

ARTICLE

Long-term telemetry study monitoring health parameters and behaviour of eastern box turtles (*Terrapene carolina carolina*) released from treatment and rehabilitation

Sarah Zurbuchen, Holly Amato, Emily Tucker-Retter, Ronald Kent Passingham & Gregory A. Lewbart

North Carolina State College of Veterinary Medicine

Abstract

In this pilot study, 16 rehabilitated eastern box turtles (*Terrapene carolina carolina*) had radio transmitters attached to their carapace and were being monitored for the life of the transmitter (average lifespan of 283 days). Turtles in this study were presented to North Carolina State University's Turtle Rescue Team (TRT), an organization that cares for wild, injured and ill turtles, to triage, rehabilitate and release them back into the wild. In the years from 2005 to 2014, 897 turtles were released after treatment, which accounted for 47.1% of admissions; this percentage remains the trend. There has never been a long-term study tracking the survival and health of turtles once released from TRT's care. At the study's conclusion, one turtle was successfully followed for the entire tracking period. Three turtles were lost after a period of torpor (a state of inactivity during the winter months). Three turtles died sometime after release (mean 86 days after release). Five turtles have not been found since their release and are believed to have travelled a significant distance from their release site. Three turtles were found once after release, but not in consequent searches. One of the radio transmitters is known to have stopped emitting a signal for unknown reasons. Most commonly, the turtles have been found motionless and unboxed. All of the turtles located were found within 722 m of their release sites. This study is the first step in developing more effective protocols to estimate the survivability and behaviour of the turtles released from rehabilitation settings.

BIO

Sarah Zurbuchen is a fourth-year veterinary student at North Carolina State University with a focus on zoological medicine and pathology. She received her undergraduate degree in Zoology from North Carolina State University with a minor in Wildlife Sciences. Throughout veterinary school, Sarah has been an active member of the Turtle Rescue Team as a case manager, Outreach Coordinator and researcher. She has also spent time learning at a Florida wildlife rehabilitation centre and working at a local veterinary ER that triages wildlife. After graduation, Sarah intends to pursue a residency in anatomic pathology specializing in wildlife disease.

Holly Amato is a second-year veterinary student at North Carolina State University with a bachelor's degree in Animal Science and Plant Biology and certification in wild plant identification from NCSU. She is currently the rehabilitation coordinator for the NCSU Turtle Rescue Team, where she has volunteered since 2017, contributing to the treatment, rehabilitation and release of turtles. In the summer of 2021, she completed a research assistantship tracking tamarin monkey groups with telemetry in the Peruvian Amazon to study group range and structure.

Dr Tucker-Retter is currently a fourth-year veterinary student at North Carolina State University College of Veterinary Medicine with an interest in aquatic animal medicine. In 2019, she completed her Ph.D. at the University of Illinois on fish ecophysiology and invasive species biology. She recently served as co-President of the Turtle Rescue Team and has worked extensively with injured turtles in clinical and research settings. Following graduation, she intends to pursue a career in fish medicine and clinical research.

Dr Greg Lewbart has a B.A. in biology from Gettysburg College, an M.S. in biology with a concentration in marine biology from Northeastern University and a V.M.D. from the University of Pennsylvania School of Veterinary Medicine (1988). He worked for a large wholesaler of ornamental fishes before joining the faculty at the NC State College of Veterinary Medicine in 1993, where he is Professor of Aquatic, Wildlife,

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Correspondence

Sarah Zurbuchen szurbuc@ncsu.edu

Abbreviations

EBT: Eastern box turtle
IACUC: Institutional Animal Care and Use Committee
GPS: Global positioning system
PCV: Packed cell volume
SD: standard deviation
TRT: Turtle Rescue Team

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and Zoological Medicine. He is a diplomate of the American College of Zoological Medicine and the European College of Zoological Medicine. Greg is the author of over 200 popular scientific articles and speaks locally, nationally and internationally. He has authored or co-authored over 30 book chapters and edited or co-edited five veterinary textbooks. Greg has made 26 trips to the Galápagos doing conservation work on a wide variety of native species.

R. Kent Passingham is a zoological Medicine Research Specialist at North Carolina State University College of Veterinary Medicine. He received his B.S. in Animal Science from North Carolina State University in 2001. He completed 10 years of research experience in haemophilia and diabetic studies at the University of North Carolina-Chapel Hill Frances Owen Blood Research Lab. He has 10 years of experience at North Carolina State University College of Veterinary Medicine in zoological research,

clinical work and teaching. He is a Turtle Rescue Team staff liaison and mentor. He is the co-author of over 10 zoologic species publications.

Introduction

A critical aspect of wildlife medicine and rehabilitation is the successful release of rehabilitated animals. This is generally defined as the effective reintegration of the animal into the wild including the exhibition of normal behaviours and future breeding success (Mullineaux 2014). One of the most overlooked aspects of the wildlife rehabilitation process is post-release monitoring (Hall 2005; Mullineaux 2014). In addition to determining patient success, post-release studies also help determine successful medical management and rehabilitation techniques that should be implemented at wildlife rehabilitation clinics. Post-release monitoring can include identification markings such as rings, bands, tags, or tattoos that are then returned to a long-term organization (Mullineaux 2014). Radio telemetry is a tracking method with the advantage of being more cost-effective than alternative technologies (Cain & Cross 2018) but comes with a high time investment that can limit the amount of temporal data that can be collected (Mullineaux 2014; Cain & Cross 2018).

Turtles in this study were presented to North Carolina State University's TRT, an organization that cares for wild, injured and ill turtles, to triage, rehabilitate and release them back into the wild. Between 2005 and 2014, 897 turtles were released after treatment, accounting for 47.1% of admissions (Sack et al. 2017). The TRT has not previously undertaken a large-scale tracking and survivability study in its 27-year history. On occasion, individual turtles have been monitored with radio telemetry (Cerreta et al. 2017).

The EBT (*Terrapene carolina carolina*) is listed as vulnerable by the International Union for Conservation of Nature and listed as a CITES Appendix II species, controlling its international trade (van Dijk 2011). In North Carolina, where this study occurs, habitat fragmentation and the building of roads are cited as the main causes of mortality for the EBT (Burge & Jones 2010). In the Southeast, it is estimated that road

mortality accounts for 5% of overall mortality among land turtles (Gibbs & Shriver 2002). Road mortality represents a large proportion of turtles that present to the TRT for vehicular trauma, accounting for 63.2% of intakes (Sack et al. 2017).

The EBT is a long-lived species. They have a life span of 25–35 years in the wild (van Dijk 2011) and do not reach sexual maturity until 7–10 years of age (Burge & Jones 2010). In species with late maturation and low overall fecundity, treatment of injuries, rehabilitation and release can be an important way to conserve the population (Paterson et al. 2021). The successful release of hundreds of turtles from the TRT each year may contribute to the native box turtle populations in North Carolina, especially in the urban Research Triangle area where many of our patients are released. In a recently published 10-year population monitoring study, it was found that the greatest decline in populations of EBTs across the state of North Carolina was in urban areas with small patches of habitat surrounded by urban development (Roe et al. 2021), describing the conditions in the Triangle region.

The aim of this study is threefold: 1) to monitor the survivability of EBTs released from TRT, 2) to assess basic health parameters and behaviour of those turtles with consideration of their presenting complaints at the clinic and 3) to initiate a sustainable tracking programme that can be used for future patient monitoring.

Materials and methods

Study participants

Sixteen EBTs released from TRT were included in this study. Turtles were selected based on several logistical parameters. The period in which turtles were tagged and released ranged from 28 May to 20 September 2021. The turtles selected for the study were deemed fit for release by both the rehabilitation coordinators and the turtle's case manager, as is standard for any release at TRT. All turtles had to have been found within one hour's driving distance of the North Carolina State Veterinary Medical Campus to facilitate regular tracking. The turtles had to live on public land or, if on private property, landowners

needed to agree to regular tracking of the turtle for the length of the study (this was the case for turtles 2021-149 and 2021-258). Turtles also needed to weigh at least 100 g to ensure the transmitter was not over 7% of their body weight, as described in similar studies in other terrestrial chelonians (Schubauer 1981; Kapfer et al. 2013; Somers et al. 2017). There were no selection criteria based on presenting injury, diagnosis, or time spent in treatment/rehabilitation.

Transmitters and placement

All procedures involving live animals were approved by the North Carolina State University College of Veterinary Medicine (NCSCU-CVM) IACUC (protocol #21-265).

Turtles were fitted with SOPR-2190 transmitters (Wildlife Materials, Inc., Murphysboro, IL) weighing an average of 5 g. Radio transmitters were placed on the fourth or third pleural scute depending on the conformation of the turtle's shell. It is noted that transmitters placed on the pleural scute, usually the 4th pleural scute, with the antenna placed towards the posterior of the turtle do not appear to affect breeding or movement (Eckler et al. 1990; Somers et al. 2017).

Transmitters were adhered with Waterweld epoxy putty (J-B Weld, Sulfur Springs, TX) and covered with original cold-weld steel reinforced epoxy (J-B Weld, Sulfur Springs, TX). This method was used based on previous telemetry projects conducted at TRT (Cerreta et al. 2017). The weight of the transmitters and epoxy was maintained below 7% of each turtle's body weight.

Physical examination and sampling

The turtles were weighed before and after the placement of the radio transmitters. Next, a standardized physical examination was performed on each turtle according to TRT protocol. A blood sample was taken to obtain a packed cell volume and total solids. 1 ml luer-slip syringes with a 25Gx5/8" needle were used and primed with sodium heparin to prevent clotting. Blood samples drawn from the subcarapacial sinus were prioritized. However, if significant lymph contamination was present (based on visual inspection of the sample colour) a sample from either the femoral, brachial, or dorsal coccygeal vein was taken based on turtle behaviour and access. Sample volume was restricted to less than 1% of the turtle's body weight. Samples were placed into heparinized micro-hematocrit tubes and centrifuged for two minutes. PCV was read on a standard hematocrit reader chart. Total solids were measured using a refractometer with a drop of plasma.

Release

Each turtle was released, if possible, within 1.6 kilometers of where it was found, at the nearest suitable habitat to its original location. In this study, 13 turtles were released at a location provided by the turtle's original finder or with the finder themselves. The authors were not able to get in contact with Turtle 2021-173's finder. Secondly, due to human error, turtle 2021-463 was mistakenly released at an incorrect location (54.7 km from his found location). Thirdly, turtle 2021-184 only had a broadly reported location of being somewhere along a 263-ha lake with no finder contact information.

Tracking

Turtles were tracked using a TRX-16 rechargeable receiver and 3-element folding antenna (Wildlife Materials, Inc., Murphysboro, IL). Each time a turtle was located in the field, its behaviour was observed and recorded based on a box turtle ethogram. An ethogram is a catalogue of all potential behaviours an animal could be displaying when observed. The ethogram was created by authors based on first-hand knowledge of box turtle behaviour and a study outlining box turtle activity budget (Dodd 2002). The turtle was then weighed on a digital kitchen scale placed on a clipboard to create a flat surface. The turtles were then examined as above. The latitudinal and longitudinal coordinates of the turtle's location were recorded.

Study conclusion

The transmitters were to be left on the turtles for an average of 250 days, allowing at least a 30-day buffer period in which to find the turtle and remove the tracker prior to the battery's death.

Statistical analysis

All statistics were performed on Microsoft Excel. F-Test two-sample for variances was performed using the analysis ToolPak.

Results

Tracking intervals

The time between tracking sessions for each individual turtle was highly variable due to the variable availability of tracking volunteers. The longest intervals between

tracking sessions occurred around torpor/winter season (mid-December-Spring). Tracking intervals averaged 29 days ($n = 29$, $SD = 18$ days) and are outlined for each individual in Table 2.

Movement

The furthest distance travelled by a turtle was by 2021-463; the animal moved 721.77 m in a 39-day period. This subject was released the farthest from its original site as he was mistakenly released at an incorrect location (54.7 km from his found location). This turtle is one that was then reliably found prior to torpor. His last movement was 172.32 m from his last location in 34 days. Overall, turtles moved an average of 104 m ($n = 20$, $SD = 161$ m) between each time they were located.

Behaviour

The majority of turtles were found to be motionless and unboxed. Two of the turtles who died during the study, 2020-295 and 2021-273, were found in dorsal

recumbency during a tracking event prior to being found dead. Turtle 2021-273 was observed in dorsal recumbency on two separate occasions prior to being found deceased, and his empty shell was also found in dorsal recumbency. Turtle 2020-295 was found in dorsal recumbency once prior to being found deceased with her empty shell prone. Further, 2020-295 was found to have had a fresh 1 cm laceration/skin flap on her right proximal neck when she was found upside down. The only other novel behaviour observed has been motionless and boxed by turtles 2021-184 and 2021-463 on 5 December and 28 December 2021, respectively.

Mortality

Three turtles were found deceased. There was no significant difference between time spent in treatment/rehabilitation and mortality ($F(1, 5) = 0.78$, $p = 0.42$). Turtles died an average of 86 days ($n = 3$, $SD = 25$ days) after release and were each found at least once prior to being found deceased. Further demographic information on these turtles can be found in Table 1.

Table 1 The presenting injury, diagnosis, days in treatment and rehabilitation, times located post-release and current location status of the 16 turtles tagged with radio transmitters for this study.

Patient ID	Sex Male (M) Female (F) Juvenile (J)	Diagnosis code	Presenting Injury	Days in treatment	Days in rehabilitation	Times located post-release	Status
2020-290	F	A	Right hindlimb traumatically amputated	16	224	1	Location unknown
2021-149	M	D	Shell lacerations	33	0	3	Transmitter malfunction
2020-295	M	A	Shell fractures, coelomic breach	53	209	5	Deceased
2021-273	F	A	Carapacial puncture	13	0	3	Deceased
2021-258	J	C	Skin shedding, dehydration	34	0	2	Location unknown
2021-159	F	A	Plastron fracture	47	0	1	Location unknown
2021-245	M	A	Cracked mandible	30	11	0	Location unknown
2021-173	M	B, E	Shell abrasions, aural abscess	24	36	1	Location unknown
2021-165	F	C, E	Nasal discharge, aural abscess	51	0	1	Location unknown
2021-252	M	F	Bridge fracture, hindlimb trauma	65	0	0	Location unknown
2021-184	F	A	Proptosed left eye, abrasion on left front leg	72	0	3	Location unknown
2021-347	M	F	Carapacial puncture, myiasis of all wounds	0	45	2	Deceased
2021-208	M	F	Fractured tomia, globe luxation, facial trauma	48	52	0	Location unknown
2021-463	M	A	Plastron abrasion	25	0	2	Location unknown
2021-181	M	F	Mandibular and maxillary fracture	39	66	0	Location unknown
2021-499	M	E	Right aural abscess	12	0	0	Location unknown

Diagnosis codes are as follows: (A) hit by car (B) dog attack (C) systemic disease (D) hit by lawn mower (E) aural abscess (F) unknown trauma. "Location unknown" refers to turtles who have not been located during their two most recent consecutive searches.

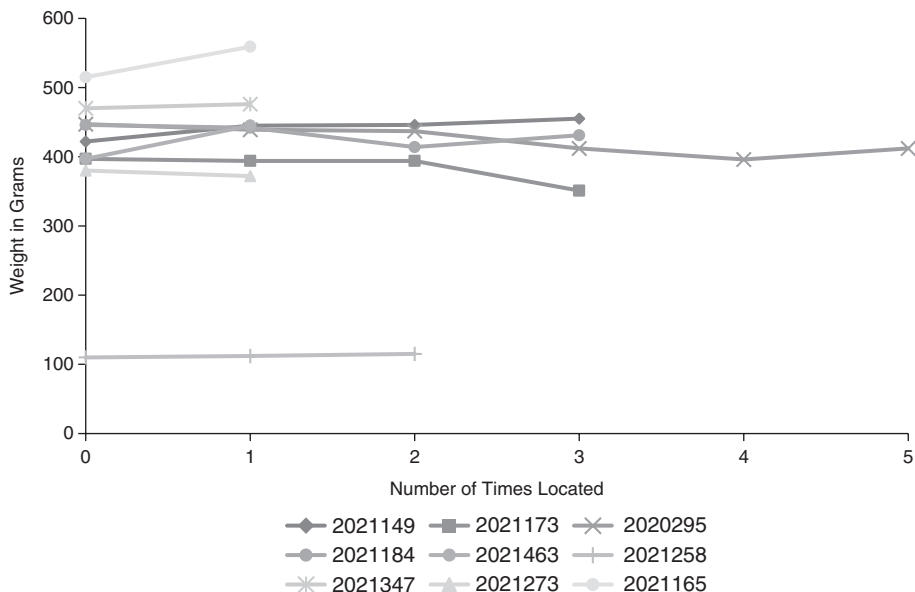


Fig. 1 A graph showing the change in weights of the turtles from release and each subsequent time they were tracked.

Table 2 The number of days between tracking sessions and outcome of that tracking session in parentheses.

Patient ID	Tracking Interval (Days since last search)						
2020-290	5 (F)	7 (NF)	6 (NF)				
2021-149	8 (F)	38 (F)	37 (NF)	35 (NF)	279 (F*)		
2020-295	3 (F)	14 (F)	13 (F)	24 (F)	12 (F)	10 (F)	37 (FD)
2021-273	9 (F)	8 (NF, S)	44 (FD)				
2021-258	5 (F)	66 (F)	189 (NF)				
2021-159	17 (F)**	44 (NF)	14 (NF)				
2021-245	30 (NF)						
2021-173	22 (F)	36 (F)	14 (F)	63 (F)	120 (NF)		
2021-165	7 (NF)	24 (NF)	4 (F)	64 (NF)	3 (NF)		
2021-252	7 (NF, S)	33 (NF, S)	41 (NF, S)	39 (NF, S)	4 (NF, S)		
2021-184	6 (F)	34 (F)	27 (F)	45 (F)	117 (NF, S)	4 (NF, S)	
2021-347	31 (F)	12 (NF)	41 (FD)				
2021-208	35 (NF)						
2021-463	39 (F)	35 (F)	125 (NF)				
2021-181	36 (NF)						
2021-499	16 (NF, S)	14 (NF)					
Mean (SD)	17.25 (±12.93)	28.08 (±28.8)	46.25 (±55.34)	45 (±15.89)	89.17 (±108.07)	7 (±4)	37

F- Found; NF-Not found, no signal detected; NF, S- Not found, signal detected; FD- Found deceased.

*-found visually, without telemetry.

**-found, but health data was not recorded.

Body weight

Overall, two of the turtles that died during the study lost weight. Turtle 2020-295 increased in weight slightly before being found deceased but lost 7.8% of body weight overall. Deceased turtle 2021-347 gained 1.3% of body weight and deceased Turtle 2021-273 lost 2.2% of body weight. As can be discerned from Fig. 1, turtle weights fluctuated throughout the study period.

Discussion

At the conclusion of this study, one turtle was successfully followed for the entire tracking period. Despite their location being known at the beginning of torpor, three turtles were lost after relocation attempts were made in the spring. Interestingly, these same three individuals (2021-173, 2021-463 and 2021-184) that were regularly found prior to the winter season are those that did not have release locations that correlated precisely with where they were found. Most notably, although turtle 2021-184 was erroneously released 54.7 km from his found location, he remained within 800 m of his release site. Traditionally, it has been believed that most turtles released away from their home range will travel great distances to return to their original habitat (Dodd 2002). Relocated turtles tend to experience higher mortality and disappearance rates than turtles that remain in their home range. This is generally attributed to increased energy use, inability to locate resources and increased chance of injury when traveling long distances (Hester et al. 2008). On the other hand, a study of translocated EBTs concluded that homing did not persist indefinitely. Of the 20 turtles in the study that showed homing tendencies, 16 still established home ranges within the translocation site which measured 5.27 km² (Cook 2004). Further, in one study, three EBTs released more than 1.6 km from capture sites did not rehome (Dodd 2002). The tendency to travel and home seems to vary greatly among individuals (Dodd 2002).

The equipment used to track the turtles detected transmitters up to about a 600-meter radius (varied greatly based on terrain and battery charge of the receiver). This made it extremely challenging to find turtles that had moved any distance from their release sites. It is hypothesized that the five turtles that were not found after release may have moved a significant distance outside of this range making it extremely challenging to locate their signal and triangulate a location. It is possible that despite our best efforts these turtles were not released close enough to their home habitat and sought to move towards their home range. It is also suspected that within box turtle populations there are a number of “transient” individuals, defined as those individuals that move continuously throughout their environment

without retracing previous routes (Kiester et al. 1982). It is unknown what percentage of turtles are true transients or their demographics (Dodd 2002). All five of the turtles in this study that were not located post-release were adult males. In a study conducted by Kiester et al. (1982), three of seven ornate box turtles radio-tagged were found to be transients and all were male. There is a hypothesis that such turtles play an important role in gene flow of the species (Kiester et al. 1982; Dodd 2002).

More manpower to track turtles very frequently post-release would allow closer tracking of individuals and could provide data on whether turtles moved a significant distance. Alternatively, the use of GPS transmitters that emit up-to-date location data could be used when the turtles are first released in order to follow their movement. However, these prefabricated devices can cost 14 times the price of radiotelemetry equipment used in this study (Cain & Cross 2018). Of course, other possibilities of loss such as predation, tracker malfunction and anthropogenic involvement should also be considered.

In the case of Turtle 2021-149, the transmitter was known to have malfunctioned. This turtle resided in the finder's yard and was seen in the yard with an intact transmitter within days of tracking attempts that yielded no signal. This turtle was re-found by the homeowners in the summer of 2022; the transmitter was removed and a complete post-study physical exam was performed. This was the only turtle that was able to be located after the winter season and was found to be in good health on physical exam.

The behaviour of the turtles found did correlate with other studies of normal wild box turtle behaviour in which turtles were found to be resting or under cover for 75% of encounters by researchers (Dodd 2002). The presence of a human observer should be considered a behaviour-altering factor. For example, an observed box turtle may stop what they were doing and watch a human as it approaches (Dodd 2002). At the time of writing, no published studies on the effect of direct human observation on turtle behaviour were found.

Two of the turtles in the study were amputees. Turtle 2020-290 had her right hindleg amputated. Unfortunately, she was only found once post-release but had normal physical examination findings. Turtle 2021-184 was enucleated in the left eye and had a left forelimb amputation. She was consistently tracked prior to torpor. She lost 3.4% of body weight but was found in good health based on physical examination at each tracking prior to torpor period.

Turtle 2021-184 was found to have lost the most weight in one period between tracking events. From 17 September to 1 October 2021, he lost 10.9% of body weight. On 3 December he was found with a good health assessment, although no weight was obtained due to a scale error. In a population of EBTs in eastern Tennessee,

it was found that turtle's weights peaked in the latter part of the summer. Weight fluctuations in the 5-month study period were not extreme, and the largest change was 10% of the turtle's body weight in 1 month (Dolbeer 1969). It is of note that the only turtle found after torpor (2021-149) increased steadily in weight over the study period with an overall gain of 38 g over an 11-month period.

The three turtles found deceased originally presented for shell fracture with coelomic breach (2020-295) or carapacial punctures (2021-273 and 2021-347). When they were found only their shell was left. Thus, it was impossible to discern the cause of death. It is unknown whether turtles died from complications of injuries for which they presented to TRT, predation, novel injury, disease or simply failing to thrive after release. One of the turtles that died was also the only turtle in the study that was kept over winter in rehabilitation. This turtle lost 7.8% of its body weight during the tracking period. It is possible it was not obtaining adequate forage or that the weight loss was due to underlying systemic disease. Considerations based on future data could include soft release into a fenced habitat with the goal of encouraging the return of normal behaviours, particularly successful foraging. This could be implemented for turtles that have been over-wintered or in rehabilitation for prolonged periods of time. However, research supporting the benefits of soft release is lacking and most current literature focuses on translocated or captive-reared turtles. In one study of ornate box turtles, soft release did not have a significant effect on growth rate or mortality of released 10-month-old, captive-reared turtles (Sievers 2015). More research is needed to assess the effects of soft release on rehabilitated turtles being introduced back into their home range.

In order to further help collect data on the turtles, it would be helpful to utilize citizen scientists. Contact information could easily be transcribed on a turtle shell for people who find the turtle to contact and report. Incidentally, during this study, we received verbal verification from citizens who had sighted turtles with transmitters. This information was relayed by owners of homes nearby who communicated with tracking volunteers. This occurred for both Turtle 2021-165 and 2021-159. Further, property owners for one of the turtles living on private property, 2021-149, had very active involvement in sightings and reports of the turtle. There is demonstrated success of numerous citizen science projects that have aided in the study of wildlife populations including eBird, iNaturalist and iSeeMammals (Sun et al. 2021). This data is encouraging, indicating implementation of a citizen scientist programme for released rehabilitated turtles in the Triangle area, NC could be promising.

Overall, due to the small sample size of the study and numerous subjects lost to follow-up after release, the results of this study mainly serve to inform methods for future post-release monitoring studies, particularly in terrestrial chelonians.

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