Federal Aid in Wildlife Restoration

W-78-R-1

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Connecting Habitat Across New Jersey (CHANJ) Assessments

Final Report

for

September 1, 2017–August 31, 2022

<u>NJ Department of Environmental Protection</u>

FISH AND WILDLIFE
ENDANGERED AND NONGAME SPECIES PROGRAM
P.O. BOX 420
TRENTON, NJ 08625

PERFORMANCE REPORT

STATE: New Jersey PROJECT NUMBER: W-78-R-1

PROJECT TYPE: Research and/or Management

PROJECT TITLE: Connecting Habitat Across New Jersey (CHANJ) Assessments

STUDY NUMBER AND TITLE: Mammals

PERIOD COVERED: September 1, 2017 to August 31, 2022

JOB NUMBER AND TITLE: Connecting Habitat Across New Jersey (CHANJ) Assessments
Prepared by: Gretchen Fowles, Brian Zarate and MacKenzie Hall

OBJECTIVE (1): Assess functional connectivity at different scales across the landscape for at least five mammal species of differing movement capabilities by August 31, 2022.

OBJECTIVE (2): Develop Road Assessments for at least 20 road segments* represented in the CHANJ mapping that bisect cores and corridors by August 31, 2022. *Funds added to this grant in 2019 ($60,000 federal plus $20,000 match) increased our goal to 35 Road Assessments.

Key Findings for Objective 1

To assess functional connectivity (observed movement/flow of organisms or genes (Keeley et al. 2021)) at different scales across the landscape, we set out to conduct gene flow assessments using DNA samples collected across the state for a minimum of 5 mammal species of various movement capabilities, and to use a variety of techniques to evaluate movement patterns of individual animals and species relative to CHANJ-mapped habitat cores, corridors, and road segments.

For the gene flow assessments, the CHANJ team enlisted more than 100 volunteers during the grant period to help with DNA sample collection. Volunteers included individuals from CHANJ partner organizations, NJDEP Fish & Wildlife (NJFW) staff, trappers, hunters, wildlife rehabilitators, USDA Animal and Plant Health Inspection Service (APHIS), and interested community members. Each volunteer was given a sampling kit for ear tip clipping, preserving, and documenting each sample collected, as well as an instruction sheet and safety vest. Emails were sent periodically to update the volunteers, call out species and areas in need of more samples, and arrange re-supplies and the transfer of samples. Our goal was to collect samples from a diversity of native terrestrial mammal species, with at least 20 samples each from both the North and South regions of the state (Fig. 1), for a total of at least 40 samples collected per species. State Route 1 served as the North/South dividing line for this study, as the highway runs roughly through the center of New Jersey between Philadelphia and New York City and is a highly urbanized human travel corridor which we suspected to be a severe impediment to wildlife movement between northern and southern NJ.

Figure 1. Map of New Jersey with State Route 1 (orange line) serving as the dividing line between the North and South regions of New Jersey. Inset images show an ear snip being sampled from a road-killed Red squirrel (at right) and a datasheet and 15ml vial with silica desiccant used for DNA sample collection.

A total of 1,669 samples representing 33 native mammal species were collected across the state (Fig. 2), mostly from carcasses of road-killed or legally harvested animals. A few scats were collected as well for this study using the Endangered and Nongame Species' (ENSP) scat detection dog. Additional Bobcat DNA samples (tissues and scat; N = 148) collected as part of a different project (W-71-R-3) were also utilized for this study. The samples were collected between 1/2017 and 2/2022, except for several shrew and vole samples collected in 2012 and 2015. All study samples were labeled, documented in a database, and mapped in GIS based on latitude/longitude coordinates provided by the volunteers or the best available information for harvested specimens (e.g., ~50% of White-tailed deer samples were obtained from hunters who reported only the municipality and deer management zone of their harvest, and ~99% of Northern river otter samples were obtained from trappers who reported the municipality, management zone and water body). All samples were sent to the National Genomics Center for Wildlife and Fish Conservation (Genomics Center) for potential genotyping.

Species	North	South	Total
American beaver	12	15	27
American black bear	74	0	74
American mink	18	15	33
Bobcat	(148)	0	(148)
Brown rat *	1	4	5
Cinereus shrew	6	2	8
Common muskrat	8	11	19
Coyote	27	38	65
Eastern chipmunk	29	8	37
Eastern cottontail	39	97	136
Eastern gray squirrel	82	57	139
Eastern mole	2	2	4
Eastern pygmy shrew	1	0	1
Gray fox	9	11	20
Long-tailed weasel	5	0	5
Meadow jumping mouse	3	0	3
Meadow vole	5	1	6
North American porcupine	12	1	13
North American red squirrel	17	0	17
Northern river otter	46	108	154
Northern short-tailed shrew	15	2	17
Raccoon	73	68	141
Red fox	47	96	143
Smoky shrew	8	0	8
Southern bog lemming	2	0	2
Southern flying squirrel	55	0	55
Southern red-backed vole	4	0	4
Star-nosed mole	6	1	7
Striped skunk	30	19	49
Unknown	4	0	4
Virginia opossum	55	53	108
Weasel sp.	1	0	1
White-footed deermouse	16	4	20
White-tailed deer	109	151	260
Woodchuck	39	39	78
Woodland vole	6	0	6
Total	866	803	1669

Figure 2. A map (left) displaying the locations of all DNA samples collected for this study (gray dots) from terrestrial mammal species across New Jersey. State Route 1 (orange line) is shown again here as the North/South dividing line. The table (right) shows the number of samples collected by species from both the North and South regions. Yellow highlighting indicates the 10 species for which gene flow assessments were done. Note, Bobcat tissues and scats were collected as part of a different project and utilized for this study. *Brown rat is not native to NJ.

From these DNA samples, gene flow assessments were conducted for 10 native mammal species, exceeding our goal. Nine species had adequate sampling (≥20 samples in both the North and South regions of NJ, with wide spatial distribution). Those species are highlighted yellow in Fig. 2 and include: Coyote, Eastern cottontail, Eastern gray squirrel, Northern river otter, Raccoon, Red fox, Virginia opossum, White-tailed deer, and Woodchuck. We also decided to assess Bobcat, even though all samples were from the North region due to the species' limited distribution in NJ. The 10 assessed species vary considerably in life history characteristics and movement capabilities, offering insights into how landscape barriers may affect their populations differently over time.

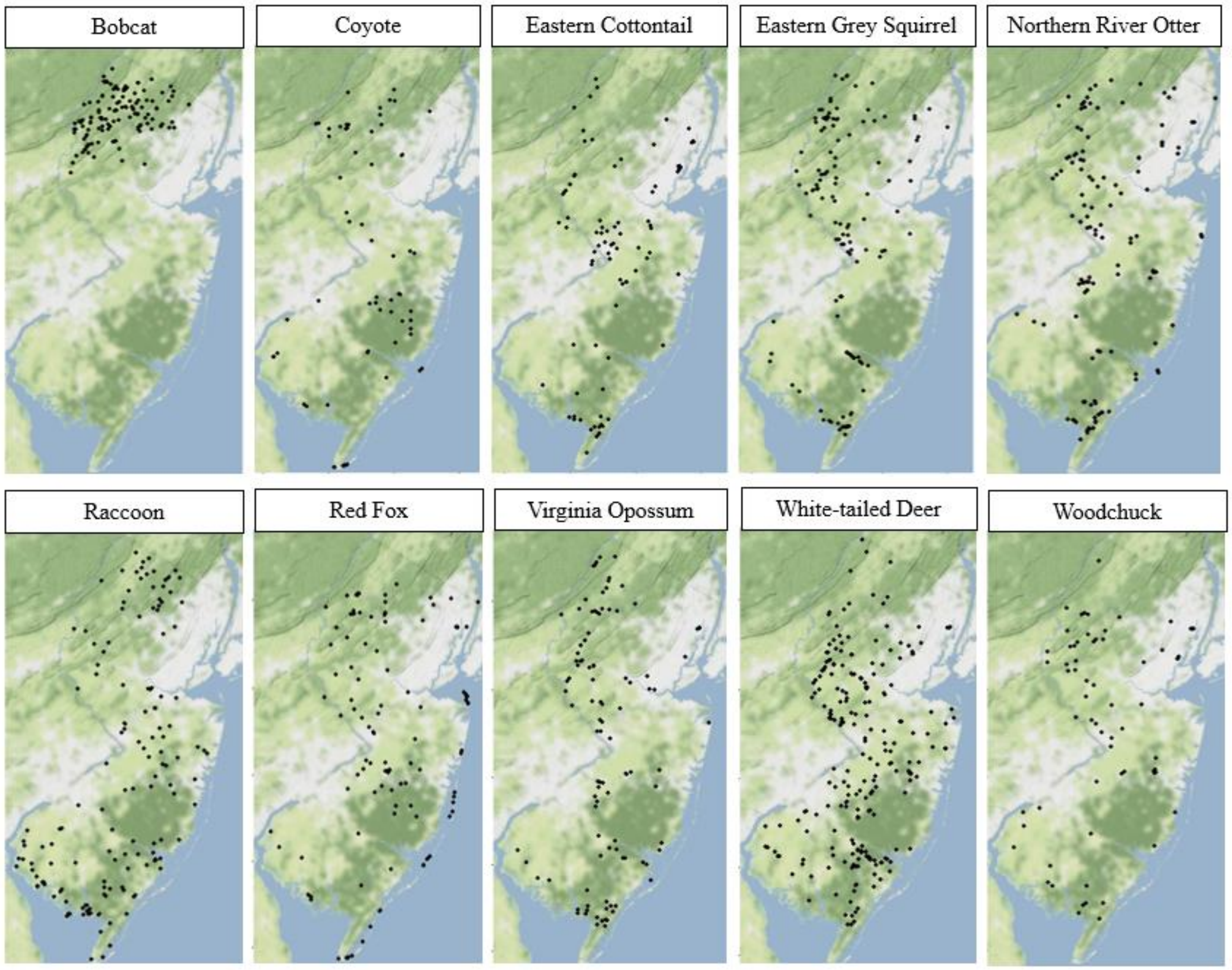

Figure 3. Maps showing DNA sample distribution for each of the 10 mammal species assessed in this study.

Samples from each of the 10 species were genotyped at 10-12 microsatellite loci (Table 1) by the Genomics Center. Only samples that amplified at 70% or more of locations were included in the analyses. Additionally, 20 individuals were omitted from the analyses because they occurred on the border with, or within, a neighboring state; these included 9 Bobcats, 6 Eastern cottontails, 2 Eastern gray squirrels, and 1 each of Raccoon, Red fox, and Northern river otter.

Table 1. The 10 mammal species genotyped for this study, with the sample number (N) and number of Microsatellite loci analyzed as markers for each species, as well as the specific Microsatellites (repetitive segments of DNA in the genome) used.

Species	N	# Loci	Microsatellites
Bobcat	148	10	Fca559, 5ca96, Lc106, Lc109,Lc111, Lc118, Fca132, Fca57, Fca43, PcoA208
Coyote	66	11	GH2137, GH2088, FH1020, FH2079, FH2096, FH2054, PEZ17, FH2001, FH2161, cxx250, FH2004
Eastern cottontail	126	10	Sol03, Sol44, 7L1D3, Sol08, Sat16, 5L1A8, Sat5, Sol30, Sol33, Sat12
Eastern gray squirrel	136	10	GR09, GR10, GR16, GR17, GR22, GR48, GR51; Scv31, Scv12, Scv13
Northern river otter	152	12	Lut782, Lut604, Lut435, Lut456, Mvis075, Tt1, Gg7, RIO07, Gg14, RIO11, Lut701, RIO17
Raccoon	135	11	Plot06, Plot10, Plot07, Plot11, Plot01, Plot02, PLOM15, PLO371, PLOM20, PLOM17, Pfl11
Red fox*	134	10	CP8, FH2004, CPH12, FH2010, PEZ17, FH2054, CXX250, FH2088, AHT130, C20253
Virginia opossum	106	11	Op03, Op38, Op16, Op46, Op30, Op39, Op31, Op41, Op36, Op48, Op02,
White-tailed deer	257	10	BM888, FCB193, RT7, Cervid.1, T7.P, BM4107, BM4208, RT9, C89_D, BM1225
Woodchuck	78	11	Bibl36, GS14, Bibl4, Bibl25, ST10, MS56, GS25, MS53, Bibl18, GS22, MS45

* Excluding barrier island samples, the sample size for Red fox was 88.

Maximum likelihood population effects (MLPE) modeling (Clarke et al. 2002) was used to assess which of the following four hypotheses best explains the genetic distances among individuals of each species:

1. **<u>Isolation by barrier</u>**: Animals on the same side of the Philadelphia-NYC highway corridor (North or South of State Route 1) are more closely related than those on opposite sides. Note: this model was not built for Bobcat since there were no Bobcat DNA samples from the South region.
2. **<u>Isolation by resistance</u>**: Genetic distance between individuals is explained by non-habitat landscape features (e.g., human modification) that affects their ability to disperse. The habitat resistance layer built as part of the CHANJ habitat corridor modeling was used to represent landscape resistance (Fig. 4). This 20m-scale product integrates habitat classification (habitat vs. non-habitat based on NJ 2012 Land Use/Land Cover Classification designations) and road usage (both road size and traffic volume) to define landscape resistance (New Jersey Division of Fish and Wildlife, 2019).
3. **<u>Isolation by distance</u>**: Genetic distance between individuals increases with geographic straight-line distance. This is a commonly observed phenomenon in natural populations, particularly those with limited dispersal. It does not take into account the additional effects of landscape elements on gene flow.
4. **<u>Panmixia/ Null hypothesis</u>**: Genetic distance between individuals is similar, regardless of where animals are located across NJ.

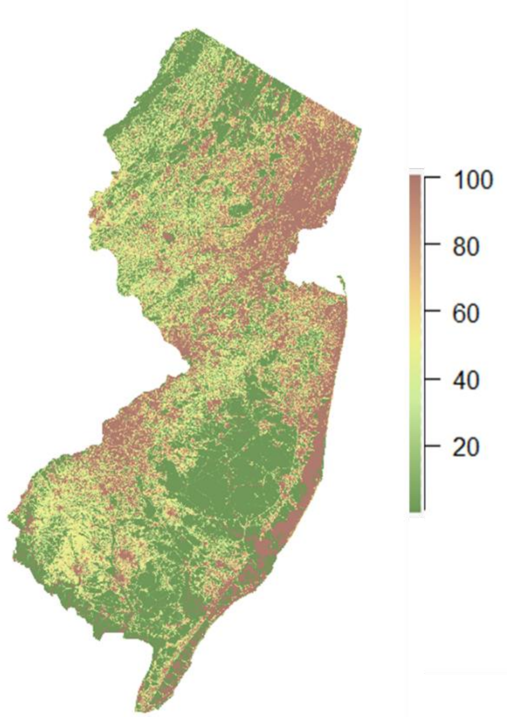

Figure 4. Map showing landscape resistance (modeled by 20m grid size) with resistance values from 1 (low resistance; green; easier for terrestrial wildlife to move through) to 100 (high resistance; red; harder for terrestrial wildlife to move through).

Pairwise genetic distances between individuals were calculated as Nei's genetic distance with the R package *adgenet* (v2.1.7; Jombart 2008) as well as Principal Component Analysis (PCA) genetic distance using methods and code from Bauder *et al.* (2021). Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to evaluate support for the hypotheses for each species. Since White-tailed deer samples had different spatial resolution (e.g., less precise location data for harvested animals than non-harvested animals), these samples were analyzed three ways: all animals, harvested animals only, and non-harvested animals only. Results for these three models did not differ, so only results for all animals together are reported. Bobcat data were analyzed two ways, once using all data collected from 2006 to the present day and again using only data from 2018 onwards. Results for these two models did not differ, so only the more recent data are reported. Finally, Red fox data were analyzed two ways, once using all samples and again excluding the barrier islands samples. Both results are reported below.

Species identifications by DNA were also done for all species where visual misidentification by volunteers may have been an issue or where the volunteer was uncertain of species ID. A secondary benefit, we figured, was the potential to discover a previously unconfirmed species in the state, though this did not happen. In two instances, Gray foxes had been misidentified as Red foxes at the time of sample collection, so those records were corrected. There had also been several samples with uncertain species identifications which we were able to clear up using DNA. For instance, the 55 flying squirrel specimens collected as part of this study were all confirmed to be the more common, Southern flying squirrels (*Glaucomys volans*); none were Northern flying squirrels (*Glaucomys sabrinus*).

The best fit model for genetic distance varied among species (Fig. 5). For 4 and possibly 5 of the 10 species tested, their genetic connectivity seems to be impacted by human modification of the landscape – either by the Philadelphia-NYC highway corridor itself, or by broadscale landscape modification as represented by the habitat resistance layer. For another 4 and possibly 5 of the species, human modification has not altered their genetic connectivity. And for one species, genetic connectivity does not seem to be altered by human modified landscape features, nor Euclidian distance, and is similar throughout the state. Results by each genetic distance hypothesis:

1. **Isolation by barrier** best explains the genetic distance in **Coyote**. The range expansion of Coyotes started in the late 1930s in NJ. Perhaps there was a significant enough population in the South NJ region prior to urbanization of the Philadelphia-NYC highway corridor in the 1960s and 1970s, and since then it has served as a barrier to the species.
2. **Isolation by resistance** best explained the genetic distance in **Eastern cottontail**, **Woodchuck**, and **Bobcat**. When the barrier island Red fox samples were included in the analyses, isolation by resistance was also the best fitting model for Red fox. When the barrier island samples were excluded, isolation by resistance and isolation by distance models performed similarly. Eastern cottontail and Woodchuck have the smallest home ranges of the 10 species analyzed, and with smaller dispersal distances it may be difficult for these animals to maintain gene flow through areas of high landscape resistance (human modification). In the case of Bobcats, their distribution is restricted to mainly northern NJ, which is why there were no DNA samples from the South region to test. But based on previous analyses of movement patterns of collared Bobcats in NJ near major roads and other studies underscoring the impact of habitat fragmentation on Bobcats, particularly females (Riley et al. 2003), this result is not surprising. It is a bit unexpected that isolation by resistance was somewhat supported for Red fox, which is often considered a more urban-adapted species.
3. **Isolation by distance** best explained the genetic distance in **Eastern gray squirrel**, **Raccoon**, **White-tailed deer**, and **Northern river otter**. In the case of Northern river otter, the high level of resistance assigned to large water bodies like lakes and ponds in the landscape resistance layer may explain why isolation by resistance was not found to be significant for this species, perhaps inaccurately. The other 3 species all have larger home range sizes and dispersal distances and/or have adapted well to urban environments and are quite abundant in NJ, which likely plays a role in their ability to maintain good gene flow despite the barriers.
4. The **Virginia opossum** population is **panmictic** according to these analyses, which means there is no pattern of genetic differentiation across the state. Perhaps the panmixia signifies a somewhat recent range expansion; opossums were very uncommon in the northern counties of NJ in the late 1860s but now are quite abundant. A study in IN where opossums are expanding also found no pattern of genetic differentiation statewide (Hennessy et al., 2014). Also, the Virginia opossum is one of the few mammals that does not occupy a fixed or permanent home range. Some adults are sedentary, but many wander - often following water courses and establishing temporary dens in new areas (Saunders 1988). This, plus their adaptiveness to urban environments and abundant population, could explain why opossums seem to have the highest gene flow of all the species tested.

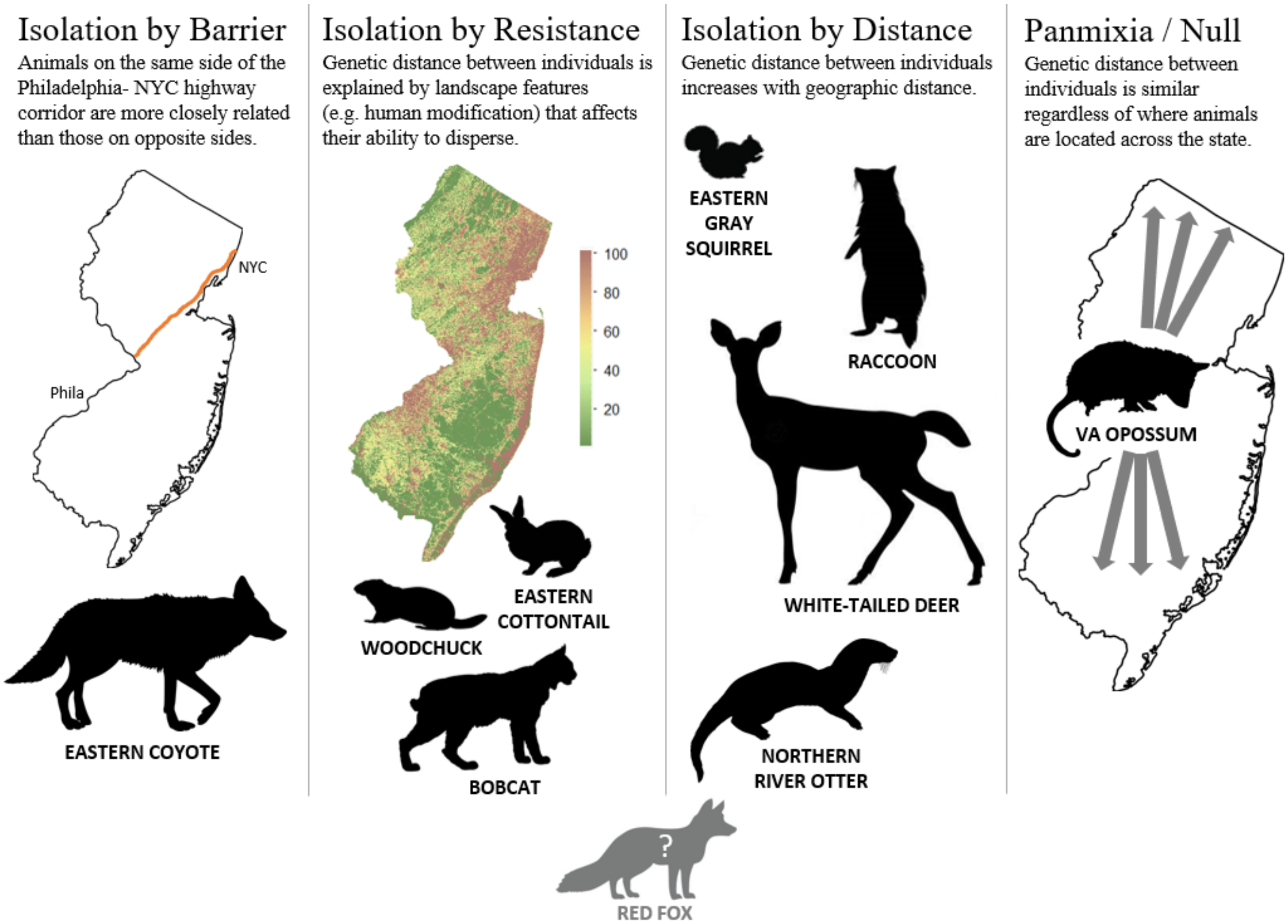

Figure 5. Results of maximum likelihood population effects (MLPE) modeling for the 10 mammal species tested, categorized by which of four hypotheses best explains the genetic distance among individuals across NJ.

Concurrent with this study period but under a separate grant (NJ T-11-T-3), we completed our development of Connecting Habitat Across New Jersey (CHANJ; www.chanj.nj.gov), a project which aims to improve functional habitat connectivity across the state. Our main CHANJ products include a mapping tool – delineating habitat cores and corridors and the roads that cut through them – and a guidance document to steer connectivity-focused land acquisition, habitat restoration and management, and road mitigation. As part of this current CHANJ Assessments study, we looked at tools and techniques for evaluating wildlife movement patterns relative to the CHANJ-mapped cores, corridors, and roads to help us assess whether areas are functionally connected and to inform mitigation strategies such as road permeability improvements. These tools and techniques included:

GPS data: Point data from GPS collars or radio telemetry studies are one of the best means of evaluating how individuals move across a landscape and interact with certain landscape features. We did not deploy collars as part of this study, nor did NJFW have any active mammal collaring projects during the grant period. Rutgers University had an active collaring project of Red fox during the study period, and the data they shared with us help to illustrate the utility of this technique. The example below (Fig. 6) shows how an individual Red fox interacted with a high-volume roadway (average annual daily traffic >10,000 vehicles) within its home range from 1/2021 to 10/2021. The fox crossed the highway several times, but it is unclear from the 2-hour interval GPS fixes whether the fox utilized existing culvert structures (shown in red) to do so or crossed over top of the road. But these data help to target a road segment for further investigation using the CHANJ tools and survey protocols described under Objective 2 to better understand whether and how to mitigate the road barrier for land mammals.

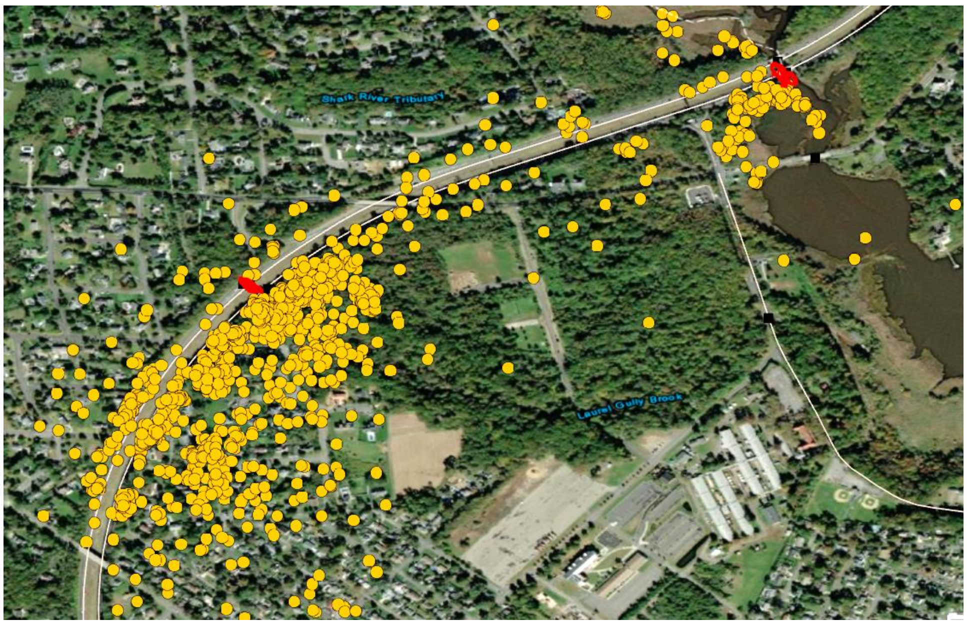

Figure 6. Movements of a Red fox (orange dots) collected by a GPS collar between 1/2021 and 10/2021 in southern New Jersey. The red areas indicate road/stream structures according to the North Atlantic Aquatic Connectivity Collaborative (www.naacc.org). Further investigation would be needed to assess the passability of these structures for terrestrial wildlife and their potential for improvement.

Camera monitoring: Infrared motion-triggered cameras are a very effective tool for noninvasively monitoring movements of wildlife through the landscape and/or in relation to features such as potential wildlife passage structures. Camera monitoring was deployed in CHANJ-mapped areas at potential crossing structures as part of this study (see Objective 2).

Scat surveys: The DNA in animal scat (from sloughed-off intestinal cells) can be analyzed to determine the species and individual animal of origin. Using a scent detection dog trained to find the scat of target species is therefore another effective, noninvasive means of confirming presence and evaluating the movement of wildlife through the landscape and/or in relation to features such as potential wildlife passage structures. Fig. 7 exemplifies how Red fox and Bobcat scat samples collected by the ENSP's detection dog-handler team can be used by themselves, or combined with other sampling methods to piece together movement data for 2 mammalian species.

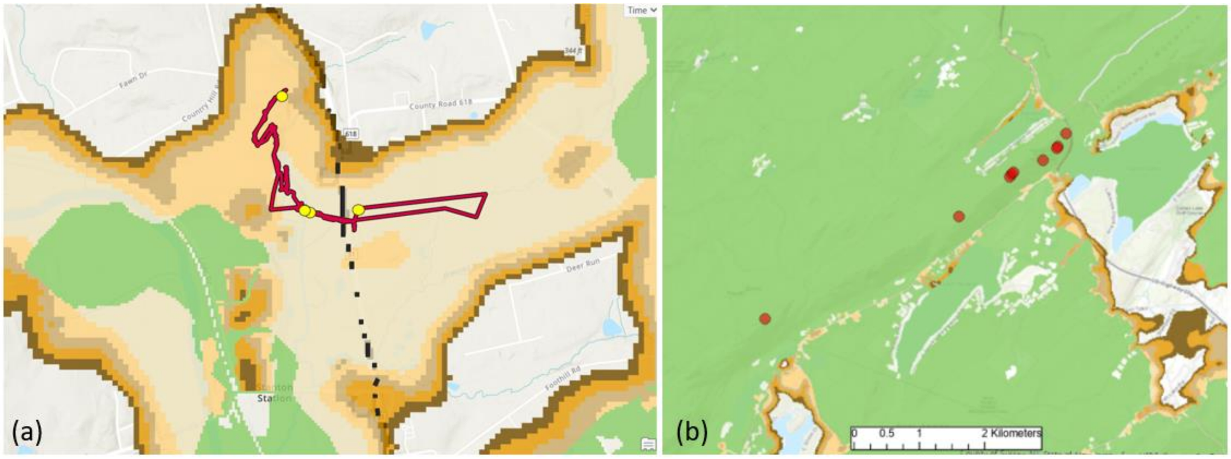

Figure 7. Maps of a) scent detection dog survey transect (red line) within a CHANJ corridor on 1/10/2020 where Red fox scats (yellow dots) from 3 individuals were found on both sides of a road barrier, and b) Bobcat scats (red dots) from the same female on 3 different sampling occasions over a 2 year period, found by our detection dog within a CHANJ core habitat in northern NJ.

<u>Roadkill data</u>: Point locations of road-killed wildlife can be very useful for quantifying the effect of roads on different species or taxonomic groups and for pinpointing road mortality hotspots potentially in need of mitigation. This project employed standardized roadkill surveys in CHANJ-mapped areas – described under Objective 2 – as well as "crowd-sourced" data collection by other NJFW bureaus, our volunteers and members of the public. Opportunistic roadkill data collection (and DNA sampling) by our gene flow volunteers was described above.

From other NJFW bureaus, we obtained records of dead-on-road Black bear, Coyote, Fisher, and other species of interest that have been solicited from the public for the past several years, each with their own separate tracking system and none having been digitally mapped previously. We converted these records to GIS (Fig. 8) and imported them into our new NJ Wildlife Tracker application, which now serves as a centralized, standardized roadkill database (as well as the ENSP's main rare species reporting method; described in NJ T-11-T grant reports).

ENSP has been maintaining a GIS database of Bobcat roadkill data and those, too, have been imported into the NJ Wildlife Tracker application. Centralizing all roadkill records into this app will enable us to more easily identify hotspots (as exemplified in Fig. 8, inset) and justify future road mitigation solutions. A tool to report roadkill is also an effective outreach method to highlight the issue of landscape fragmentation and prompt people to notice the roadkill that most have become complacent to. As we roll out NJ Wildlife Tracker more broadly in 2023 and solicit roadkill data from the public, we expect the database to grow considerably.

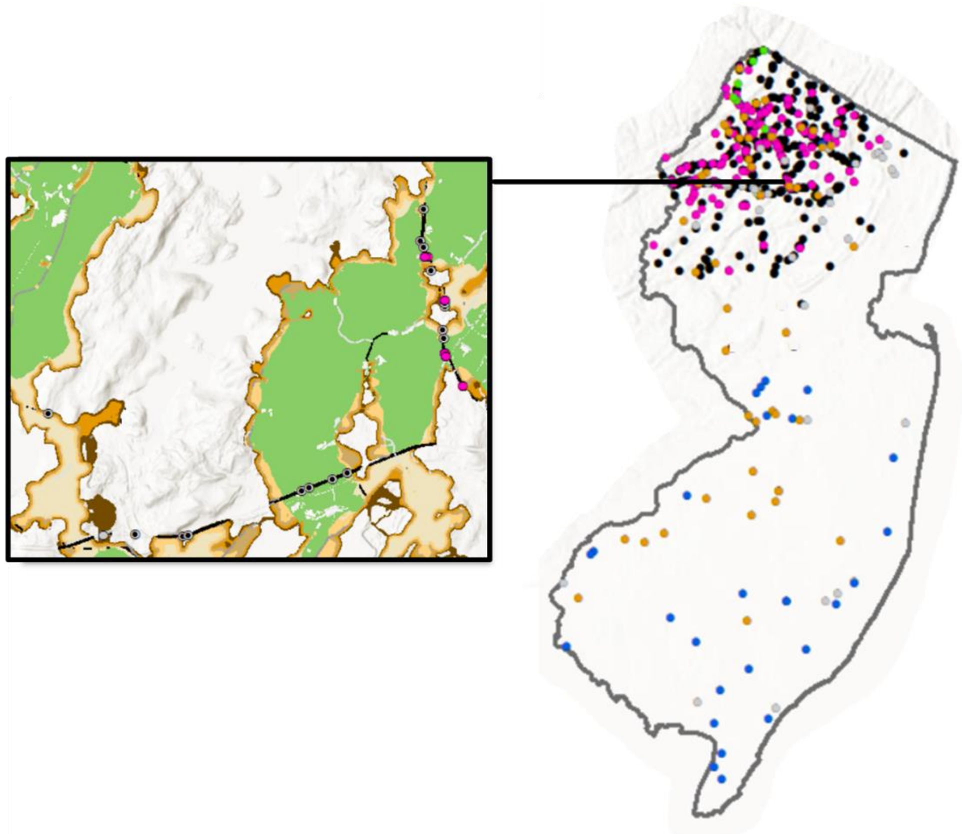

Figure 8. Roadkill records (dots) of Black bear (black), Coyote (grey), Northern river otter (blue), Fisher (green), Beaver (brown) and Bobcat (pink) from NJFW from 2000 to 2022 that are now centralized in the NJ Wildlife Tracker database. Inset, a zoomed-in example of apparent roadkill hotspots for high-mobility species between 2017 and 2022 along CHANJ-mapped road segments in northern NJ.

Key Findings for Objective 2:

For this objective, we set out to develop Road Assessments for at least 35 road segments bisecting CHANJ-mapped cores and corridors, incorporating GIS analyses, barriers to wildlife movement observed, NAACC culvert survey results, roadkill data, camera monitoring results, and recommendations for improving connectivity as needed. During the grant period, 533 unique CHANJ Road Segments were assessed by at least one survey method (Fig. 9). A total of 33 CHANJ Road Segment Reports were developed using information from 172 unique CHANJ Road Segments. We decided to include multiple Road Segments within most Reports due to the short length and/or adjacency of many Segments. Contents of the Road Segment Reports are detailed near the end of this section.

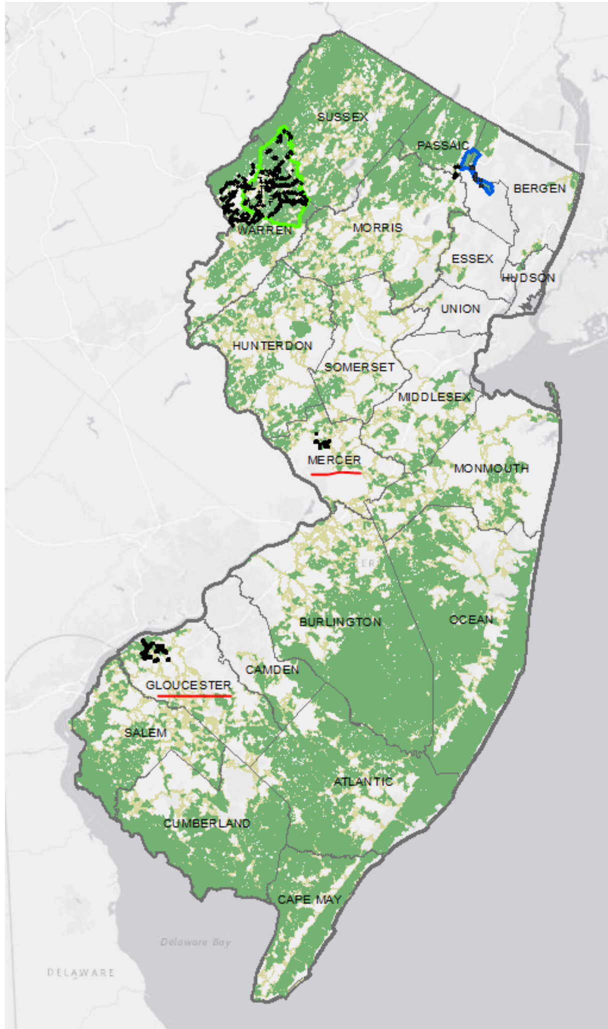

Figure 9. The 533 CHANJ Road Segments assessed (black lines) within the pilot areas of Bobcat Alley (green outline), High Mountain (blue outline), Mercer County and Gloucester County, NJ, overlaid on the CHANJ core and corridor mapping.

The ENSP CHANJ team worked with The Nature Conservancy in New Jersey (TNC) during the first reporting period to delineate two pilot areas for road assessment work in northern New Jersey, dubbed Bobcat Alley and High Mountain. Given the extensive road networks within these pilot areas, baseline GIS analyses were done to prioritize Road Segments for assessment based on certain criteria; for example, whether the road occurs in a CHANJ core or corridor (required), intersects a stream, or has protected land on one or both sides. TNC staff assessed a total of 387 CHANJ Road Segments, representing at least 10% of the CHANJ Road Segments in the two pilot areas.

ENSP seasonal field staff assessed an additional 146 CHANJ Road Segments in separate areas from TNC, in the central (Mercer County) and southern (Gloucester County) areas of the state. Segments selected in these areas were based on occurrence within a CHANJ-mapped area and ability to safely survey by vehicle.

The methods used to assess Road Segments included:

<u>Standardized transect surveys for on-road mammals</u>: We adopted a standardized CHANJ Roadkill Survey Protocol and reporting form (Appendix I) for this project. Transect surveys entailed driving or walking each Road Segment at least once weekly for a minimum 6-month timeframe and recording information during each survey, including the date and time of the survey, the number and species of animals found alive-on-road or dead-on-road (or that no animals were observed), the location of animals observed within the transect, and a voucher photograph of each. Surveys were generally conducted from April through November. A total of 12,764 transect surveys were completed along 533 unique CHANJ Road Segments during this project:

- 2018: 3,646 surveys were conducted by TNC staff.
- 2019: 1,780 surveys were conducted by TNC staff. Segments monitored in 2018 were repeated in 2019 to collect a larger dataset across at least 6 months.
- 2020: 4,180 surveys were conducted by TNC staff and 3,158 surveys were conducted by ENSP seasonal staff. All Road Segments monitored in 2020 were new to the study.

A total of 210 alive-on-road or dead-on-road mammal species were recorded along the monitored CHANJ Road Segments during the survey period of 2018-2020 (Table 2). There were 16 different mammal species observed and recorded in addition to 12 individual unknown mammal species that could not be identified to species-level based on the amount of decomposition or limited remains at the time of detection.

Table 2. Mammal species observed along monitored CHANJ Road Segments during the years 2018-2020. Observations include both alive-on-road and dead-on-road animals.

Mammal Species	**Number Observed (2018-2020)**
EASTERN CHIPMUNK	54
EASTERN MOLE	1
LEAST SHREW	1
STAR-NOSED MOLE	1
WHITE-FOOTED MOUSE	2
COMMON RACCOON	23
EASTERN COTTONTAIL	7
EASTERN GRAY SQUIRREL	64
LONG-TAILED WEASEL	3
RED SQUIRREL	1
STRIPED SKUNK	1
VIRGINIA OPOSSUM	17
WOODCHUCK	13
COYOTE	1
RED FOX	3
WHITE-TAILED DEER	6
UNKNOWN	12
TOTAL	**210**

Additional road-killed mammal data was collected opportunistically as part of the actions described under Objective 1 above, including as part of the DNA collection (gene flow) element and via community reports of priority mammal species obtained from NJFW's Bureau of Wildlife Management.

Motion-triggered camera monitoring: Our goal was to deploy cameras at a minimum of six (6) existing culverts and/or bridges beneath CHANJ Road Segments to assess frequency and diversity of wildlife using the structures. In total across all project years, our collaborators at TNC deployed cameras either singly or in pairs at 24 locations: 19 within Bobcat Alley (14 in CHANJ Road Segments), 4 within High Mountain (2 in CHANJ Road Segments), and 1 in a separate area of interest in Sussex County under Rte. 15 (Fig. 10). Not all camera stations were deployed in CHANJ areas, as other potential crossing areas of interest were also investigated. More than 112,000 photos were processed, resulting in well over 1,000 instances of native mammals of at least 18 species interacting with the stream crossings.

In April 2020, the ENSP CHANJ team collaborated with NJ Department of Transportation (NJ DOT) to install 6 cameras at two locations in the Sourland Mountains region of central NJ where wildlife mitigation measures were recently implemented within CHANJ-mapped cores/corridors under a state highway (Rte. 31) in Hunterdon County and a county road (CR 518) in Mercer County (Fig. 10). NJ DOT purchased the cameras and contracted a consulting firm to process the photos using ENSP's camera monitoring protocol. ENSP collaborated on the placement of the cameras and resultant data was shared with ENSP to incorporate into the Road Segment Reports. More than 1,600 wildlife events were captured in or around these crossings, representing 9 native mammals species (White-tailed deer and Red fox being most common).

Figure 10. Yellow squares on the map show locations where motion-triggered cameras were used to monitor pass-through or avoidance of a bridge or culvert by terrestrial mammals during this project. At right are examples of motion-triggered camera setup and wildlife images captured, from top to bottom: Neha Savant (TNC) with a camera deployed in Bobcat Alley in Year 1; a Bobcat tries to navigate but does not pass through a stream culvert lacking dry passage; a White-tailed deer and fawns willingly pass through the same culvert, showing their tolerance for "wet feet" compared to many other land mammals.

<u>NAACC culvert inventory</u>: The ENSP CHANJ team enlisted three contractors to perform stream culvert assessments within Bobcat Alley, High Mountain, the Sourland Mountains, and other targeted CHANJ-mapped areas of New Jersey. These contractors are trained and certified to collect and manage data following the North Atlantic Aquatic Connectivity Collaborative (NAACC) protocols for assessing passability of road-stream crossings for terrestrial wildlife. In addition, four ENSP hourly mammal technicians became certified and performed culvert assessments for this project. A total of 642 culvert assessments for terrestrial wildlife passage were completed under this project (Table 3).

Table 3. NAACC culvert surveys done by contractors and staff to assess terrestrial wildlife passage within CHANJ-mapped focal areas.

Organization	**CHANJ Focal Area(s)**	**# Assessments**
Montclair State University	Bobcat Alley, High Mt., Sourland Mts	260
Barnegat Bay Partnership	Barnegat Bay watershed & I-195	145
Raritan Headwaters Association	Sourland Mountains corridors	119
NJFW ENSP	Various	118

In the course of this project, at least 24 new individuals were trained and certified as NAACC Lead Observers (L1), adding to New Jersey's capacity and momentum for this work.

To help coordinate efforts among a growing number of survey partners, and as a tool for other connectivity advocates across the state, a "NAACC Culvert Inventory of New Jersey - Terrestrial Organism Passability" data layer was integrated into our CHANJ Web Viewer (Fig. 11). When culvert assessments are completed in New Jersey and approved within the NAACC database, they are now instantly reflected in the CHANJ mapping. This NJ NAACC data layer is also now available as a GIS feature service.

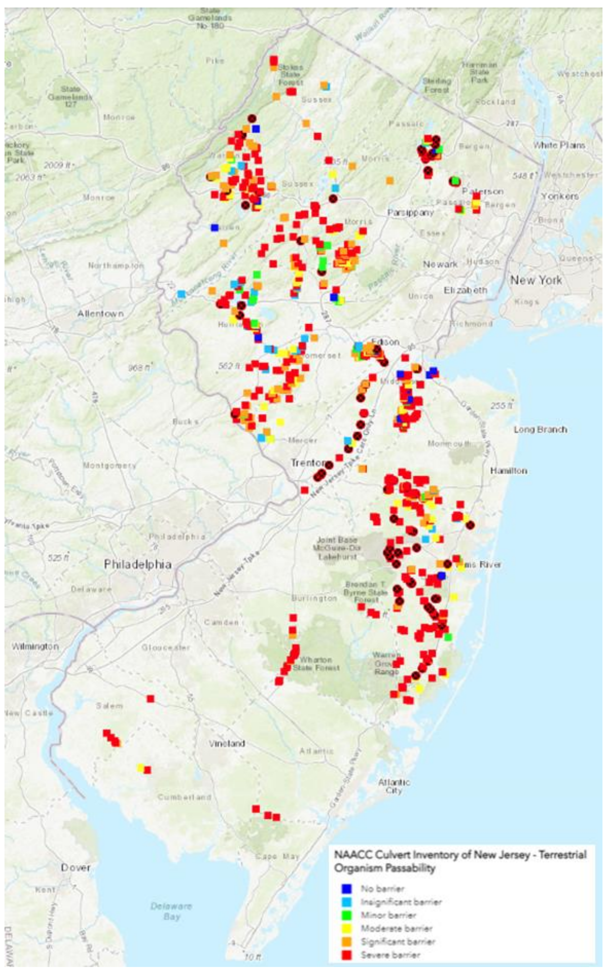

Figure 11. The NAACC Culvert Inventory of New Jersey - Terrestrial Organism Passability data layer, available via our interactive CHANJ Web Viewer, shows all NJ stream crossing assessments completed to-date (N=798) with their terrestrial passability scores.

To date, 798 structures have been assessed across NJ, with 642 of these occurring in CHANJ-mapped areas. From the NAACC scores, 77% of these structures in CHANJ-mapped areas pose a “moderate,” “significant,” or “severe” barrier to terrestrial wildlife passage, while 23% were found to be “insignificant, “minor,” or “no” barrier to terrestrial wildlife passage (Fig. 12).

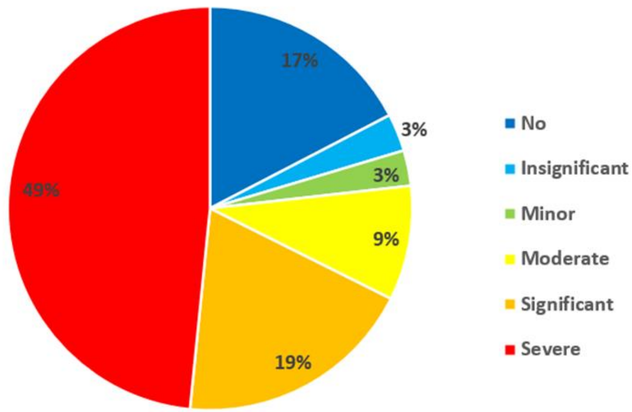

Figure 12. Summary of NAACC stream-crossing assessment results within CHANJ mapped areas, N = 191 and 451 in Cores and Corridors respectively, by terrestrial wildlife barrier category, for all NJ surveys combined (N=642).

<u>CHANJ Road Segment Reports</u>: Going into this project, we identified the need for a standardized template to compile and present information about the various CHANJ Road Segments being assessed. An early deliverable for this project was to create a CHANJ Road Segment Report template, in a format that is useful for communicating with transportation planners and conservation partners about the need for mitigation at a location, and as a basis for a grant proposal. The outline for our Road Segment Report template includes:

Chapter 1. Segment Description & Assessment
- 1.1. Location, Extent, and Description
- 1.2. Potential Wildlife Barriers, Adjacent Land Ownership, Land Use, and Human Activity
- 1.3. Crossing Structure Inventory
- 1.4. Transportation Plans

Chapter 2. Wildlife Use & Survey Results
- 2.1. Wildlife Observations and Landscape Project Habitat Values
- 2.2. Road Surveys for Wildlife
- 2.3. Camera Monitoring

Chapter 3. Road Mitigation Recommendations

Chapter 4. Road Mitigation Construction Details and Documentation
- 4.1. Conceptual Designs
- 4.2. Engineering Plans
- 4.3. Project Costs
- 4.4. Construction Photographs

Chapter 5. Post-construction Monitoring and Evaluation
- 5.1. Monitoring
- 5.2. Evaluation

Again, 533 CHANJ Road Segments were monitored during this project. A total of 33 CHANJ Road Segment Reports were developed using information from 172 unique CHANJ Road Segments. Multiple Road Segments are included in most Reports due to the short length and/or adjacency of many Segments. In multi-segment Reports, information on individual Road Segments remains itemized and available, such that the lumping of Segments does not result in the loss of Segment-specific data. In most cases, Reports are limited to Chapters 1 and 2 since road mitigation is not yet planned. An example of a CHANJ Road Segment Report is included as Appendix II. These Reports may eventually be made available via a layer within the CHANJ Web Viewer.

Conclusions and Recommendations:

CHANJ was launched publicly during this grant period, and its mapping tools are already serving as a useful filter for our efforts and those of our collaborators by focusing resources on areas with the most potential for connectivity gains. Deliverables under this grant both stand alone as valuable outputs to assist in forming next steps or providing evidential baselines, but also provide platforms to seek new opportunities, partnerships, and additional funding to expand and continue work.

The first objective of this project starts at a broad scale, with the goal of understanding whether human modification of the landscape (e.g., habitat fragmentation) is affecting the genetic connectivity of native terrestrial mammals across New Jersey. The second objective takes a closer look at ground-level road features and road-wildlife conflicts (e.g., roadkill, lack of safe passage across road barriers) and various tools we can use to inform road mitigation priorities, ultimately improving functional habitat connectivity across the state.

Together, results, data, and products from both objectives can inform where conservation action is highest priority and in greatest need, and where action may be most effective. For example, "hotspots" from roadkill data for species identified in Isolation by Barrier or Isolation by Resistance can be prioritized for road mitigation or habitat restoration work in an attempt to increase gene flow for those local populations, especially to ensure there is functional connectivity across all guilds of species between northern and southern NJ. NAACC scoring within hotspots can help us identify whether existing structures may already be suitable to pass terrestrial mammals, perhaps just with the addition of barrier fencing to funnel target species to those structures.

Below, we highlight objective-specific conclusions and recommendations:

Objective 1 Conclusions:

- With help from more than 100 participants, 1,669 DNA samples were collected representing 33 native mammal species across the state, enabling the first gene flow analysis across multiple terrestrial mammals in NJ. Our crowd-sourced method of using volunteers to opportunistically sample road-killed animals by clipping a small (¼") ear tip and placing it in a 15ml vial with silica desiccant at room temperature proved to be an effective sampling technique. More than 99% of samples collected this way had good quality DNA and amplified well for inclusion in the analyses.

- We now have a genetic database with precise geospatial data across a variety of native mammal species, collected over a 5-year period, for the first time that may be useful for other projects in the future.
- The results of the gene flow study indicate that human modification may be altering genetic connectivity for multiple species across NJ – including for carnivores like Coyote and the state-endangered Bobcat. This finding is consistent with studies from other states (e.g., Crooks 2002) and reinforces the use of large carnivores as indicators of landscape connectedness (Beier et al. 2008), presumably because these animals' large area requirements and dispersal distances make them particularly vulnerable to habitat fragmentation. The DNA results also indicate that landscape resistance/fragmentation may be affecting the dispersal ability of species with small home ranges as well, including Woodchuck and Eastern cottontail.
- Other results from the gene flow study suggest that urban-adapted species with varying home range sizes, like White-tailed deer, Raccoon, and Eastern grey squirrel, are less influenced by habitat fragmentation. Northern river otter should be re-assessed using a more appropriate resistance layer for this aquatic/terrestrial adapted species. Virginia opossum showed panmictic genetics, perhaps explained by this animal's nomadic nature and tolerance for human modified habitats.
- Each DNA sample was first identified to species, which corrected a few mis-identified specimens and gave us the opportunity to test for presence of less-likely/range-edge species that are easily confused with more common species. This step did not result in any noteworthy "finds" (e.g., no New England cottontails or Northern flying squirrels, etc.), but it does represent a low-effort screening tool.
- The gene flow component of this project had the secondary benefit of helping jump-start the roadkill portion of NJFW's new NJ Wildlife Tracker application and to centralize existing roadkill records from different bureaus of our agency. Close to 2,000 roadkill records were imported into NJ Wildlife Tracker between the samples collected as part of the gene flow study (N = 681) and the records maintained by NJFW (N = 1297).

Objective 1 Recommendations:

- Continue to collaborate with the National Genomics Center for Wildlife and Fish Conservation analyzing landscape connectivity via genetic analyses to better understand the functional connectivity of NJ's landscape. For example, we can further explore gene flow in Coyotes by adding samples from our neighboring states, PA and NY, to gain a broader context. It would also be beneficial to utilize different regional resistance layers in each species' gene flow analysis to avoid edge effects, rather than removing samples. And, testing other resistance layers that depict potential landscape barriers in different ways may be informative, particularly for semi-aquatic animals like Northern river otter.
- Continue to collaborate with bureaus in our agency as well as outside organizations and academia to analyze mammal GPS collar, scat, and camera data in relation to the CHANJ mapping to help understand the functional connectivity of the landscape.

Objective 2 Conclusions:

- A total of 12,764 road transect surveys were completed along 533 unique CHANJ Road Segments during this project. Motion-triggered cameras were used to monitor terrestrial mammal behaviors at 26 stream crossing locations along roads, far exceeding our goal of 6 locations. Our contractors and staff completed a total of 642 NAACC culvert assessments for terrestrial wildlife passage under this project, illuminating problem areas for wildlife mobility within CHANJ-mapped cores and corridors and underscoring the need for connectivity enhancements (e.g., 77% of the NAACC-assessed structures in CHANJ-mapped areas pose a "moderate," "significant," or "severe" barrier to terrestrial wildlife passage). Results of these road transect, camera and culvert surveys are summarized in 33 Road Segment Reports so far, putting large datasets into a digestible and quantitative format.
- Objective 2 of this project led the ENSP CHANJ team to adopt a standardized Roadkill Survey Protocol for NJ. The protocol is included as an Appendix to the CHANJ Guidance Document (released in 2019), where it will continue to be useful to us and other CHANJ implementers. An updated version is found in Appendix I of this report. Likewise, the Road Segment Report template and the 33 Reports we created will facilitate information processing and sharing with transportation agencies and other roads-wildlife collaborators as we begin to propose more road mitigation projects.
- NJ DOT participated in and contributed funds for a camera monitoring project for the first time through this project, establishing their capacity and willingness to continue to do so in the future, in part because of the power of photos.
- This project has greatly expanded NAACC capacity and the number of certified Lead Observers across New Jersey, adding at least 24 new certified individuals. It has also added a strong focus on CHANJ-mapped areas and terrestrial wildlife passability among NAACC surveyors. In fact, this project accelerated the release of the NAACC terrestrial passability module in 2018 and has served to pilot the new protocols for the region.
- Success in the first half of this project led to us requesting and receiving additional grant funds ($80,000 total, including $20,000 in match) to cover more stream culvert surveys, roadkill surveys, and Road Segment Reports by contractors/partners in CHANJ-mapped areas of NJ.
- The NAACC terrestrial connectivity data layer has been integrated into our CHANJ Web Viewer, so that as assessments are completed and approved within the NAACC database, they are instantly reflected in the CHANJ mapping. Site-based information is therefore readily available to the public and to connectivity advocates for identifying and tackling connectivity challenges/opportunities.

Objective 2 Recommendations:

- Roll out the NJ Wildlife Tracker application and solicit roadkill data from the public to expand our database. The data will help to 1) engage the public in this issue of habitat connectivity, 2) gain support for wildlife mitigation where roadkill data suggest a need, particularly for at-risk species or for larger animals that pose a significant safety risk for motorists, and 3) identify "hotspot" areas that warrant further study. Roadkill data, too, are useful records of species occurrence and distribution for our agency.

- Encourage our own agency as well as interested universities, conservation groups and community members to utilize the standardized methodologies for roadkill and camera monitoring and to participate in NAACC, as a means to engage them in habitat connectivity efforts while broadening our data stream.
- Create a new data layer to make Road Segment Reports accessible through the CHANJ Web Viewer.
- Collect data at more CHANJ-mapped locations using the tools and methods from this project. Continue developing Road Segment Reports for CHANJ Road Segments of interest to help prioritize and communicate specific areas of need, such as key areas through the Route 1 corridor and/or specific structures where wildlife passage improvements could have a positive impact on functional connectivity for terrestrial wildlife. This information will have us well-poised to seek funding for road mitigation projects when available (e.g., from new Infrastructure Act programs).

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