Progress report – Summer 2016

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The overarching goal of this project is to assess how the anthropogenic landscape impacts the population of a mesopredator, the bobcat (*Lynx rufus*). Apex predators have been largely absent in the northeastern United States for much of the last century due to imprudent but successful eradication efforts. In recent decades, efforts to conserve or reintroduce populations of apex predators have increased in popularity and success, particularly in the American west. However, the acceptability of predator rewilding efforts to people in more densely populated areas, such as New England, is a major challenge. As such, mesocarnivores have become increasingly important agents of ecosystem balance, especially in human-dominated landscapes (Prugh et al. 2009). A greater understanding of the interplay between human and bobcat populations may lead to better functioning ecosystems in an increasingly anthropocentric world.

Study system

Bobcats (*Lynx rufus*) in the New England Region (NER) and specifically in New Hampshire (NH) are a unique study system for anthropogenic impacts on wildlife population dynamics. The demography of bobcats in NER have been influenced by varying hunting and trapping efforts, prey availability, land use, and habitat fragmentation (Litvaitis et al. 2006). The NER represents the northern limits of bobcat range, which is presumably expanding northward in the face of climate change. Furthermore, the human impact on the landscape is growing. NH is the fastest growing state in the northeast United States (Johnson 2012), and ranks second among US states in percentage of residents living in a wildland-urban interface (Martinuzzi et al. 2015). Bobcats have been protected from hunting and trapping in NH for 25 years but are harvested in all surrounding states, creating the potential for distinct source/sink areas and a dynamic dispersal environment. Their extensive range, general habitat requirements, and dispersal ability qualify bobcats as an umbrella species for many taxa, including mammal, bird, and amphibian species of concern (Cushman and Landguth 2012). A better understanding of this species' dispersal landscape and response to human impacts will lead to better connectivity conservation and mitigation programs throughout the region.

Sample collection

Through collaborations with state and provincial wildlife agencies and licensed hunters and trappers, tissue and/or hair samples were collected from bobcats in Maine, Massachusetts, New Hampshire, Vermont, and Quebec, Canada (Table 1). NH does not have a bobcat harvest season, so the majority of samples from this state came from vehicle mortalities or incidental kills collected by NH Fish and Game Department. As such, these samples were collected year-round. Samples from other states were collected during or shortly after their respective winter harvest seasons (Table 1). Tissue and hair samples were stored at -20°C. Dry hair samples were stored in paper envelopes, as moisture buildup can degrade hair samples over time.

Table 1 Bobcat samples collected (as of April 2016) for contemporary genetic analyses.

	n	Years	M:F	Sample harvest dates
VT	168	2013-2015	1.14	Dec. to Feb.
NH	164	2010-2015	1.46	Year-round
QC	33	2013-2015	2.67	Nov. to Dec.
MA	16	2012-2015	0.86	Nov. to Mar.
ME	11	2014-2015	1.50	Nov. to Feb.
Total	**392**	**2010-2015**	**1.54**	-

My dissertation focuses on the following four measures to assess the effects of a human-altered landscape on bobcat populations. The current progress report provides information of work completed to date, and plans for the remainder of 2016. Detailed procedures and data analyses will be presented in the dissertation proposal.

Contemporary population genetics of New England and Quebec bobcats

To assess the current genetic status of bobcats in NER, DNA was extracted from tissue samples using a Qiagen DNeasy Tissue Kit. A suite of 13 bobcat, Canada lynx (*Lynx canadensis*), and domestic cat (*Felis catus*) microsatellite loci were used to genotype each individual (Table 2; Menotti-Raymond et al. 1999, 2005, Carmichael et al. 2000, Faircloth et al. 2005).

Table 2 Microsatellite loci used in population genetic analyses.

Locus Name	Fragment Size	Allelic Richness	Observed Heterozygosity	Reference Heterozygosity	Reference
BC1AT	292-304	7	0.72	0.73	Faircloth et al. 2005
BCD1T	249-279	4	0.74	0.41	Faircloth et al. 2005
BCE5T	236-256	7	0.64	0.76	Faircloth et al. 2005
BCH6T	176-186	11	0.85	0.50	Faircloth et al. 2005
FCA008	118-142	7	0.56	0.90	Menotti-Raymond et al. 1999
FCA023	132-144	7	0.67	0.81	Menotti-Raymond et al. 1999
FCA045	136-166	9	0.60	0.55	Menotti-Raymond et al. 1999
FCA082	224-244	6	0.77	0.78	Menotti-Raymond et al. 1999
FCA126	114-144	9	0.75	0.71	Menotti-Raymond et al. 1999
FCA149	116-138	7	0.75	0.71	Menotti-Raymond et al. 1999
FCA391	188-212	12	0.81	0.81	Menotti-Raymond et al. 1999
FCA740	316-348	8	0.80	0.75	Menotti-Raymond et al. 2005
Lc111	120-150	8	0.63	0.59	Carmichael et al. 2000

The analytical focus is on identifying the effects of roads on the genetic structure of the bobcat population; specifically, to determine if roads are a significant barrier to dispersal as has been found in bobcat populations elsewhere (Lovallo and Anderson 1996, Poessel et al. 2014, Serieys et al. 2014).

A hierarchical analysis conducted in Structure (Pritchard et al. 2000) and Geneland (Guillot et al. 2005) revealed a significant subdivision in the bobcat population, especially along a SW to NE axis closely aligned with Interstate 89 (Fig. 1). Not all interstates in the region were barriers and some of the highest traffic-volume roads did not appear to be barriers, so more analysis needs to be done to determine if I-89 is an actual barrier or if it is coincidentally aligned with another feature that affects population structure (i.e., urban development; Fig. 2). It does appear that allelic richness decreases as urban development increases (Fig. 2), which could at least partially account for the observed structure. It may also mean that large undeveloped areas acting as reservoirs to maintain bobcat genetic diversity are equally important as connectivity across high-volume roadways.

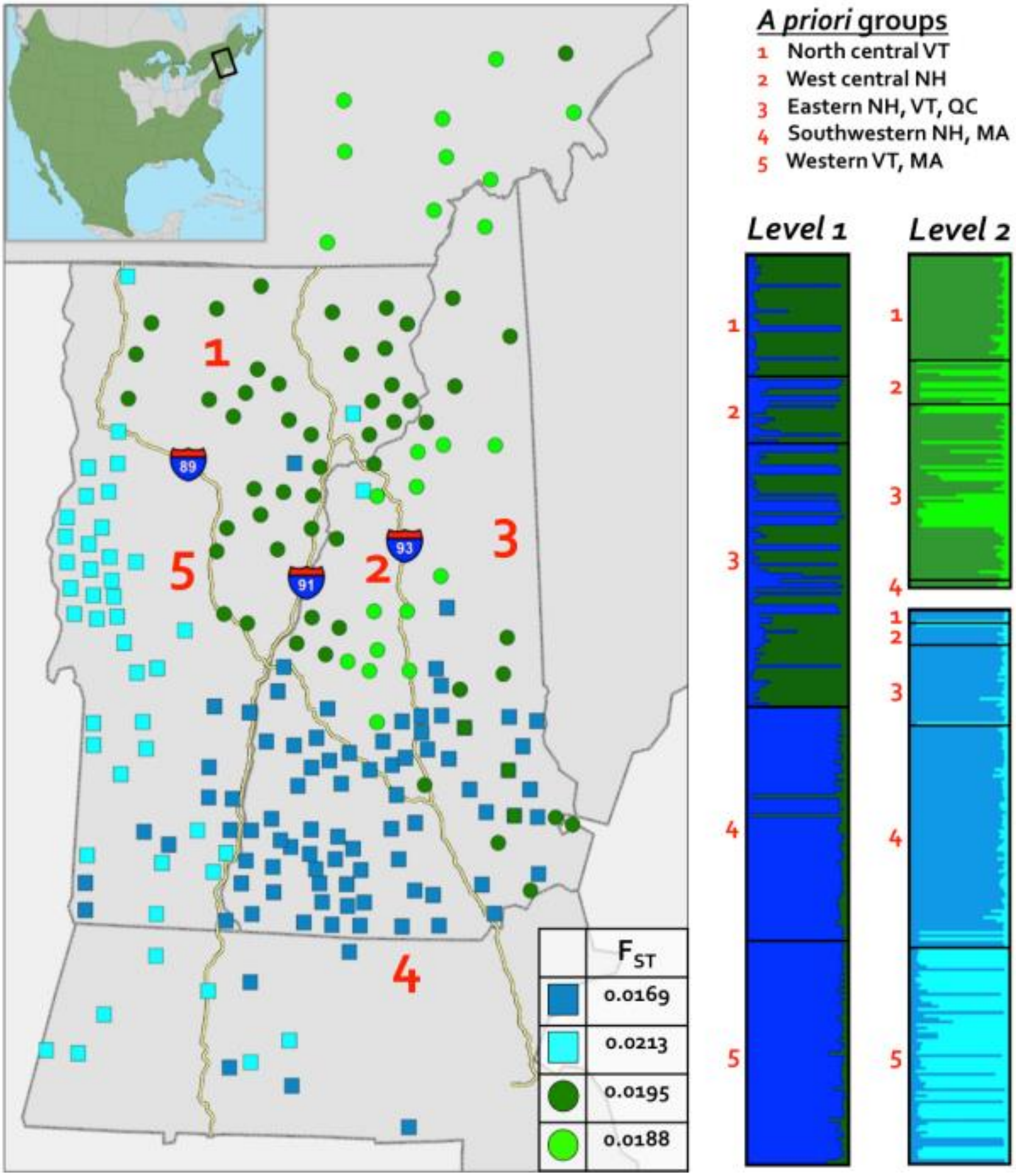
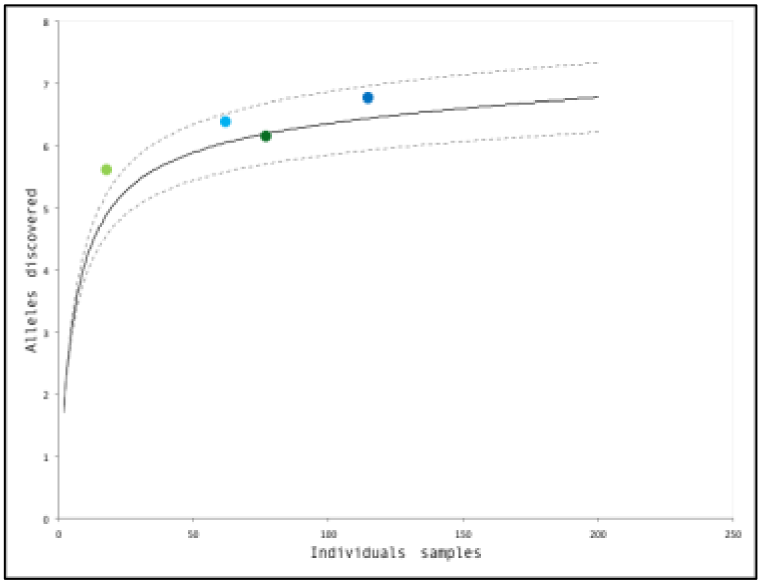
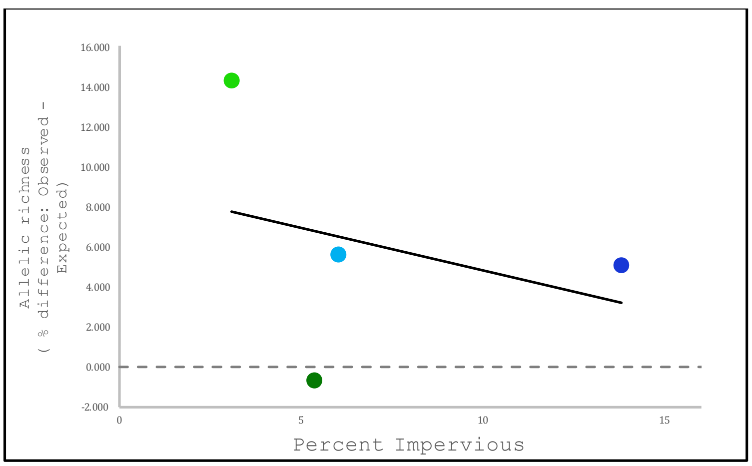


Figure 1 Population genetic subdivision inferred from 286 individuals at 13 microsatellite loci. Numbers represent areas separated by interstates and hence *a priori* predictions of subdivision.



a.



b.

Figure 2 a) Rarefaction was performed in ADZE (Szpiech et al. 2008) to determine if allelic richness in each subpopulation differed from total population expectations. Dashed lines represent 95% confidence interval. b) Percent difference from expected allelic richness was correlated to percent impervious surface, an urban development metric.

The SW to NE division was consistent even when the data were reanalyzed in sex- and generation-specific groups. Structure discovered two demes within each group (red and blue; Fig. 3). A probability of belonging to either deme was calculated for the entire landscape using inverse distance weighting based on the probability of each individual belonging to its assigned deme. The possibility that this major geographical division may be the result of edge of range dynamics or a boundary between the *L. rufus* subspecies *(L. rufus rufus* and *L. rufus gigas)* (Reding et al. 2012) will be tested.

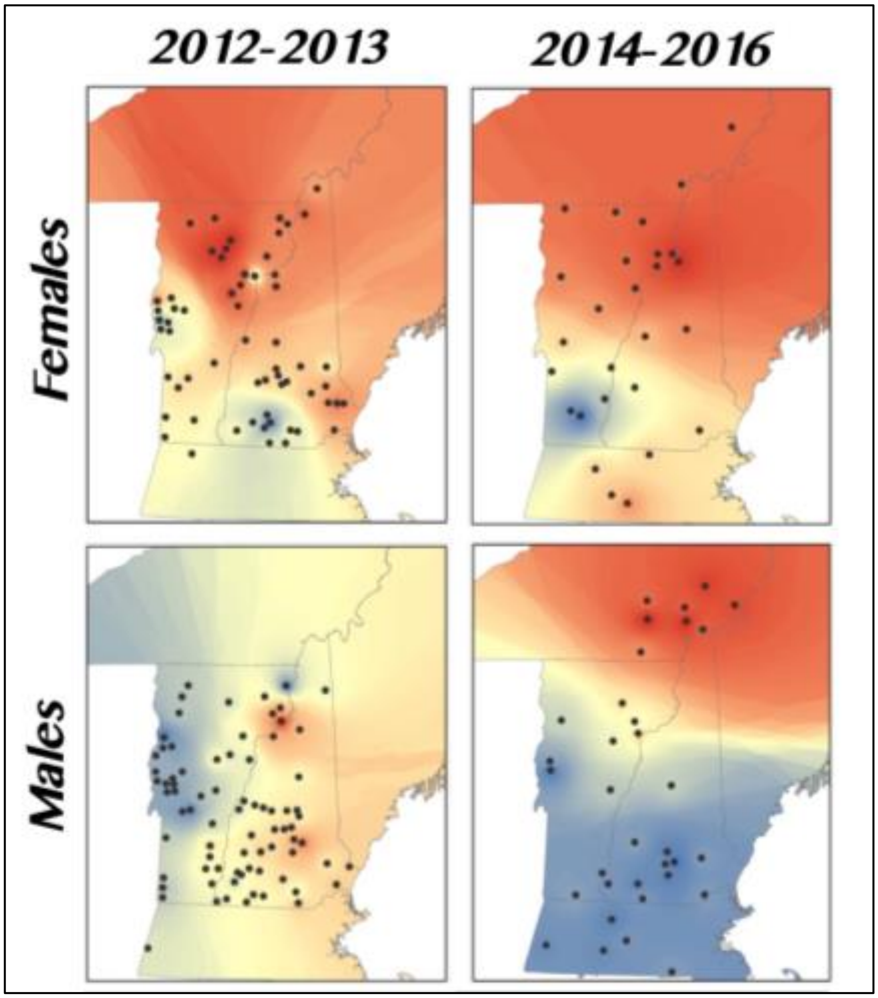


Figure 3 Interpolated probability of belonging to either the blue deme or red deme shows a fairly consistent boundary extending from north-central VT toward southeast NH. These smaller groups mimic the pattern seen in the data set as a whole.

Because the status of bobcats differs in NH (protected) from surrounding states (harvested furbearers), successful dispersal events may be occurring disproportionately out of NH. If so, regional bobcat conservation (i.e., long-term population viability) in New England may rely heavily on conservation efforts and suitable habitat in NH. Preliminary data suggest that wildlife management units (WMUs) in NH, especially in southern NH, are a source area for dispersing bobcats primarily entering southern VT and Quebec. These analyses, and all population genetic analyses, will be rerun once the data set is complete. Furthermore, since WMUs are primarily designated for white-tailed deer biology, they may not be an appropriate spatial unit for bobcats. The size and arrangement of regional spatial units will be altered to better align with bobcat biology, which may alter results.

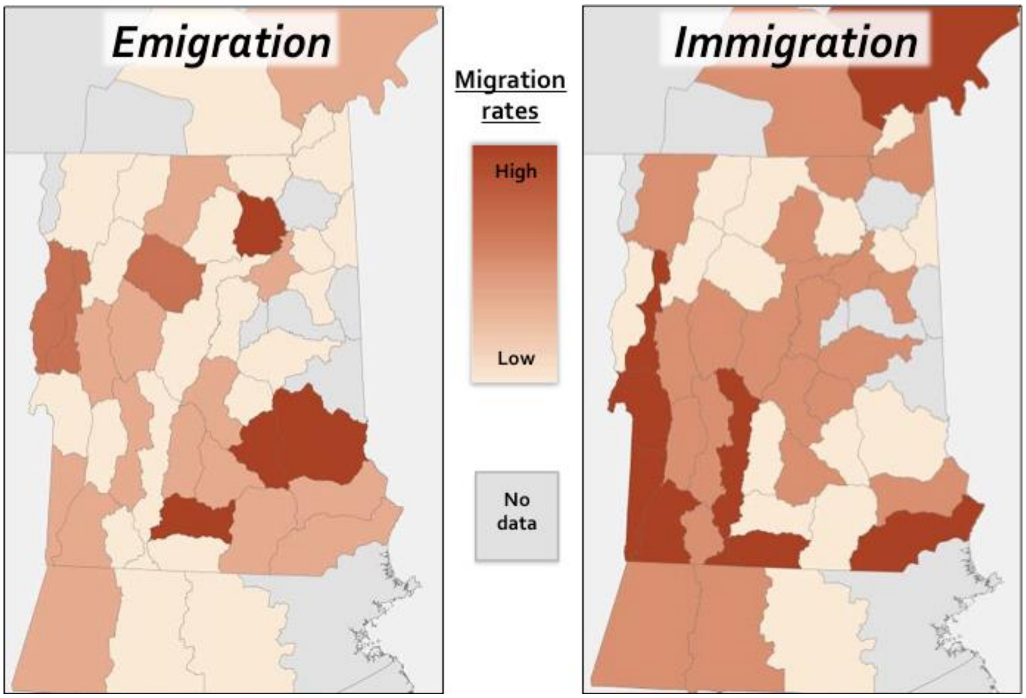


Figure 2 Bobcat immigration to and emigration from wildlife management units as determined in BAYESASS v.3.0 (Wilson and Rannala 2003).

Historic population genetics of New Hampshire bobcats

A unique collection of bobcat skulls will allow for comparisons of population dynamics and genetic structure across spatial and temporal gradients. The late UNH professor Clark Stevens collected, studied, and catalogued nearly 600 bobcats surrendered for bounty payments in NH between 1951 and 1964. Of those samples, 180 have complete skulls and metadata including date of capture, town of capture, sex, weight, and age class. Skulls were prepared by boiling and for much of the past 50+ years have been stored *en masse* in cardboard boxes at room temperature.

Thus far, with the help of an undergraduate lab tech, the DNA extraction protocol for these skulls has been optimized specifically taking into account their history of preparation, storage, and handling, all of which are not ideal for the extraction of high quality DNA. To date, genomic DNA from 46 individuals has been successfully extracted. In addition to more extractions, current work includes optimizing a genotyping protocol and identifying a set of microsatellite loci that will reliably amplify highly fragmented DNA. These data will be used in a population genetic analysis similar to that of the contemporary samples. These analyses will allow for temporal and spatial comparisons of bobcat populations across an anthropogenic development gradient.

*** At this time, the following two objectives are in early stages only ***

Spatial and temporal patterns in the diets of New England bobcats

A potential factor contributing to the rebounding of bobcats in New Hampshire is a changing prey base. Historical evidence suggests a strong link between bobcats and lagomorph populations (Litvaitis et al. 1986*a*, *b*). However, the dramatic decline in NH of the New England cottontail (*Sylvilagis transitionalis;* Litvaitis 1993) and climate-induced range shifts in snowshoe hare (*Lepus americanus*; Sultaire et al. 2016) have forced bobcats to seek an alternate dependable food source. Ultimately the shift away from a lagomorph-heavy diet may have benefited bobcats, as the mean adult weight has significantly increased in the last half century (Fig. 5).

Figure 3 Mean weight of adult bobcats has significantly increased since 1964 ($p < 0.001$; * $p < 0.05$).**

Results of a citizen science-based camera survey suggest increasing synurbization of NH bobcats (see tinyurl.com/NHbobcats). Images show bobcats taking advantage of anthropogenic landscapes and stalking prey under backyard bird feeders, previously considered a rare occurrence in New England for a reclusive predator. At this point, it is not possible to relate observed synurbization to neither a) a documented increase in bobcat density and consequently a lack of highly suitable "wild" home ranges, nor to b) a behavioral response to a novel and less energetically costly prey source. Synurbization may also be season-dependent, as bobcats are more limited in their movement when snow is prevalent and may preferentially use more developed areas in winter. However, by analyzing the link between anthropogenic landscapes and bobcat diet more information will be gained on how bobcats respond to pressures from human development.

Hair samples collected from hunter-harvested and roadkilled bobcats from NH and VT will be used in a dietary stable isotope analysis (SIA; Fig. 6). Hair samples carry the isotopic signature of prey items ingested during the active growth of the hair, thus represent a dietary average since the last molting period (Kelly 2000). Bobcats are habitat generalists (but see Peers et al. 2012), and thus, their diet reflects the wildlife community of their surroundings, which can be heavily influenced by anthropogenic effects. It is hypothesized that the isotopic signature will vary in relation to anthropogenic and natural variables (i.e., development, snowfall), as well as with time of year.

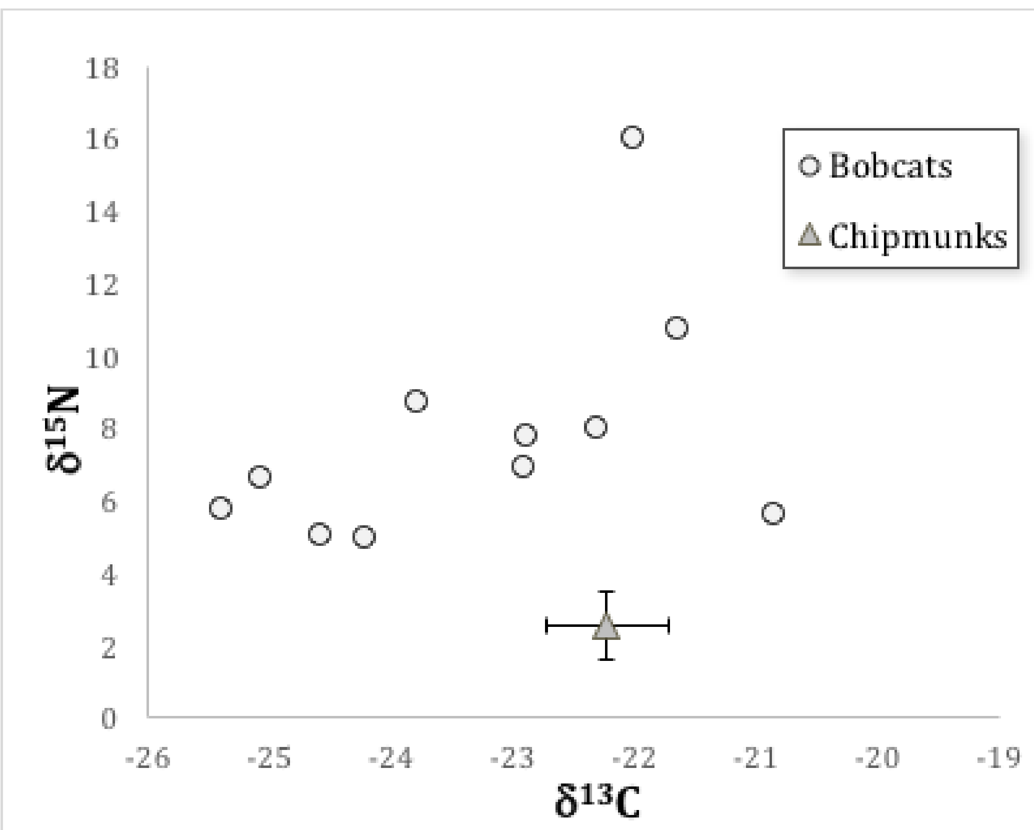


Figure 4 Preliminary $\delta^{13}C$ and $\delta^{15}N$ data for bobcats and one potential prey item. Chipmunk isotope values were calculated from 71 hair samples collected in Bartlett Experimental Forest, NH (R. Stephens, unpublished data).

Representative key prey will be tested to determine a prey-characteristic isotopic signature. Bayesian statistics will be used to estimate the proportion of each prey in bobcat diets in a particular location. Potential prey items include deer, cottontail rabbit, snowshoe hare, grey squirrel, red squirrel, muskrat, small mammals, and turkey. Specific focus will be placed on turkeys because of their currently rapid expansion in NH (Walski 2015) and citizen-captured images of bobcats feeding on turkey under backyard bird feeders. It is possible that turkeys represent a critically important prey species for a likewise expanding bobcat population. An additional benefit to the historic skull collection (see above) is that the stomach contents of each cat were recorded. These data will be compared to contemporary diets inferred from isotopic data, allowing a deeper temporal component to the diet analysis.

Anthropogenic impacts on chronic stress in New England bobcats

Glucocorticoids (GCs) are steroid hormones released into the bloodstream when an animal experiences stress. Chronic stress produces an overabundance of GCs – especially cortisol – in the system, potentially leading to decreased immune function, greater susceptibility to disease and parasites, inhibition of growth and tissue repair, and lower fecundity (McEwen 2001). Cortisol is incorporated into growing hair proportionately to systemic levels, hence this tissue can be used as a temporal record of stress levels in mammals (Davenport et al. 2006, Macbeth et al. 2010, Mastromonaco et al. 2014, Lafferty et al. 2015). Bobcats molt in spring and fall. Thus, hair cortisol data will represent stress levels during approximately two months in either spring (mating and dispersal) or fall, depending on the time of collection.

In collaboration with Dr. Tom Foxall, a mammalian physiologist at UNH, immunoassays will be used to determine levels of cortisol in bobcat samples from across an urbanization gradient while controlling for factors that may influence chronic stress (age, sex, reproductive status, relative health, etc.). It is hypothesized that individuals living in areas of greater anthropogenic influence will exhibit higher cortisol titers because of greater stress levels experienced in human-altered landscapes. The magnitude of this difference may inform conservation efforts and community planning in areas of high conservation value.

Dissemination of Results

Publications

Litvaitis, J.A., G.C. Reed, **R.P. Carroll**, M.K. Litvaitis, J. Tash, T. Mahard, D.J.A. Broman, C. Callahan, and M. Ellingwood. 2015. Bobcats *(Lynx rufus)* as a model organism to investigate the effects of roads on wide-ranging carnivores. Environmental Management. Doi: 10.1007/s00267-015-0468-2.

Reed, G.C., J.A. Litvaitis, C. Callahan, **R.P. Carroll**, M.K. Litvaitis, D.J.A. Broman. In review. Modeling landscape connectivity for mesocarnivores using expert opinion, radio telemetry, and bobcats (*Lynx rufus*) as a focal species: how well do they work?

Reed, G.C., J.A. Litvaitis, M. Ellingwood, P. Tate, D.J.A. Broman, A. Sirén, **R.P. Carroll**. In review. Describing habitat suitability of bobcats (*Lynx rufus*) using several sources of information obtained at multiple spatial scales.

Conference presentations

2015 European Congress of Mammalogy, Stockholm, Sweden – Poster
2015 Northeast Association of Fish and Wildlife Agencies, Newport, RI – Oral
2015 Univ of New Hampshire Graduate Research Conference, Durham, NH – Oral
2014 GIS Day, University of New Hampshire, Durham, NH – Oral
2014 Society for Conservation Biology, Missoula, MT – Poster
2014 Communities of Inquiry Conference, Manchester, NH – Oral
2014 Univ of New Hampshire Graduate Research Conference, Durham, NH - Poster
2014 Northeast Association of Fish and Wildlife Agencies, Portland, ME – Poster

Community and K-12 outreach presentations

2016 Jaffrey Conservation Commission/Jaffrey Children's Library, Jaffrey, NH
2016 Cottrell-Baldwin Lecture Series, Hillsborough, NH
2015-16 Little Harbor Elementary School
2015 NH EPSCoR Ecosystem Computing Challenge
2015 Harris Center for Conservation Education, Hancock, NH
2014-15 NH EPSCoR Wildlife Treks and Stream Safari

Teaching

Since arriving at UNH, I have taught lab sections for courses in wildlife ecology, mammalogy, and environmental policy, helping to create or revise the curriculum and lab activities in each course. I have also given guest lectures in an introductory wildlife ecology course and designed an intensive conservation corridor learning module for a wildlife senior capstone class. I am currently enrolled in the Cognate in College Teaching, a program sponsored by the UNH Center for Teaching and Learning that trains graduate students in the art and science of teaching in higher education.

Tentative Timeline

May – June 2016:	Cognate in College Teaching – 2 courses, May 23- June 10 IsoCamp, University of Utah, Salt Lake City, UT – June 12-28 *An intensive course in stable isotope ecology taught by some of the leaders and originators of the field.*
July – Dec 2016:	Complete genotyping of contemporary samples Continue historic skull DNA extraction and genotyping (completed by January 2017) Prey sample collection for SIA (hair/feather) SIA sample preparation and submission (bobcat/prey hair, bobcat skulls); Cortisol assay pilot study
Oct 2016:	Dissertation proposal to committee
Nov 2016:	Proposal defense
Jan 2017:	Written comprehensive exams

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