway. Two of these hatched and the hatchlings were released.

Field technicians also came across 18 (7 Blanding's Turtles, 9 Midland Painted Turtles, 1 Snapping Turtle, 1 Map Turtle) recently deceased turtles where viable eggs were extracted and captively incubated for release at the Georgian Bay Turtle Hospital. One unique situation involved a gravid Snapping Turtle whose injuries were fatal and after careful consideration and liaising with our veterinarian, the decision to medically euthanize the individual was made. Her eggs were then extracted and incubated, leading to successful hatching and release of her babies.

There is still room for improvement in increasing the proportion of individuals 'hit by a car' that make it to treatment. Faster access to local treatment is likely to result in improved outcomes. The Georgian Bay Turtle Hospital has received a rehabilitation license and was able to treat a few turtles with minor injuries, as well as the snakes mentioned above. However, our resources are still limited and we need to send the more serious cases on to the Ontario Turtle Conservation Centre in Peterborough. We hope to continue to expand our capabilities for turtle treatment in the coming years so we are able to take on more difficult cases.

We found another 680 turtles, 560 snakes and 1251 amphibians that were already dead (or dying) on the road or roadside. Ten individuals (1 Midland Painted Turtle, 2 Blanding's Turtles, 1 Massasauga Rattlesnake, 5 other snakes and 1 amphibian) were found near death, having previously been hit by a vehicle, with no chance of survival from treatment. These were field euthanized upon discovery, after receiving authorization from Jeff Hathaway or Kelsey Moxley. Over the past few years we have seen increasing numbers of dead turtles (as well as snakes and amphibians) near or on roads. This may be a result of our intensive road mortality walking surveys, completed biweekly and now weekly for snake surveys, or it may be a result of us extending the project further to areas where we have had little effort in the past. Other factors may play a role. Section 10 discusses road mortality in more detail, with respect to our efforts to mitigate it through targeted road surveys.

During the field season, tissue samples of most dead species at risk individuals were collected by field technicians and frozen. Tissue samples or whole carcasses are currently housed in our freezer pending transfer to Dr. Stephen Lougheed at Queen's University. We are currently writing a joint proposal with Dr. Lougheed to support a PhD student who will use a genomic approach to studying Eastern Massasauga ecology in the Georgian Bay population.

10. Road Mortality Mitigation

Since 2013, we have had a growing presence in Simcoe-Muskoka and beyond, with an

extensive amount of effort spent patrolling roads in search of turtles- especially nesting females. This allows us to monitor road mortality, assess hotspots, guard female turtles while nesting, and excavate eggs along the road shoulders. While these activities are individually important, we have been working towards a larger goal- to directly mitigate turtle road mortality through our presence on the roads during critical time periods. With enough search effort, we should be able to find more alive turtles, and prevent them from being hit. This effort may take the form of driving cars, bicycling, or walking, and may be done by paid staff or trained volunteers. The logic is simple. If we drive a road once a year, the odds of finding a live turtle are low, but the odds of finding dead turtles are much higher, since dead turtles are persistent- carapace pieces may be easily found months after death. If we drive the same road once a month, the odds of finding both live and dead turtles will increase. However, the number of dead turtles found will not increase in direct proportion to survey effort, as dead turtles found on one trip will not be available to find later. More effort will likely result to some degree in finding more dead turtles as it may increase the odds of finding some since not all dead turtles persist on the road for long periods of time. Some are removed by scavengers or people. However, this relationship should decrease with increasing search effort after some threshold. More effort should equal more live turtles, and nearly all live turtles found are then prevented from becoming dead turtles through careful watch. Note that this is not 100% successful- we have had turtles run over and killed despite our efforts.

As the search effort scales up, the trends should become noticeable. If a road is driven multiple times per day, we should find the number of live turtles increasing and the number of dead turtles leveling off. This may be best examined using the proportion of alive/injured/dead turtles found, as more effort will, to some extent, increase the numbers of all categories. Note that roads of different characteristics (traffic volume, speed, surfacing) are likely not comparable, as the search effort relative to the odds of a turtle being killed are different. For example, on a gravel road with little traffic, a turtle may have a low chance of being killed even if we rarely survey that road. However, on Highway 11, a turtle may have a high chance of being killed even if we drive up and down the highway incessantly. We simply cannot get to it fast enough to prevent mortality with such a high volume of traffic.

We have not yet examined this approach for snakes, though we suspect that it will be less effective since snakes are not persistent along roads compared to nesting turtles, or compared to dead turtles. Alive snakes tend to keep moving, and dead snakes are more likely to be completely removed by scavengers, rendering them undetectable later.

We are presenting here for the first time, some analysis of this effort. We stress that this remains a preliminary evaluation of data from 2013-2021. Road-associated observations of alive/injured/dead turtles from April 1 to July 15 were used. This captured the time period of most road surveys, with consistent effort within each year, but varying somewhat between years. Following nesting season, we do not specifically patrol roads so the search effort would be too variable. A future refinement will be to use the specific dates of nesting season, which varies with the weather, to reduce the chance of skewing the results due to varying search effort. Turtle

observations included in this analysis consisted of turtles on the road surface or road shoulder, or in a dry ditch, if they were observed as part of a road survey. Any observations resulting directly from a hotline call, the activities of community volunteers, or specifically targeted walking surveys (see section 4.1) were excluded, to focus only on the effects of routine surveys that are similar across years and roads.

Also, the search effort values used so far are qualitative rather than quantitative, being divided into categories of low, medium, high and very high, with each road being assigned a value based on our estimation of how much search effort it had. In general, a low value would indicate that the road was driven once per week, medium would be several times per week, high would be once or twice per day, and very high could be 3-5 or more times per day. This is applied to the whole road, despite some segments of the road being driven much more than others. Another refinement will be to quantify the search effort based on our location tracking of each time. This will allow a total number of trips to be calculated for each road segment. With those caveats in mind, some results are included here. Figure 6 shows the proportion of alive/injured/dead turtles, for a subset of 6 roads with similar characteristics. All are two lane, paved roads with 80 km/h speed limits. At low search effort, about ⅔ of turtles found were dead, with ⅓ found alive. As the search effort increases, these proportions equalize and then nearly reverse.

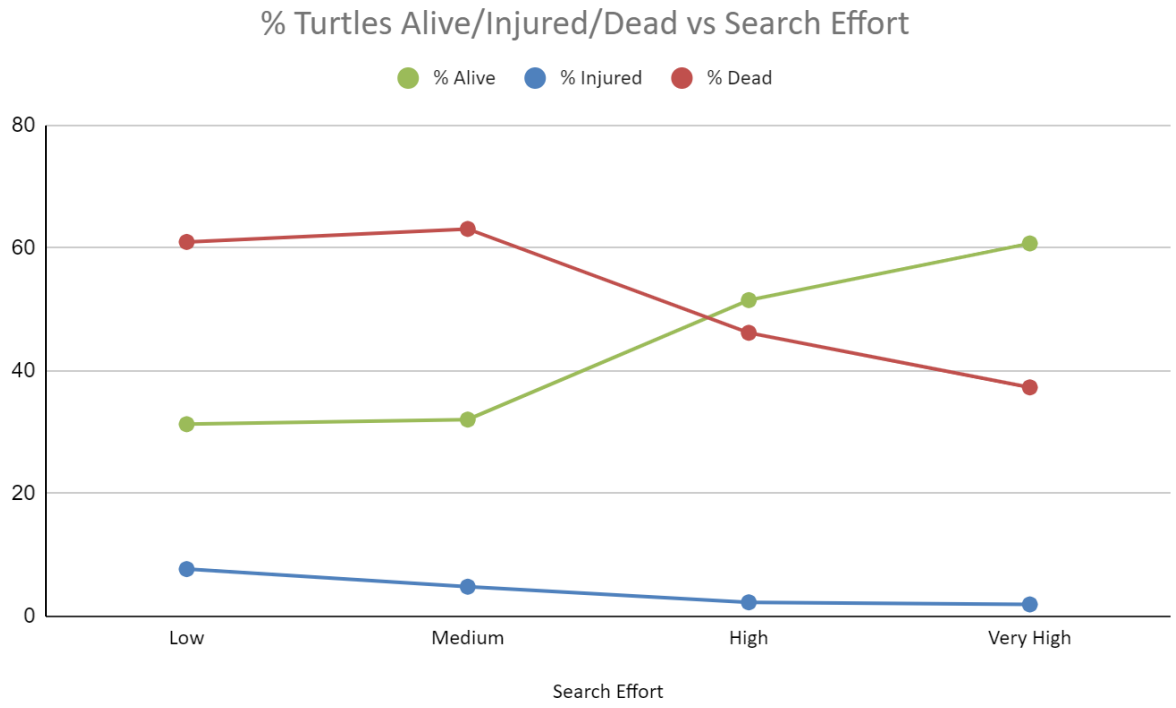


Figure 6. Proportions of alive, injured, and dead turtles found on six roads from 2013-2021 based on search effort.

This is the pattern we hoped to see. Also, the proportion of injured turtles dropped. These results suggest that the threat of road mortality, along roads with these characteristics, can be mitigated by the presence of trained personnel who can move and chaperone turtles. This is especially important for these paved, two lane roads, which are numerous and are a significant source of mortality for adult turtles. Installing exclusion fencing or other mitigation structures on all of these roads would be a Herculean task, with enormous costs. Although we think that fencing is an excellent approach, it is not going to occur on these roads in any reasonable time frame. We suggest that our approach is more cost effective, more flexible, and faster to enact, and can be used effectively to mitigate road mortality.

The results from individual roads suggest that search effort by trained staff and volunteers can make a difference for turtles along these roads. Figure 7 shows the average number of turtles encountered on Housey's Rapids Road during years of Low, Medium, and High search effort, while Figure 8 shows the proportions of Alive, Injured and Dead. As the search effort increases, more turtles, and more alive turtles, are found.

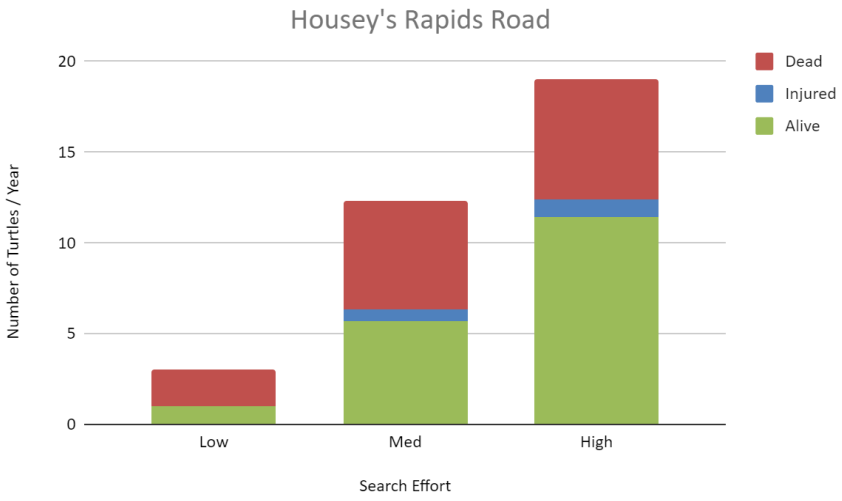


Figure 7. Graph showing the number of turtles encountered per year based on search effort for Housey's Rapids Road.

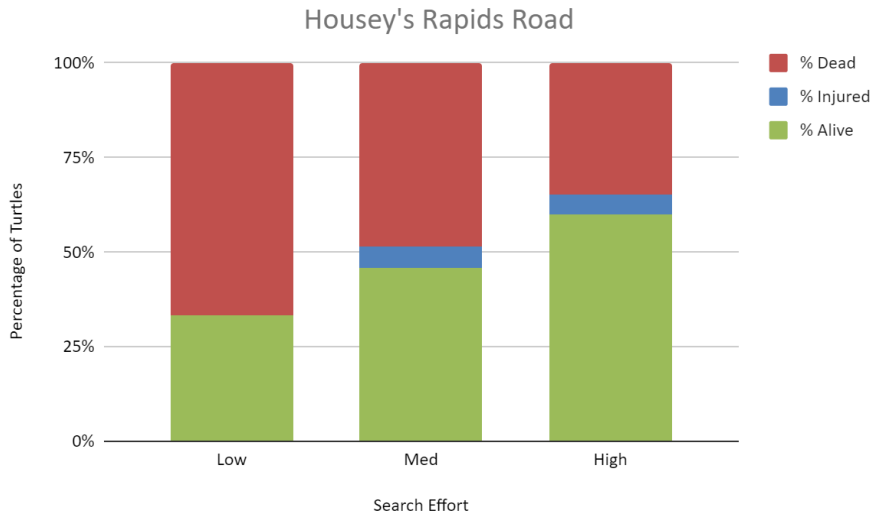


Figure 8. Graph showing the percentage of turtles encountered based on search effort for Housey's Rapids Road.

Upper Big Chute Road (Figures 9 and 10) shows a similar trend, as did the other four roads used in the pooled analysis.

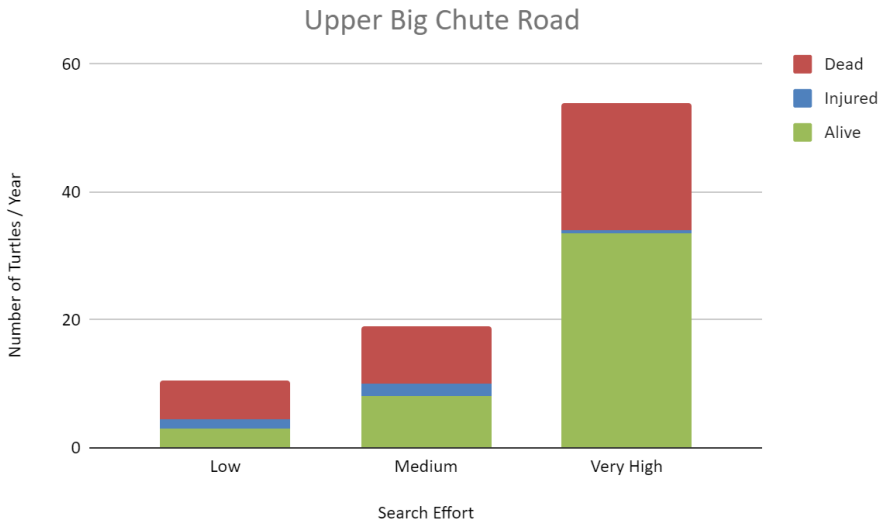


Figure 9. Graph showing the number of turtles encountered per year based on search effort for Upper Big Chute Road

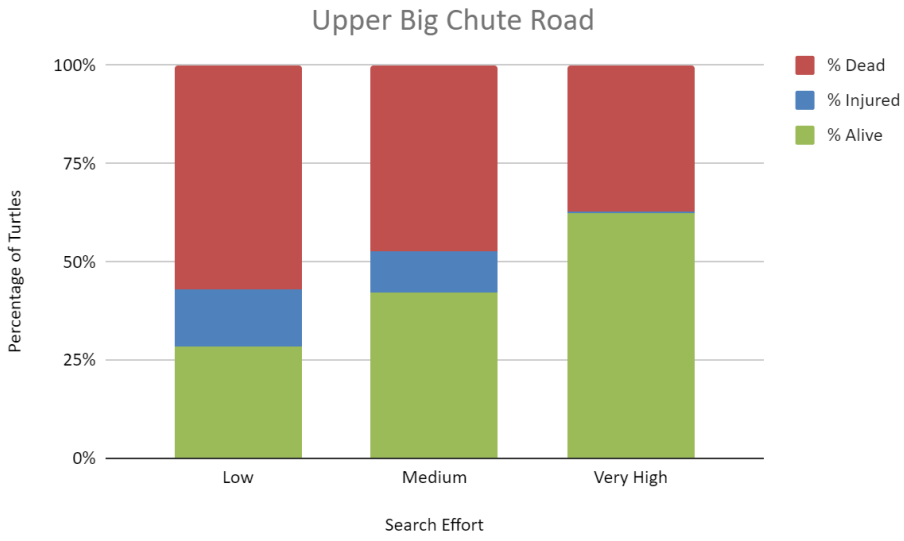


Figure 10. Graph showing the percentage of turtles encountered based on search effort for Upper Big Chute Road.

Doe Lake Road's results (Figures 11 and 12) are the most dramatic, though there is only one year with Medium search effort and no years with Low effort. With Very High search effort, 75% of turtles are found alive.

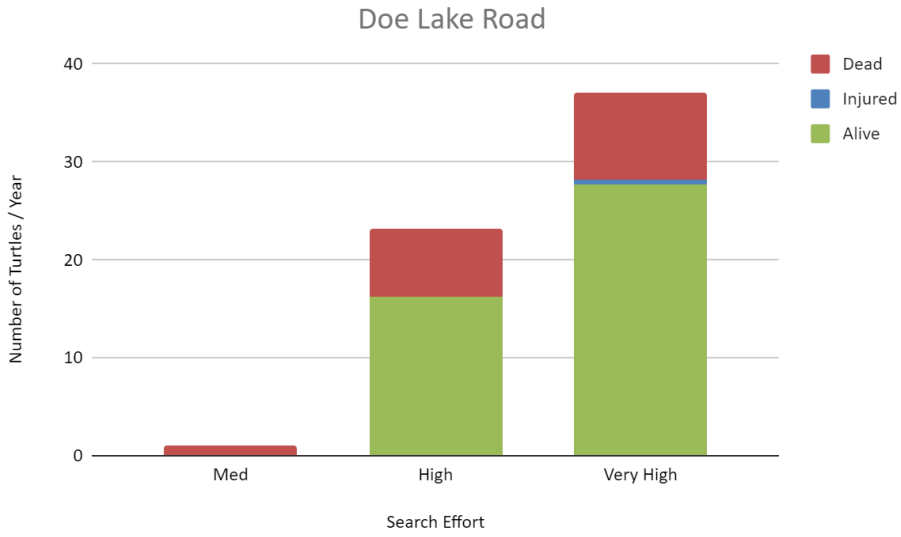


Figure 11. Graph showing the number of turtles encountered per year based on search effort for Doe Lake Road

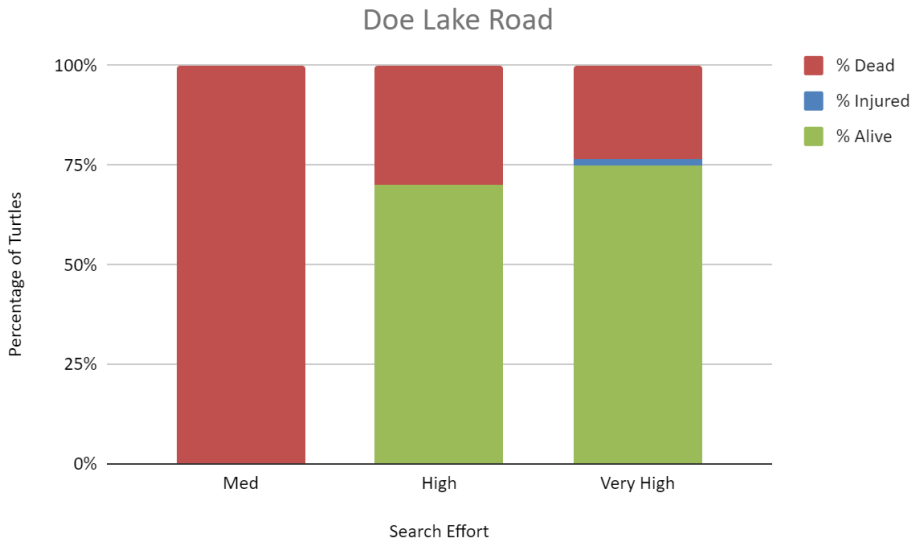


Figure 12. Graph showing the percentage of turtles encountered based on search effort for Doe Lake Road.

In contrast, for Highway 11, which is partially controlled access, with heavy traffic and a 90 km/h speed limit (with many cars exceeding this), our results are not encouraging. While the trend is present, as shown in Figure 13, it is slight at best and still approximately 75% of turtles encountered are dead. We do not expect that this kind of road mortality mitigation can be effective on a highway of this nature. We believe that exclusion fencing would be the only effective solution for such highways.

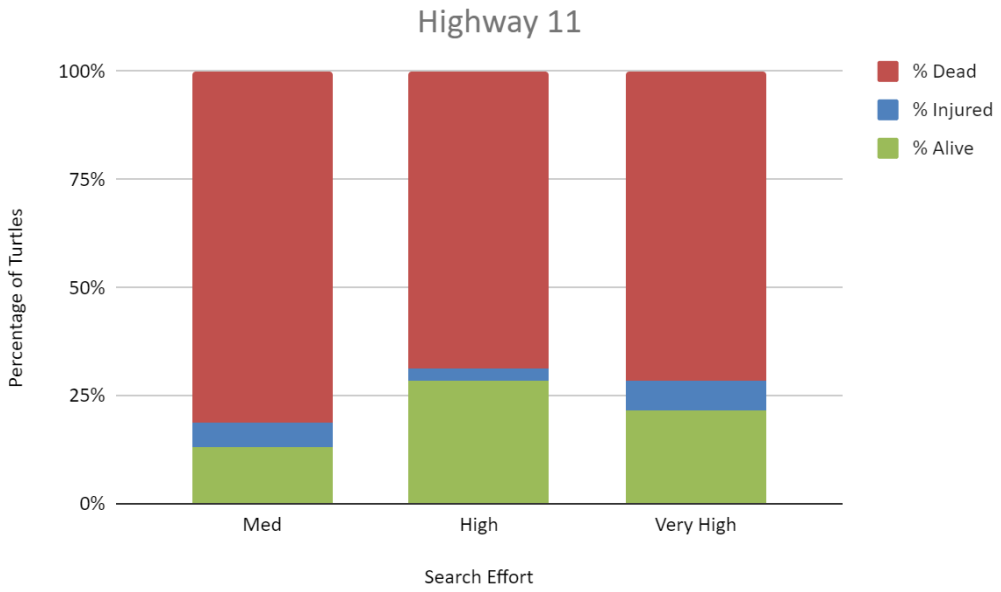


Figure 13. Graph showing the percentage of turtles encountered based on search effort for Highway 11.

And further, for gravel roads, with low traffic, there appears to be little generalized need for mitigation efforts though it would likely be useful in specific hotspots. The number of dead turtles encountered on such roads is quite low. Figure 14 shows one example of such a road. As mentioned previously, there may be other reasons to survey these roads, such as to monitor known nesting sites or other hotspots, capture and mark species at risk, etc. but it is not needed for general mitigation purposes.

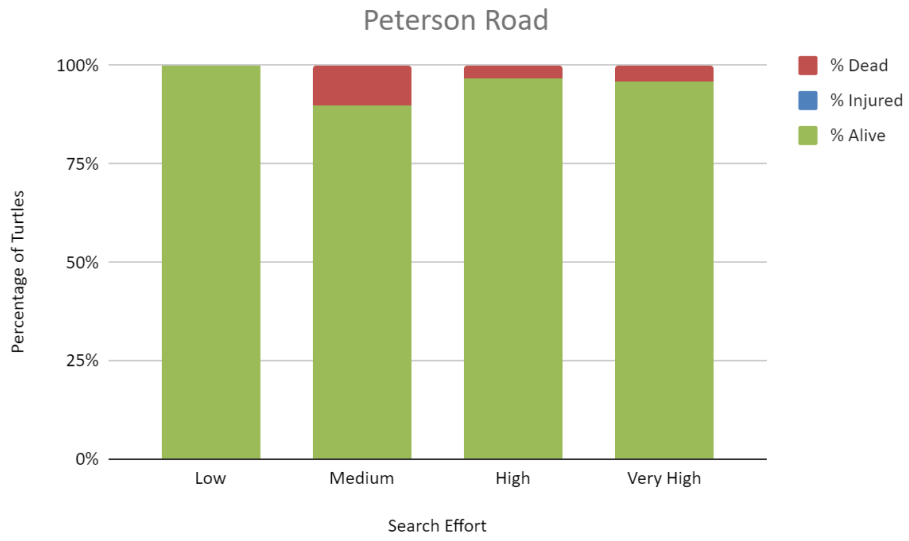


Figure 14. Graph showing the percentage of turtles encountered based on search effort for Peterson Road.

We look forward to refining this analysis further through quantifying search effort more accurately, and excluding dates when survey activity would not have been consistent such as between the end of each year's nesting season and July 15. We also plan to add in the effects of public hotline calls and community volunteer activities, as these would add to the overall conservation effort occurring on these roads. Further, we are undertaking a cost-benefit analysis to compare the cost of this effort with the potential cost of installing and maintaining fencing along these roads.

Given the promising results of this preliminary analysis, we hope to continue to increase search effort along the roads of central Ontario through recruiting and training more community volunteers, collaborating with other conservation organizations, and adding more staff teams to cover new roads in new areas.

11. Research Partnerships

As the project completes its ninth season, there is a large and growing dataset available to better understand the ecology and conservation of species at risk reptiles in central Ontario.

Field Projects Coordinator, Kelsey Moxley is working towards utilizing this data. Currently Kelsey is working with a former staff member to complete a population demography for central Ontario Spotted Turtle populations; one of which is believed to be one of the largest populations in Ontario. This will include population density, sex ratios, body size, etc. of these populations and compare them to their southern population counterparts.

Additionally, Kelsey, Maddie Kellett (former staff member) and Scott Gillingwater have

written a manuscript for submission on facial colouration differences between male and female Blanding's Turtles. This will aid in sex identification of Blanding's Turtles without the need to capture or handle the individuals. Also, there is no published data on their facial colouration, and we hope to fill this knowledge gap. Our examination into the sexing of Blanding's Turtles using facial colour and patterning has been completed and a scientific paper has been written regarding our findings. It is now in the submission and review process, which we hope will be completed in early 2022.

One of our past conservation technicians has continued to develop a predictive model to determine which nights of nesting season will be the most active. With the use of the START Project dataset, this technician is analyzing which parameters (i.e. air temperature, precipitation, etc.) may best predict turtle nesting behaviour. If any positive correlation prevails, construction of a predictive model will occur, using the correlated factors to help predict which nights during nesting season will be the busiest. This will aid with the deployment of field teams to target the most active and effective areas within the larger survey area. Specifically for this project, this predictive nesting model would help to reduce driving and fuel consumption, as there would be less reason to send as many field teams out during less active times. On a larger scale, this would aid conservation efforts across the province.

One of our staff utilized nesting behavioural data for Blanding's Turtles from the 2019-2021 nesting seasons (May-early July), to investigate the optimal conditions under which we find nesting females of this species. Through this technician's analysis, we found that Blanding's Turtles nest more frequently when air temperatures are above 19 ℃ and recent precipitation (within 24 hours of nesting) had no influence over nesting behaviours. These findings can help us forecast when the nesting activity for Blanding's Turtles will escalate, allowing us to plan our nesting survey efforts more effectively in the future. We intend to extend this analysis with data from future years of the START Project to confirm these initial findings and further refine our forecasting ability.

We are continuing to work with Dr. Chantel Markle, of McMaster University, who has published a paper on 'fingerprinting' Blanding's turtle plastron markings with photographs from our dataset. While plastron patterning can be used to identify individuals, doing this manually is very time consuming once many turtles are involved. We used a digital image recognition process to automate this process. This paper can be found online at https://cwbm.ca/using-the-blandings-turtle-emydoidea-blandingii-plastron-as-a-fingerprint-photo-identification-of-an-endangered-species/. The citation for this paper is:

> Markle Chantel E, Law Timothy, Freeman Hope C.A, Caverhill Brennan, Davy Christina M, Hathaway Jeff, McNeil Jeffie, Moxley Kelsey, Richer Sarah and Chow-Fraser Patricia. Using the Blanding's Turtle (*Emydoidea blandingii*) Plastron as a 'Fingerprint': Photo Identification of an Endangered Species. Canadian Wildlife Biology & Management. 10:2 p.47-60.

We are now working towards building a large scale image bank of Blanding's Turtles which can hopefully be used to identify Blanding's Turtle individuals in the field through this image recognition software, which may, in the future, alleviate the need for shell notching to mark individuals. This system is now operational and the results are impressive with 90% accuracy within the top 3 matches identified and 99% within the top 10. We hope to pursue field trials for this system in 2022. Additionally, we are working with Chantel towards determining features dictating wetland suitability and occupancy across the surveyed region for Blanding's turtles. With the addition of her own data, she believes this is the largest wetland survey dataset (at 415 unique wetlands) for species at risk turtles in the Georgian Bay watershed.

Early in 2021, a paper was published in the open-source journal Genome about our previous work with Scott Tarof and Steven Crookes on using environmental DNA to detect Blanding's turtles using a portable rapid sampling process. This paper can be found online at (https://cdnsciencepub.com/doi/full/10.1139/gen-2020-0043). This work demonstrated tremendous accuracy at detecting Blanding's turtles in a wetland during winter by extracting, analyzing and matching DNA from 1 L water samples collected from various field sites. The START project data and resources to enable sampling were pivotal to the success of this collaborative effort. The citation for this paper is:

> Tarof SA, Crookes S, Moxley K, Hathaway J, Cameron G, Hanner RH. Environmental DNA bioassays corroborate field data for detection of overwintering species at risk Blanding's turtles (*Emydoidea blandingii*). Genome. 2021 Mar;64(3):299-310. doi: 10.1139/gen-2020-0043. Epub 2021 Feb 4. PMID: 33538216.

We are currently writing a joint proposal with Dr. Stephen Lougheed at Queen's University to support a PhD student who will use a genomic approach to studying Eastern Massasauga ecology in the Georgian Bay population. This student will likely be one of our current field technicians, Meg Britt.

We are also currently developing a joint project for an M.Sc. student with Dr. Jacqueline Litzgus at Laurentian University to undertake our previously delayed Spotted Turtle radio-telemetry work at our best site for this species in Muskoka. Partial funding for this work has been secured, though a suitable candidate has not yet been identified.

12. Conclusion

We believe the Saving Turtles at Risk Today (START) Project and the Conservation Action, Research and Education about Snakes (CARES) Project have both continued to expand successfully. We hope to continue and improve upon the use of conservation tools, new technologies, and effective community engagement to slow or reverse species at risk population declines through threat mitigation, habitat protection, boosting recruitment and public education. We further hope to amass a long-term dataset for reptiles and amphibians, specifically focusing on species at risk turtles and snakes in central Ontario, to enable a better understanding of

populations, habitat usage, threats, and conservation actions that may be undertaken. We continue to discuss the future goals of the project with several key researchers and partners. By welcoming academic input into the project, we aim to continue to gather useful data and conduct valuable research to aid in the conservation of turtles and snakes in the area, and across Ontario.

We plan to attract additional graduate students and funding as the project moves forward, and are especially seeking candidates for projects specifically involving the radio-tracking of adult spotted turtles and juvenile Blanding's turtles, and Massasauga rattlesnake ecology and genomics, and genomic analysis of parental lineage in Blanding's and Spotted turtles across the study area.

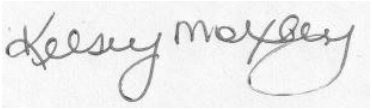
We have confirmed funding with the Rogers Foundation through the Canadian Wildlife Federation for one more year. We also plan to apply for other significant funding programs and the outcome of these applications will define how the project proceeds in 2022 and beyond.

Overall, we feel that the START Project has been a resounding success throughout the past 9 years, and specifically this year as described above. The second year of the CARES Project has gone extremely well. We look forward to the continued development of our reptile conservation efforts.

Sincerely yours,

Jeff Hathaway, START Project Director

and



Kelsey Moxley, Field Projects Manager