NORTH AMERICAN BOX TURTLE CONSERVATION COMMITTEE

7TH NORTH AMERICAN BOX TURTLE CONSERVATION WORKSHOP

Elachee Nature Science Center

Gainesville, Georgia

September 29 - October 1, 2023

From the Hosts:

Dear Workshop Participants,

Welcome to the 7th North American Box Turtle Conservation Workshop! We are delighted for you to join us in Gainesville, Georgia.

This workshop is an opportunity for us to come together, share our collective knowledge, research findings and conservation initiatives, exchange ideas, and forge meaningful connections with experts and newcomers who are dedicated to the preservation of North American box turtles in their native landscapes across the United States and Mexico. By bringing together diverse perspectives and expertise, we aim to inspire innovative approaches and collaborative efforts to tackle the pressing challenges faced by this well-known and beloved group of turtles.

The 7th North American Box Turtle Conservation Workshop features 16 oral and 7 poster presentations representing a wide variety of topics related to North American box turtle research and conservation, including ecology, epidemiology, genetics, field and laboratory studies, repatriation and augmentation, and conservation practices. This year we are pleased to bring to you keynote speaker, Dave Collins of the Turtle Survival Alliance and Association of Zoos & Aquariums' Saving Animals from Extinction (AZA SAFE) American Turtle program, who broadly examines the threats to box turtles and presents solutions through "Recovering American Box Turtles in a Beleaguered Landscape: Reasons for Hope."

Highlighting this triennial workshop are two experiences you will not want to miss. The North American Box Turtle Conservation Committee has teamed up with the Turtle Survival Alliance and their Drink Beer. Save Turtles.® program, and with event host Liquid Nation Brewing, to bring you an evening social of comradery, box turtles, and brews on Saturday, September 30th. There, you will also be able to view our poster session and engage in discussion with their presenters. Proceeds from this event will support the Lucille F. Stickel Box Turtle Research Award, which supports conservation projects and research on North American box turtles.

On Sunday, October 1st, we welcome you to join us for an immersive experience at Tumbling Creek Woods on the campus of the University of North Georgia, where we will observe Eastern Box Turtles that are part of an ongoing research effort. This is a great opportunity to see box turtles in the North Georgia piedmont and leave with applicable knowledge for your studies.

As hosts, it is our goal that you will leave the 7th North American Box Turtle Conservation Workshop with new knowledge, insights, and inspiration for your continued research and conservation efforts for this charismatic group of North American turtles. Additionally, as hosts, we would like to thank all those who made this workshop possible, including the members of the North American Box Turtle Conservation Committee, the University of North Georgia, the Elachee Nature Science Center, the Turtle Survival Alliance, the North Carolina Zoological Society, Liquid National Brewing, the numerous student volunteers, and, of course, you, the presenters and attendees.

Sincerely,

The North American Box Turtle Conservation Committee
Matthew Allender, Colin Barnett, Sandy Barnett, Gama Castañeda, Jordan Gray, John Groves, Natalie Hyslop, Jennifer Mook, and Ann Somers

Workshop hosts:

Workshop support provided by:

Cover photo: Eastern Box Turtle (*Terrapene carolina carolina*) by Jamie on Flickr

Friday Schedule

2:00 - 9:00PM

Registration

Registration begins at the Elachee Nature Science Center. Feel free to enjoy the outdoor trails and begin submitting items for the Lucille F. Stickel Fund auction.

5:00 - 9:00PM

Evening Social

Registration continues at the Elachee Nature Science Center. Appetizers and drinks will be available for evening socializing. Participants are welcome to hang out on the patio and explore the animal room at Elachee Nature Science Center.

2:00 - 9:00PM

Donations for Silent Auction benefitting Lucille F. Stickel Fund

In honor of the life and work of Lucille F. Stickel, the North American Box Turtle Conservation Committee has established the Lucille F. Stickel Box Turtle Research Award. This award supports conservation projects and research on North American Box Turtles in the following categories: natural history, ecology, conservation, reproduction, and epidemiology.

** Donations are accepted to be auctioned off in a silent auction on Saturday. All proceeds go to the Lucille F. Stickel Fund. Cash & checks, and Venmo for credit cards, will be accepted for winning bids. **

Saturday Schedule

8:00 AM

Registration & Auction Submissions

Attendees are invited to check in, socialize, and begin surveying the silent auction items.

8:30 AM

Opening Address by John Groves

Welcoming comments and keynote speaker introduction.

8:45 AM

Keynote Address by Dave Collins

9:30 AM

Morning Break

Snacks and beverages will be available. Please submit bids for the silent auction items.

10:00 AM

Morning Session

12:00 PM

Lunch

Lunch will be provided.

1:00 PM

Afternoon Session #1

2:35

Afternoon Break

Snacks and beverages will be available. Please submit bids for the silent auction items.

2:50 PM - 4:15 PM

Afternoon Session #2

15 MINUTES AFTER LAST TALK FINISHES

Silent Auction Awards

Final bids will be accepted for 15 minutes after the completion of the last talk.
Please check your bids to see if you won!

5:00-9:00 PM

Evening social and Poster Session at Liquid Nation Brewery

The poster session will take place at Liquid Nation Brewing, a family-friendly, local microbrewery, where, in collaboration with the Turtle Survival Alliance, we will host a Drink Beer. Save Turtles.® event. Join us for an evening of education and socializing!

Sunday Schedule

9:00AM - 1:00PM

Exploration and Informative sessions at UNG - Tumbling Creek

Attendees are invited to explore the Tumbling Creek Woods at the University of North Georgia. Radio telemetry techniques will be demonstrated.
Lunch will be provided from 12:00 PM - 1:00 PM.

Oral

8:30-8:45 **Introduction**
John Groves

8:45-9:30 **Recovering American Box Turtles in a Beleaguered Landscape: Reasons for Hope**
Dave Collins, *Keynote Address*

9:30-10 Break

10:00-10:15 **Left Holding the Box: Creating a Genetic Database to Return Seized Box Turtles to the Wild**
Louisa Collins*, JJ Apodaca, and Alex Krohn

10:20-10:35 **Genetic Parentage of Hatchling Eastern Box Turtles (*Terrapene carolina carolina*) in a Mixed Population of Translocated and Resident Turtles**
Ryan J. Rimple*, Brian M. Shamblin, Kurt A. Buhlmann, Olin E. Rhodes, Michel T. Kohl, and Tracey D. Tuberville

10:40-10:55 **Sample Storage Conditions, Sex, and Individual Identity Affect Assays of Innate Immunity in Eastern Box Turtles**
Bradley D. Johnson, Thomas M. Kay, Brigham J. Anderson, Brian L. Dobbels, Daren A. Glore, Jacob T. Penrose, and Bradley E. Carlson*

11:00-11:15 **The Cloacal Microbiome in Eastern Box Turtles (*Terrapene carolina carolina*): Characterizing Demographic and Environmental Associations**
Steven Kimble*, Jace Geiger, Matt Allender, and Laura Adamovicz

11:20-11:35 **An Omnivore's Dilemma: Using Stable Isotopes to Examine the Foraging Ecology of Two Eastern Box Turtle Populations in North Carolina**
John H. Roe* and Kathryn Davis

11:40-11:55 **Multi-Year Analysis of Parentage in a Captive Eastern Box Turtle Population: Implications of Using Captive Assurance Colonies for Reintroduction Programs**
Tracey D. Tuberville*, Stephanie Foertmeyer, and Joseph C. Mitchell

12:00-1:00 *Lunch*

1-1:15 **Examining the Reproductive Ecology of the Ornate Box Turtle (*Terrapene ornata*) in Western Nebraska**
Keetan Munsell

1:20-1:35 **Repeatability and Distribution of Behavior Types over Time and across Populations in Free Ranging Adult and Juvenile Ornate Box Turtles (*Terrapene ornata*)**
Abigail Trautman

1:40-1:55 **Demographics and Movement Patterns of Adult and Headstarted Juvenile Eastern Box Turtles (*Terrapene carolina*) near the Northern Limit of the Species Range in Massachusetts**
Julie Lisk, Bryan S. Windmiller, and Cara McElroy*

2:00-2:15 **Box Turtle Races at Fairs and Festivals: A Conservation Threat to North American Turtles?**
Alex Heeb* and Christine Light

2:20-2:35 **Impacts of Introduced Plants on Habitat Use of Woodland Box Turtles (*Terrapene carolina carolina*)**
Kiyoshi Sasaki*, Noah C. Wilkes, and Brooke T. Darr

**Presenter*

2:35-2:50 *Break*

2:50-3:05 **Assessing the Health and Behavioral Effects of Turtle Racing on Eastern Box Turtles (*Terrapene carolina*)**
Michelle Weaver*, John Hewlett, Sharon L. Deem, Jamie Palmer, Maris Brenn-White, Kathleen Apakupakul, Alex Heeb, Christine Light, Christine Casey, and Andrea Darracq

3:10-3:25 **Co-development of Best Practices for Woodland Box Turtle (*Terrapene carolina carolina*) Conservation in Northern Virginia**
Maxwell Earle, Jessica Meck, Travis Gallo, and Thomas Akre*

3:30-3:45 **Habitat Loss of the Endemic Turtles of Cuatro Ciénegas, Coahuila**
Gamaliel Castañeda Gaytán*, Craig Stanford, Jorge Ernesto Becerra-López, Arturo Carrillo-Reyes, Sara Isabel Valenzuela-Ceballos, Miguel Borja and Italia García

3:50-4:15 **Ill-Effects of Confiscation Events in Box Turtles (*Terrapene* sp.) and Possible Solutions to Combat Disease Events**
Matthew C. Allender*, Maris J. Daleo and Laura A. Adamovicz

Poster

5:00-9:00 **Preliminary Analysis of Fecal Samples Collected from Eastern Box Turtles (*Terrapene carolina carolina*) in Northeast Georgia**
Alisha M. Paul*, Natalie L. Hyslop, Evan C. Lampert, J. Tom Diggs, and Jennifer L. Mook

Microhabitat Use of Eastern Box Turtles (*Terrapene carolina carolina*) in Northeastern Georgia
Natalie L. Hyslop* and Jennifer L. Mook*

Field and Lab Behavioral Consistencies and Mirror Responses of Ornate Box Turtles (*Terrapene ornata*) in Nebraska
Brianna Wilson* and Samantha Kim

A Determination of Burrow Characteristics as Predictors of Ornate Box Turtle (*Terrapene ornata*) Inhabitancy in Western Nebraska
Zoe Edlund* and Timothy Speer

Movement Ecology of Ornate Box Turtles (*Terrapene ornata*) across Different Life Stages
Timothy Speer

Testing for Top-Down Ecological Effects of Eastern Box Turtles
Elijah A. Greene*, Justin T. Lewis* and Bradley E. Carlson

Has the Eastern Box Turtle Adjusted to City Living? Habitat Selection of the Eastern Box Turtle (*Terrapene carolina carolina*) Across a Developmental Gradient
Emma Rogers* and Kyle Barrett*

A Comparison of Corticosterone Levels over Time and across Populations in Ornate Box Turtles (*Terrapene ornata*)
Samantha Kim

**Presenter*

Oral

Recovering American Box Turtles in a Beleaguered Landscape: Reasons for Hope

DAVID COLLINS
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It's easy to be depressed when looking at the conditions facing one of our most familiar and favored North American turtles, the box turtle. They have suffered affronts through both historic and recent times. The loss and fragmentation of their habitat began when the first humans arrived in North America and has accelerated ever since, representing undoubtedly the greatest cause for their decline. As both humans and turtles vied for the same preferred environments, the turtle was clearly the loser. As woodlands, wetlands and grasslands were converted for human purposes, not only was the physical space lost, but with that came a myriad of human threats from bulldozers and automobiles, lawnmowers, dogs, and kids, and the list goes on. At the same time, these small, colorful, and endearing animals were coveted as pets and tens of thousands of them entered the domestic and international pet trade, first legally and now as the most numerous species in the illegal trade in North American turtles.

So, where is their reason for hope?

In searching for that answer, two things emerged and converged.

First, Jane Goodall's Four Reasons for Hope; the amazing human intellect, the resilience of nature, the power of young people, and the indomitable human spirit.

And second, the many individuals, organizations, and institutions, many represented at this Workshop, actively engaged in box turtle conservation that amplify each of those four reasons.

Oral, Keynote Address

Left Holding the Box: Creating a Genetic Database to Return Seized Box Turtles to the Wild

LOUISA COLLINS*, JJ APODACA, AND ALEX KROHN
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The illegal collection of freshwater turtles and tortoises, whether for consumption or for the pet trade, has become one of the most imminent threats to wild turtle populations including North American box turtles, which have become the most poached turtle in the United States. In recent years there have been strong efforts to dissuade collections and to seize illegally caught turtles. However, seized turtles are often not returned to their native habitat and instead are housed indefinitely in zoos and other facilities. As a result, most poaching events have the same effect on wild populations, whether or not they are intercepted by law enforcement. In an effort to bridge the gap between confiscations and returning turtles to the wild, we have developed a range-wide genetic database to identify origins of confiscated box turtles. Our data shows that confiscated box turtles can be repatriated to general regions without disrupting historical patterns of gene flow, allowing flexibility in where wildlife managers should release confiscated turtles. This also provides a powerful tool to law enforcement, both in the prosecution of poachers and in the identification of poaching hot spots. Given the breadth of our box turtle sampling effort, we also give recommendations on best practices for collecting genetic samples from box turtles.

Oral

Genetic Parentage of Hatchling Eastern Box Turtles (*Terrapene carolina carolina*) in a Mixed Population of Translocated and Resident Turtles

RYAN J. RIMPLE[1,2,*], BRIAN M. SHAMBLIN[2], KURT A. BUHLMANN[1], OLIN E. RHODES[1], MICHEL T. KOHL[2], AND TRACEY D. TUBERVILLE[1]
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Translocation is a conservation tool increasingly used in the recovery of at-risk species. For Eastern Box Turtles (*Terrapene carolina carolina*), a species in decline throughout their range, translocation could assist in mitigating some of the greatest threats facing the

species, including displacement via habitat destruction and collection for the illegal pet trade. While there are numerous examples of box turtle translocations in the scientific literature, few have involved the release of confiscated or formerly captive turtles. Additionally, post-release monitoring has primarily focused on survival and spatial ecology. To our knowledge, post-translocation reproduction and genetic parentage have not been quantified. Post-release reproduction is essential for translocated animals to contribute to the demographic recovery of the resident population. In addition, genetic integration via reproduction can also quickly increase the genetic diversity of populations that may be impacted by genetic isolation or inbreeding. In 2020 and 2021, we translocated two groups of box turtles to the Savannah River Site (SRS) in South Carolina, including confiscated turtles (n=208) and surrendered long-term captive (LTC) turtles (n=35). We collected genetic samples from all translocated turtles and all sympatric resident turtles that were encountered during our post-release monitoring (n>75). In 2022, we monitored 13 confiscated, 15 LTC, and 8 resident females for reproduction. We obtained 119 eggs from 33 clutches, then collected genetic samples from all offspring and unhatched but viable eggs (n=89). We genotyped all samples at 14 polymorphic microsatellite loci, and used the program COLONY to assign parentage to offspring using a maximum likelihood approach. We will report results of these initial analyses to determine the degree of genetic integration between resident and translocated Eastern Box Turtles following translocation.

Oral

Sample Storage Conditions, Sex, and Individual Identity Affect Assays of Innate Immunity in Eastern Box Turtles

BRADLEY D. JOHNSON, THOMAS M. KAY, BRIGHAM J. ANDERSON, BRIAN L. DOBBELS, DAREN A. GLORE, JACOB T. PENROSE, AND BRADLEY E. CARLSON*

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Immune function is critical for animal survival, and assays of immune performance in wild animals are increasingly used in ecological and conservation research. Innate immunity is the branch of the immune system that provides a generalized and fast-acting defense against pathogens. Two common assays of innate immune function are the bacterial killing assay and the hemagglutination assay. Blood or plasma samples for these assays can be stored under varied conditions, and this may influence assay performance. Additionally, environmental conditions during sample collection or phenotypic characteristics of individual animals may drive variation in assay performance. In this study, we collected repeated samples of plasma from wild Eastern Box Turtles (*Terrapene carolina carolina*) in Indiana, USA, performing immune assays after subdividing the plasma for storage for 24-48 hours in the refrigerator (4° C), one to two weeks frozen (-80° C), or three months frozen. We also collected data on morphology, temperature, behavior (boldness), and corticosterone ("stress hormone") levels. We found that freezing samples for up to two weeks preserved immune function slightly better than refrigeration for up to 48 hours, and long-term freezing impaired assay performance. We also found that male turtles had lower bacterial killing and higher hemagglutination than females, bacterial killing and hemagglutination were correlated with each other, and individuals tended to differ consistently from each other in immune function. These findings suggest that future work should use samples frozen for up to a few weeks, and individual and sex-based differences in immune function should be incorporated into further studies.

An Omnivore's Dilemma: Using Stable Isotopes to Examine the Foraging Ecology of Two Eastern Box Turtle Populations in North Carolina

JOHN H. ROE* AND KATHRYN DAVIS

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Box turtles (*Terrapene* spp.) are generalist omnivores that play potentially important roles of seed and spore dispersal and nutrient cycling in forest ecosystems. While box turtles can eat a wide variety of foods, little is known about how diet varies among individuals within a population or how populations in different environments vary in diet. Here, we examined sources of variation in diet within and between two populations of Eastern Box Turtles (*Terrapene carolina carolina*) using carbon (^{13}C) and nitrogen (^{15}N) stable isotopes sampled from claws, and compared isotope values of turtle tissues to that of locally available food sources to determine the primary foods consumed. Isotope values did not vary between sexes or populations in Coastal Plain and Sandhills regions. ^{15}N values varied from 1.1 ‰ to 8.6 ‰ and ^{13}C values from -24.8 ‰ to -21.0 ‰ among individuals, a range spanning two to three trophic levels. Both ^{15}N and ^{13}C values increased with body size, suggesting adults and juveniles may be foraging on different resources. Turtles sampled multiple times had similar isotope values over time, indicating repeatable (i.e., consistent) food sources over a period of several years within individuals. Animal and mushroom food sources represented the majority (45% and 44%, respectively) of diet incorporated into turtle tissues, while fruits represented only 11% of dietary incorporation. While both populations were generally omnivorous with a

wide trophic niche, our results indicate individuals may specialize in eating only a subset of resources. Dietary variation and specialization among individuals may have important ecological, evolutionary, and conservation implications, especially when concerning how local resource management practices may impact food resource availability and abundance in forest ecosystems.
Oral

Multi-year Analysis of Parentage in a Captive Eastern Box Turtle Population: Implications of Using Captive Assurance Colonies for Reintroduction Programs

TRACEY D. TUBERVILLE[1]*, STEPHANIE FOERTMEYER[2], AND JOSEPH C. MITCHELL[3]
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The illegal wildlife trade is a growing threat to North America's turtle fauna, particularly for species in high demand such as members of the genus *Terrapene*. Although law enforcement efforts are increasingly focused on seizing turtles illegally collected from the wild, state wildlife agencies are then tasked with determining whether confiscated turtles can be safely released back into the wild. Due to disease concerns, these turtles have historically been either euthanized or relegated to a lifetime in captivity. However, there is growing interest finding ways that confiscated turtles can best contribute to wild populations and one potential mechanism is by maintaining them in captive assurance colonies and releasing their offspring. In many assurance colonies, adults are maintained communally and allowed to breed freely. However, in most cases, little is known regarding the parentage of—and thus the genetic diversity of—resulting offspring for potential use in future reintroduction efforts. Here we present the results of a multi-year analysis of parentage in a captive Eastern Box Turtle (*Terrapene carolina carolina*) population. The captive population consisted of 25 adults (10 females, 15 males) displaced from a development site near Richmond, Virginia, USA and maintained in a semi-natural outdoor enclosure in a suburban neighborhood near the collection site. Adults were monitored for mating and nesting behavior, and nests from known females were collected for incubation indoors. During 2004-2006, we collected 112 hatchlings representing 25 clutches. In 2007 we obtained blood samples from all the adult breeders and the head-started juveniles, which we genotyped at 5 species-specific polymorphic microsatellite markers. Parentage analysis was performed using GERUD and Cervus software programs. We will present our findings with box turtles and discuss the implications for assurance colony and reintroduction program management for eastern box turtles and other turtle species.
Oral

Examining the Reproductive Ecology of the Ornate Box Turtle (*Terrapene ornata)* in Western Nebraska

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Understanding the reproductive ecology of any species is vital for determining intraspecific variation in individual fitness, modeling population growth or persistence potential, and developing effective conservation strategies. The reproductive ecology of Ornate Box Turtles (*Terrapene ornata*) including a full examination of the pattern of their egg bearing period and reproductive output is relatively understudied. The goal of this study was to specifically address the mating behavior, egg bearing cycles, body condition fluctuations, and nesting behavior of free-ranging Ornate Box Turtles via radio telemetry and ultrasonography. To do this we closely monitored a population of 32 female turtles in Nebraska over two consecutive years with each turtle being monitored for embryonic development on a four-day cycle during the presumed egg-bearing period. Individual female movements were tracked daily using radio telemetry, then an ultrasound (IBEX EVO II) was used to assess the egg bearing status, internal condition, and clutch size of that female. Our results enabled us to better describe the egg bearing period of Ornate Box Turtles and their nesting, mating, and overall movement behaviors when reproductively active. This data can be useful in understanding individual variation in reproductive output, fitness, and ultimately aid in targeted conservation plans without performing an invasive procedure.
Oral

Repeatability and Distribution of Behavior Types over Time and across Populations in Free-Ranging Adult and Juvenile Ornate Box Turtles (*Terrapene ornata*)

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As studies continue to investigate inter-individual variation, it is becoming increasingly evident that individual animals have their own unique behavior type (personality; repeatable behaviors over time and context) that persists throughout their life. Behavior is simply different forms of motion, and a behavior type influences which motions (behaviors) are most likely to be on display. Behavior types can influence resource acquisition, predation risk, and mating opportunities and thus can directly influence the fitness of any given individual. In this study, we examine the behavior types of free-ranging adult Ornate Box Turtles (*Terrapene ornata*), including their repeatability and variation both within and across populations. In addition, we examine, for the first time, the behavior types of free-ranging juvenile Ornate Box Turtles and compare their behavior type and distribution to their adult counterparts. We found the juveniles, like the adults, to be extremely consistent and repeatable in their behavior types and that populations varied in their average behavior types. Understanding animal personality can have important implications for how we manage species, the individuals therein, and their habitat. Understanding how different behavior types within a population interact and potentially promote population persistence is important, especially for species of conservation concern, such as the Ornate Box Turtle.

Oral

Demographics and Movement Patterns of Adult and Headstarted Juvenile Eastern Box Turtles (*Terrapene carolina*) Near the Northern Limit of the Species Range in Massachusetts

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Eastern Box Turtles (*Terrapene carolina carolina)* reach the northern edge of their range in New Hampshire, just north of the Massachusetts boundary. In Massachusetts, eastern box turtle populations are largely concentrated in the southeast and Connecticut River Valley. Although there have been scattered reports of individual eastern box turtles in northeastern Massachusetts, populations were thought to be unsustainable in this area, until we began to explore a potentially regionally significant population in 2018. Below, we detail five years of field data on the 21 unique wild Eastern Box Turtles (8 males, 10 females, and 3 juveniles) we have discovered in this northeastern Massachusetts population. We used radio telemetry to monitor the movement and behavior of 15 of these adults, including potentially nesting females in June. We located and protected 22 nests, which yielded a total of 75 hatchlings. A subset of these hatchlings (30, in two cohorts) were captive-reared for ~21 months and radio-tracked for up to 3 years post-release in their natal habitat. Overall, our results support previous regional studies which suggest that eastern box turtles on the northern edge of the species range exist at very low population densities and wander extensively when compared to more southerly populations. Both adult and captive-reared juvenile Eastern Box Turtles demonstrated high annual survival rates (100% and 94.7% respectively.) The growth, survival, and movement data of our post-release captive-reared juveniles may help inform the design and implementation of future headstarting programs. Our results suggest that headstarting juveniles may be a highly effective management strategy to augment declining populations of Eastern Box Turtles at the northern edge of their range. We note that since Eastern Box Turtles occur at low densities in northern Massachusetts, populations are difficult to find and census, and those populations are particularly vulnerable to local extirpation. As a result, we hypothesize that many extant populations of eastern box turtles in northern Massachusetts and New Hampshire have not been mapped and are therefore not protected by state-level endangered species regulations.

Oral

Box Turtle Races at Fairs and Festivals: A Conservation Threat to North American Turtles?

ALEX HEEB* AND CHRISTINE LIGHT
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Turtle conservationists have only recently become aware of the cryptic phenomenon known as turtle races in the United States. Once thought to be one-off oddities, a 1997 Kansas survey demonstrated that these events were much more common than was previously realized. Building off that research, through a thorough search of electronic newspapers, social media outlets and search engine

records we discovered that there are over 600 turtle races being held at fairs and festivals across 30 states. Oklahoma, where turtle races appear to have originated as part of a 1920s festival, and Kansas were particularly notable, accounting for nearly half the national total. To better quantify how many box turtles are being impacted, a citizen science project was launched in 2020 with the aim of dispatching a volunteer to all 600 races. Data collection throughout the summer and fall of 2021, following a one-year delay due to the COVID-19 pandemic, focused on quantifying how many turtles are collected, how many species are being impacted, and examining husbandry parameters. We will present preliminary results and discuss conservation concerns that arise due to direct collection, relocation of turtles, disease and the use of endangered and protected species. Additionally, we will discuss why turtle races have not developed a "conservation ethic" like other wildlife sports, and if these events can be converted for the benefit of turtle conservation.
Oral

Impacts of Introduced Plants on Habitat Use of Woodland Box Turtles *(Terrapene carolina carolina)*
KIYOSHI SASAKI*, NOAH C. WILKES, AND BROOKE T. DARR
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Introduced plants typically form dense, often monotypic, thickets. By doing so, introduced plants may affect the resources and conditions on which resident animals depend, thereby having the potential to displace resident animals from areas otherwise suitable as habitat. However, behavioral response of *Terrapene* species to introduced plants remains largely unknown. This study examines the impacts of introduced plants on habitat selection of Woodland Box Turtles (*Terrapene carolina carolina*) and determines mechanisms of impacts. Habitat selection will be estimated based on radio telemetry data from 2020 to 2022. To identify environmental factors underlying habitat selection, prey availability and microclimate conditions (temperatures and humidity) will be quantified within and outside patches of introduced plants. Understanding factors affecting habitat selection helps develop targeted habitat management strategies.
Oral

Assessing the Health and Behavioral Effects of Turtle Racing on Eastern Box Turtles (*Terrapene carolina*)
Michelle Weaver[1]*, John Hewlett[1], Sharon L. Deem[2], Jamie Palmer[2], Maris Brenn-White[2], Kathleen Apakupakul[2], Alex Heeb[3], Christine Light[3], Christine Casey[4], and Andrea Darracq[1]
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Turtle racing involves the public capturing and using largely wild-caught box turtles (*Terrapene* spp.). Turtle races can be standalone events or a part of larger family events (e.g. festivals). Turtle races are legal in most states because of a lack of regulations and/or funding to enforce regulations that may exist. We lack information on the potential effects (e.g. disease transmission, survival, movements, and general health) of races on the turtles that could help support the implementation of regulations. Our objective is to understand the short-term and long-term health and behavioral effects of turtle races on Eastern Box Turtles (*Terrapene carolina*). We collected 29 box turtles from a race held in Kentucky. We completed physical exams, including collecting blood samples and oral/cloacal swabs, on each turtle. We quarantined the turtles for two weeks and then, following negative PCR test results for Ranavirus (FV3), released 19 turtles (weight > 400 g), with attached transmitters, onto a national wildlife refuge in Kentucky. We also captured eight native box turtles from the same release location and followed the same procedure done with the race turtles. Since release, we have been tracking turtles weekly and have completed four total health evaluations. We are currently completing laboratory analyses associated with testing for other diseases besides ranavirus. We present here the preliminary data on health metrics, disease surveillance, movement, and survival.
Oral

Co-development of Best Practices for Woodland Box Turtle (*Terrapene carolina carolina)* Conservation in Northern Virginia

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The Woodland Box Turtle (*Terrapene carolina carolina)* was once common and widespread across the Mid-Atlantic region, but apparent population declines have followed the rapid development that has sprawled across the region over the last several decades. Today, populations appear to remain in some patches of working landscapes that are the common land use of exurban and rural areas in the region. Within these mixed-used agricultural mosaics, the major threats to population viability appear to be habitat loss and decay, mortality from roadway vehicles and agricultural machinery, and poaching for the pet trade. Taken together, these threats place a premium integrating ecological information on distribution, density, demography and dispersal into working landscape management. In response to this need, we began a collaboration with a regional private landowner conservation network to co-develop best practices for working landscape management for the Woodland Box Turtle. Our goals were the following: 1) optimize detection of box turtles under a range of environmental and surveillance conditions, 2) reveal relationships between landscape and patch features and occupancy, abundance and demographic distribution, 3) reveal seasonal patterns of movement and habitat use, 4) co-develop best management practices for conservation on working landscapes, and 5) co-develop the Woodland Box Turtle as a widespread and durable touchstone for stakeholder-based private lands conservation. To achieve these goals, we first used scientific investigation on private lands to provide an ecological foundation of box turtle knowledge *and* a socio-political foundation of stakeholder engagement with the ecology of box turtle conservation. With this socio-ecological foundation in place, we will then use co-development of best practices with the landowner network to inform, socially market, and monitor and adapt conservation science and practice. This presentation will examine and discuss methods, results, and relevant observations from the first season of socio-ecological field work, as well as goals and lessons for next steps and adaptation of future directions. These include coordination with the regional landowner network and partners, connection with the local landowners, box turtle population survey techniques and results, and germane socio-ecological observations for box turtle ecology and conservation.

Oral

Habitat Loss of the Endemic Turtles of Cuatro Cienegas, Coahuila

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Cuatro Cienegas contains a wetland of international importance for its biodiversity and for being the only habitat for three species of turtles (*Terrapene coahuila, Trachemys taylori* and *Apalone spinifera atra*). In 2012, Torres-Vera evaluated the extent of wetland for the year 1977, finding an area of 330 ha, which had been reduced to 73 ha by the year 2000. Given this reduction, we updated the wetland estimate considering Landsat satellite images for the years 1986 and 2019. The analysis included calculation of the Normalized Differential Vegetation Index and the modified Normalized Differential Water Index as well as supervision of Google Earth images and field verification. We found a reduction in the area of wetland of 59.1%, a reduction in the number of pools of 65.9% and an average reduction in the size of each pool of 47.1% over a period of 33 years. Previous population data of one of the three species of turtles, Coahuilan Box Turtle (*T. coahuila*), from 148 ind/ha in 1968 to 3.1 ind/ha in 2012, suggest a high-risk condition for the existence of turtles of Cuatro Cienegas. The reduction of the wetland places these three species as among the most restricted and most threatened species in Mexico.

Oral

Ill-effects of Confiscation Events in Box Turtles (*Terrapene* sp.) and Possible Solutions to Combat Disease Events

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Infectious diseases affecting the respiratory system have been associated with significant morbidity and mortality in Eastern Box Turtles (*Terrapene carolina carolina*). Recently, eastern box turtles have experienced population declines due in part to the illegal wildlife trade and its association with the potential spread of infectious diseases, yet the impact of diseases in wildlife confiscations is not thoroughly described. The Association of Zoos and Aquariums' (AZA) Saving Animals From Extinction (SAFE) North American Turtles program with experts in non-profits, academia, government, and zoological institutions partnered to develop a comprehensive plan, including health assessment and disease surveillance with the goal of releasing turtles in a similar plane of health to turtles in the receiving population. During confiscation events, individuals are swabbed for between 4-24 pathogens simultaneously. Fortunately, these procedures were in place because in July 2021, 96 Eastern Box Turtles were confiscated by the US Fish and Wildlife Service while en route to Asia and were relocated to three zoos. From July – August, 40 turtles died from a Ranavirus outbreak in which 33 of 36 tested turtles (92%) were qPCR positive for frog virus 3 (FV3), 100% were positive for *Mycoplasma* sp., 80% were positive for *Terrapene* herpesvirus 1 (TerHV1), and 60% were positive for *Terrapene* adenovirus. All tested turtles were co-infected with at least two pathogens. The remaining 56 turtles naturally brumated over winter in 2021 and were found dead in May 2022 with remains too autolyzed for informative necropsy. In the fall of 2022, another 19 turtles were confiscated, detected with FV3 on intake, and surviving individuals are currently at the Wildlife Epidemiology Lab to characterize and identify options for long-term survival and viral persistence. The prevalence of a fatal infectious disease within the first 2 years of this program highlights the urgent threat to releasing animals without surveillance in place and has significant implications for long-term confiscation planning.

Oral

Poster

Preliminary analysis of fecal samples collected from Eastern box turtles (Terrapene carolina carolina) in Northeast Georgia

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Eastern Box Turtles (*Terrapene carolina carolina*) have experienced significant declines in their populations and are considered a species of concern. Studies have shown that eastern box turtles are omnivores; however, for species conservation, further information is necessary on the components in their diet across their geographic range, including in areas with invasive plant species. Therefore, it is essential to examine fecal contents to observe potential food items and parasites that may be present. We collected and analyzed 147 fecal samplings from 59 individual Eastern Box turtles that were radio-tracked from 2013 to 2022 in northeastern Georgia. Upland habitats at our site included area dominated by native hardwood-pine mixed uplands, as well as areas with canopies dominated by either non-native Chinese Privet (*Ligustrum sinense*) or by a privet-pine mix (primarily Loblolly Pine [*Pinus taeda*]). Aquatic habitats included freshwater shrub wetlands created by beaver activity along a permanent creek, and multiple, unconnected seepage wetland areas dominated by either privet (over and understory) or native woody and herbaceous vegetation. Each fecal sample was manually sorted to find consumed specimens that could be identified. There was a total of 42 samples that had roundworms, 64 had mollusks, 76 samples contained insect parts identifiable to orders, 10 samples contained unidentifiable insect parts, 5 samples had crustacean body parts, and 72 samples had seeds and 99 had other plant materials. Our results provide additional information necessary to help understand eastern box turtle's diet in northeast Georgia, that is also in an area with a prominent invasive plant species.

Poster

Microhabitat use of Eastern Box Turtles (*Terrapene carolina carolina*) in Northeastern Georgia

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Eastern Box Turtles (*Terrapene carolina carolina*) are a primarily terrestrial species, native to the eastern United States, that has experienced population declines throughout its native range largely due to habitat loss and alterations and collection for the pet trade. Understanding spatiotemporal variation in organismal use of specific resources is vital to conservation and restoration efforts. To investigate microhabitat use in EBT in northeastern Georgia, we analyzed active season and overwintering habitat use of an EBT population monitored using radio telemetry from 2013-2022. We measured microhabitat vegetation and substrate composition in a 1.5m diameter circular plot around turtle locations. Total understory (<150 cm in height) vegetation cover and basal area were also recorded. Upland habitats at our site included area dominated by native hardwood-pine mixed uplands, as well as areas with canopies dominated by either non-native Chinese Privet (*Ligustrum sinense*) or by a privet-pine mix (primarily Loblolly Pine [*Pinus taeda*]). Aquatic habitats at our study site included freshwater forested/shrub wetlands created by beaver activity along a permanent creek in addition to multiple, unconnected seepage wetland areas dominated by either privet or native woody and herbaceous vegetation. For active season use, we analyzed microhabitat data for individuals with ≥10 microhabitat plots (n = 33 turtles; 17 M and 16 F) resulting in 1471 locations. We collected overwintering microhabitat data on 37 individual turtles (22 M; 15 F) for 8 overwintering periods between 2013-2022. We retained the individual turtle and season for all analyses. Patterns of microhabitat use in active seasons showed that turtles used an average of 35% understory vegetation cover (± 0.14), an average basal area of 80 ft2/ac (± 21 ft2/ac), and 60% canopy cover (± 14%). During brumation, average understory cover was 14% (±16%), average basal area was 106 ft2/ac (± 37 ft2/ac), and average canopy cover at locations was 41% (± 18%). Data analysis is ongoing and further results and their implications will be discussed.
Poster

Field and Lab Behavioral Consistencies and Mirror Responses of Ornate Box Turtles (*Terrapene ornata*) in Nebraska

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Animal personality is significant on the individual level, as it has been linked to many important aspects of the individual's ecology. For example, differences in personality have been linked to how the animal interacts with their environment and their performance in certain tasks such as resource acquisition, social interactions such as mating and territory defense, ranging and philopatry, and navigation. Certain behavior types can be isolated and scored in assays, which can then be translated to personality. Animal personality is made up of five axes (behavior types): boldness, aggression, sociality, activity, and exploration. In this field study, we investigated personality traits (boldness and activity) of Ornate Box Turtles (*Terrapene ornata*) within a population in Western Nebraska. We also explored Ornate Box Turtle behavior when exposed to a mirror as a preliminary foray into potential social behaviors exhibited by this species. These field assays, aside from our mirror assay, are designed to be directly comparable to lab assays historically completed on this same population of turtles over the past eight years. The results of our field assays were compared to lab assay data on the same individual turtles to examine similarities and differences of behaviors across environmental contexts to measure the strength of personality traits within and across individuals. Personality should be considered in conservation, as different personality types will respond uniquely to different environmental contexts and associated management tactics employed by conservation agencies.
Poster

A Determination of Burrow Characteristics as Predictors of Ornate Box Turtle (*Terrapene ornata*) Inhabitancy in Western Nebraska

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Ornate box turtles (*Terrapene ornata)* use a wide array of cover types for a variety of different benefits including thermoregulatory purposes, refuge from predators and potentially wildfire, and for brumation. As important as burrows are for this species, they typically do not dig their own burrows. They are known to partake in a form of kleptoparasitism, sharing or using holes and burrows that other animals have dug. In this study, burrows found to have turtles in them in the recent past (2022-2023), were surveyed for a number of

different characteristics, including temperature and size measurements, and were borescoped on a rotating basis to capture burrow occupancy otherwise impossible to ascertain without using destructive measures. The goal of this survey was to determine the characteristics of a burrow Ornate Box Turtles prefer, if any, and whether inter- or intra- species cohabitation occurs within these burrows. This study will benefit overall understanding of Ornate Box Turtle burrowing habits, which, in turn, should help assist future conservation, rehabilitation, and reintroduction projects for this species.
Poster

Movement Ecology of Ornate Box Turtles (*Terrapene ornata*) across Different Life Stages

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Understanding the movement ecology of individuals/populations is critical for predicting future needs and success in any species, especially as viable habitats become scarce, and climates continue to change. The movement ecology of many species has been extensively studied (particularly in mammals); however, other taxonomic groups including many ectotherms have not been well documented. One such ectotherm is the Ornate Box Turtle (*Terrapene ornata*), which has rarely had its movement ecology examined across multiple life stages, especially at the western edge of their range. In this study, we employed a two-pronged approach to investigate the movement ecology of reproductively active females (determined via ultrasonography, mating, and nesting observations) and males (determined via mating observations) compared to adults with no known follicular/embryonic development or mating/nesting observations. In addition, we also examined the movement ecology of juveniles (ranging from 35g to 250g) to capture the full extent of movement patterns as they relate to the various life stages of an Ornate Box Turtle. By comparing the movement ecologies of these different groups our findings are helpful in understanding the habitat needs, space-use, and day-to-day behaviors of Ornate Box Turtles throughout various life stages.
Poster

Testing for Top-Down Ecological Effects of Eastern Box Turtles

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Turtles and tortoises are among the most threatened groups of animals and may play important ecological roles. The Eastern Box Turtle (*Terrapene c. carolina*) is in decline, yet little is known about its effects within its ecological community. This research aims to test for effects of box turtles on vegetation in their habitat and behavioral patterns of favored prey (three species of terrestrial snails). We created exclusion pens in a forest occupied by box turtles to measure the effect of turtle herbivory over a span of two years. Initial results suggest no differences in plant density or species richness between exclusion, control, and reference plots. This may be because of low turtle densities and low rates of feeding on vegetation. This experiment will be extended to produce a larger dataset. To see whether box turtles affect the behavior patterns of terrestrial snails, we ran two laboratory experiments with land snails. First, we tested snail climbing behavior in response to filter paper saturated with water and different combination of scents, including box turtle, injured conspecifics (crushed snails), and a reptile that is not a natural predator of land snails (*Pantherophis spiloides*). Next, we conducted a choice experiment where snails could select to spend time on filter paper wetted with water or with combinations of scents listed above. Results for this study are forthcoming and will be reported.
Poster

Has the Eastern Box Turtle Adjusted to City Living? Habitat Selection of the Eastern Box Turtle (*Terrapene carolina carolina*) Across a Developmental Gradient

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Urbanization poses a significant threat to wildlife through fragmentation, degradation, and loss of habitats. Species response to urban development is variable, from species that thrive in urban areas to species that avoid them. Eastern Box Turtles (*Terrapene carolina*

carolina) are known to live and reproduce in urban areas; however, reports of population declines throughout the species' range have raised concerns. Urban studies on the Eastern Box Turtle commonly address causes of direct mortality such as road strikes, but rarely address how the built environment impacts the behavior of the species. The objective of this study is to determine the effect of urban development on the habitat selection of the Eastern Box Turtle in Clemson, South Carolina. We are tracking 14 individuals twice weekly during the active season (April – October) via radio telemetry in habitats along a developmental gradient ranging from residential neighborhoods to primarily forested habitat within the Clemson Experimental Forest. In addition to recording the location of telemetered turtles we are collecting the habitat data on temperature, humidity, canopy cover, vegetation cover and structure, leaf litter depth, and distance to water and nearest built structure. Data are collected at the turtle's location as well as at random points between 5-10 and 50-60 meters away to determine habitat selection at two spatial levels. We will use a linear mixed model to evaluate the best predictors of habitat use among the measured variables. These values will then be regressed against percent urban cover at each site to determine resource selection within the home range (3rd order selection) across a developmental gradient. Preliminary habitat use data indicates turtles may be selecting for habitat that offers higher humidity and canopy cover compared to ambient values. These factors may drive selection because of their importance to physiological regulation. We will use the results from this study to determine if urban development is affecting the habitat selection of the Eastern Box Turtle and inform land management practices for private landowners to promote the persistence of the species in urban areas.

Poster

A Comparison of Corticosterone Levels over Time and across Populations in Ornate Box Turtles (*Terrapene ornata*)

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Stress has been shown to be directly linked to fitness. Often stressed animals have reduced reproductive output, are more susceptible to disease and parasitism, and are often outcompeted by others (both within and across species). Stress hormones are highly conserved across taxonomic groups with corticosterone (produced by the adrenal gland), the primary stress hormone found in most vertebrate ectotherms. During stressful events corticosterone is released into the bloodstream and thus levels of corticosterone in the bloodstream have regularly been measured as an estimate for the overall stressed state of the animal. In this study, we collected fecal samples of Ornate Box Turtles (*Terrapene ornata*) across populations and over time. ELISA kits were used to measure individual corticosterone levels. Corticosterone levels of individual turtles were averaged by population and compared to determine if certain populations appeared more stressed, on average, than others. At the individual level, we compared corticosterone levels to other aspects of the animal, including their size, sex, and personality traits (determined via several different behavior assays). In addition, we also examined corticosterone levels of turtles across multiple years to determine whether individuals were consistently stressed or not. Our study provides guidance for future noninvasive monitoring of corticosterone levels in Ornate Box Turtles and can be used as a way to monitor overall population and individual health conditions.

Poster