Parasitology Research (2019) 118:2257–2262
https://doi.org/10.1007/s00436-019-06351-5

HELMINTHOLOGY - ORIGINAL PAPER

Gastrointestinal parasites of the New England cottontail rabbit (*Sylvilagus transitionalis*) and eastern cottontail rabbit (*Sylvilagus floridanus*) in the Hudson Valley, New York

Christopher M. Whipps[1] · Emily J. Gavard[1] · Jonathan Cohen[1] · Sadie J. Ryan[1,2]

Received: 7 January 2019 / Accepted: 9 May 2019 / Published online: 8 June 2019

Abstract

The New England cottontail rabbit (NEC, *Sylvilagus transitionalis*) population has decreased dramatically in New York, USA, and the role of parasites in limiting the population has never been examined. The closely related and sympatric eastern cottontail rabbit (EC, *Sylvilagus floridanus*) was introduced into the range of NEC by humans and is currently thriving. This study aimed to investigate gastrointestinal parasites of the NEC and the EC and compare their parasite communities. Fecal pellets from 195 NEC and 125 EC were collected from the Hudson Valley, New York, in the winter of 2013–2014. Centrifugal fecal floats were performed in Sheather's sugar solution, and parasite ova and cysts were examined microscopically to identify gastrointestinal parasites present. For all pellets combined ($n = 320$), 91% were found to harbor at least 1 parasite species, with *Eimeria* species being the most common. Genetic analysis of pellets using microsatellite DNA identified 248 individual rabbits, with parasite prevalence (94%) similar to the prevalence estimate based on all pellets (91%). EC samples had a significantly higher ($p < 0.05$) parasite species richness (1.73, range 0–4) than NEC (1.20, range 0–3). EC and NEC shared 3 moderate to high (9–89%) prevalence parasites, in which EC prevalence was consistently higher. One parasite species was only found in NEC, and two were only found in EC, but the majority of these were of low abundance, precluding further statistical analyses.

Keywords *Sylvilagus transitionalis* · *Sylvilagus floridanus* · New England cottontail · Non-invasive genetics · Eimeria

Introduction

The New England cottontail rabbit (NEC), *Sylvilagus transitionalis*, is an early successional forest (e.g., shrub thicket) obligate lagomorph that is native to the Northeastern United States (Litvaitis 2001). Extensive clearing of the northeastern landscape for agriculture beginning in 1875 and the later abandonment of those farms led early successional forest to dominate the landscape by 1950 (Litvaitis 1993; Trani et al. 2001). The maturation and human development of early successional forest has led to a drastic reduction in NEC population size since 1960 (Linkkila 1971; Jackson 1973; Fenderson et al. 2011). Additionally, a closely related competitor, the eastern cottontail rabbit (EC), *Sylvilagus floridanus*, was introduced in the range of NEC in the 1930s by humans and is now thriving there (Chapman and Morgan 1973). The NEC is classified as "vulnerable" by the International Union for Conservation of Nature (IUCN), with its population size declining by 50% or greater across its range over the last 10 years (Barry et al. 2008). In New York, the NEC is listed as a species of "Special Concern" (NYDEC 1999).

Eastern cottontails were introduced to the Northeastern United States in the 1930s for hunting (Probert and Litvaitis 1996). They are able to use smaller and more fragmented landscapes than NEC, as well as those modified by human use (Smith and Litvaitis 2000; Litvaitis 2001). Eastern cottontails tend to exploit new early successional habitat more quickly than NEC (Litvaitis et al. 2008) and are generally not displaced by NEC (Probert and Litvaitis 1996); thus, the two

This study represents the first parasitological study of cottontail rabbits in New York.

Responsible Editor: David S. Lindsay

✉ Christopher M. Whipps
cwhipps@esf.edu

[1] Department of Environmental and Forest Biology, SUNY-ESF, State University of New York College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, NY 13210, USA

[2] Department of Geography and Emerging Pathogens Institute, University of Florida, Gainesville, FL 32611, USA

species are found to co-occur in many areas with EC being more abundant. NEC and EC are very similar in appearance and genetic confirmation is typically necessary for species identification.

Parasites of EC have been studied since the late nineteenth century and are relatively well known (Jacobson et al. 1978; Bertolino et al. 2010; Tizzani et al. 2014). Nematodes found in previous studies with the greatest prevalence include *Passalurus ambiguus*, *Trichostrongylus calcaratus*, *Obeliscoides cuniculi*, *Dermatoxys veligera*, and *Vexillata noviberiae* (Clancy et al. 1940; Erickson 1947; Wiggins et al. 1980). Cestodes found in greatest prevalence include *Mosgovoyia* spp. and *Cittotaenia* spp. and the most commonly found trematode found in EC in Pennsylvania was *Hasstilesia tricolor* (Wiggins et al. 1980). On the other hand, NEC gastrointestinal parasites have not been studied in depth, and not at all since the major decline of the species. A study in 1940 found that NEC in Connecticut carry *O. cuniculi*, *P. ambiguus*, *Cittotaenia variabilis*, *Taenia pisiformis*, and coccidia (Clancy et al. 1940). Given the current concerns for conservation of the NEC, our understanding of the parasites of this species, and their potential impacts, is woefully lacking. The concern is that infectious diseases, parasites among these, may be contributing in part to the decline of the NEC.

Our objective was to undertake a survey of the gastrointestinal parasites of the native NEC and invasive EC in the Hudson Valley, New York. Specifically, we sought to determine what parasites are present, and whether the same parasites occur in both host species. Our results would serve as a baseline for further study of the role of parasites in the population dynamics of these sympatric competitors.

Materials and methods

This study was conducted in conjunction with our seasonal cottontail rabbit monitoring, in the Hudson Valley, New York, at nine sites located in Putnam, Dutchess, and Columbia counties (Fig. 1). Between 11 December 2013 and 27 February 2014, 320 fecal pellets were collected from transects spanning each site, 50 m apart to reduce non-independent samples. Samples were collected within two to five days following each snowfall, to prevent DNA degradation and facilitate parasite egg recovery (Kovach et al. 2003). Immediately following collection, half of the pellets collected in each sample were stored at − 20 °C, and the others at 4 °C for subsequent DNA analysis and fecal flotations, respectively. Host species identification was carried out using a restriction fragment length polymorphism analysis targeting the mitochondrial DNA (Kovach et al. 2003). Briefly, a quarter of a rabbit pellet was digested and extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Valencia, California) according to manufacturer's instructions. The target sequence (D-loop of mitochondrial DNA) was amplified using primers of Kovach et al. (2003) and digested with NlaIII restriction endonuclease (New England Biolabs, Ipswich, Massachusetts). Any individual sample producing a cutting pattern not clearly identified as EC was also cut with BfaI restriction endonuclease (New England Biolabs) to distinguish EC from NEC (Litvaitis and Litvaitis 1996). To obtain individual identities for pellet samples identified as New England cottontail, 10 microsatellite loci from Fenderson et al. (2011) and King et al. (2017) were used (INRA16, INRA326, Lsa1, Lsa8, Sat12, StrQ2, StrQ15, StrQ18, StrQ32, StrQ46). These were amplified and run in multiplex, using a multiple tubes approach (Frantz et al. 2003), re-amplifying each sample until the same genotype was observed at least twice or until amplification at that locus had been repeated five times (Cheeseman et al. 2019). If a consensus genotype could not be determined after five amplifications, those samples were removed from further analyses. The sex-specific marker INRA326 was scored as a binary (M/F); this marker was included in determination of individual identity but otherwise not included in analyses. PCR products were analyzed using a 3730xl 96-Capillary Genetic Analyzer (Applied Biosystems, San Francisco, CA), and alleles were manually scored using Peak Scanner 1.0 (Applied Biosystems, Foster City, CA). A similar approach was taken with pellets identified as EC, but using the following 12 microsatellite loci of King et al. (2017): StrQ2, StrQ7, StrQ11, StrQ15, StrQ18, StrQ20, StrQ26, StrQ32, StrQ41, StrQ43, StrQ44, StrQ46.

For parasitological analysis, centrifugal fecal floats were performed, using the pellets stored at 4 °C, in Sheather's sugar solution (1.33 specific gravity, determined by densitometer). On average, floats were performed using 2.2 g of pellets, but where pellet piles did not permit, we exhausted the samples available to us. Microscopic examination was used to identify and count parasite species in each sample. Parasite ova and apicomplexan oocysts were photographed and identified morphologically. Ova were categorized based on size and overall appearance. Subsequently, each sample was compared with existing records of cottontail parasites (Clancy et al. 1940; Levine 1968; Wiggins et al. 1980; Schoeb et al. 2007) and assigned to the lowest possible taxonomic rank. For *Eimeria* species observed, most of the oocysts were unsporulated, making morphological identification to species difficult. Typically, identification would rely on morphological measurements of sporulated oocyst features (Duszynski and Wilber 1997; Bertolino et al. 2010), but these could not be observed in our samples, and we categorized them all as *Eimeria* spp. for the purposes of this study.

Parasite species richness, S (Whittaker 1972), was calculated for each rabbit sample. Within the two species EC and NEC, S was found to be non-normally distributed, so differences between the distributions were assess using a non-parametric Mann-Whitney *U* test (equivalent to a Wilcoxon rank sum test with continuity correction) in program R (R

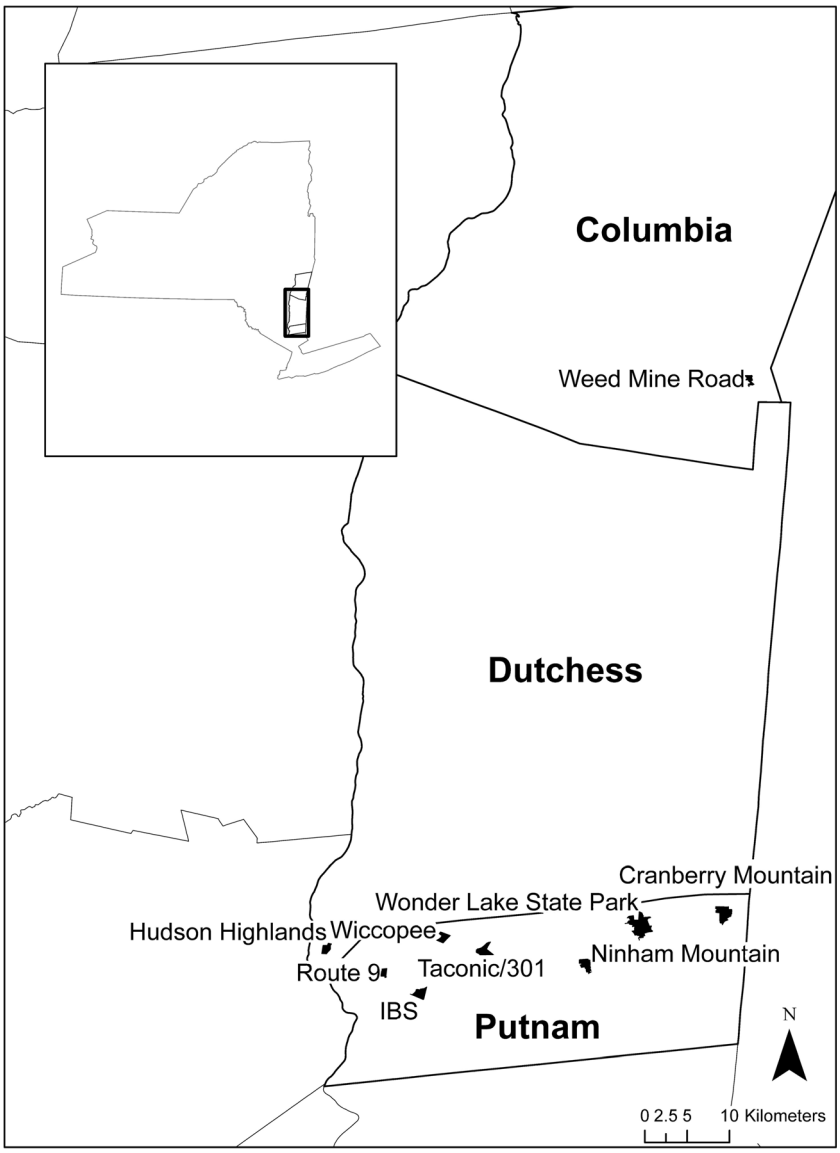

Fig. 1 Locations of nine field sites in the Hudson Valley, New York

Core Team 2013). Parasite prevalence was calculated as the proportion of hosts infected with a particular parasite species (Bush et al. 1997); mean prevalence and confidence intervals are reported in Table 1. Mean counts per sample were calculated based on average counts (of eggs, oocysts, etc.) per all hosts, both infected and uninfected. To achieve this, the total counts were divided by the total number of samples.

Results

Of the 320 cottontail fecal samples, 125 were identified as EC, and 195 were NEC. Two hundred and ninety-two of the 320 cottontail fecal samples (91%; 95% CI 87.6–93.9) were found to have at least one species of parasite. On average, 2.2 g of cottontail pellets were used in each fecal float. Of the 28 fecal samples that were not found to contain any parasites, 13 of the floats used less than 1 g of feces. Based on individual genetic identification using microsatellite DNA, the 320 pellets represent 248 rabbits, with 139 NEC and 109 EC. Overall, 232 of 248 rabbits (93.6%; 95% CI 89.8–96.0) had at least one parasite. Ova were categorized based on previous studies of cottontail rabbits, range of dimensions, and overall appearance. More than six species of parasites were detected in the sample set (Table 1), including five nematode species, and several protozoan parasite species (different *Eimeria* species). Evaluating the parasite prevalence based on rabbits rather than samples resulted in similar estimates of prevalence (Table 1). The average parasite species richness of all samples was 1.40, with an average richness of 1.20 in NEC (range 0–3) and 1.73 in EC (range 0–4). Shapiro-Wilk tests for normality showed that neither EC nor NEC distributions of S were normal (NEC: $W = 0.769$, $p < 0.05$; EC: $W = 0.823$, $p < 0.05$). A Mann-Whitney U test indicated the two were significantly different ($W = 17{,}197$, $p < 0.05$).

Table 1 Parasite prevalence (*P*) based on sample (all pellets) and prevalence by rabbit (as determined genetically), and mean counts per sample (MC/s) with overall range of counts. Values were calculated for both New England cottontail (NEC) and eastern cottontail (EC). Mean counts are determined across all hosts (infected and uninfected)

Parasite species	Cottontail species	*p* (sample) No. samples (%, 95% CI)	*p* (rabbit) No. samples (%, 95% CI)	MC/s (range)
Nematoda				
Obeliscoides cuniculi	NEC	18 (9.2%, 6–14%)	14 (10%, 6–16%)	0.83 (0–26)
	EC	20 (16%, 11–24%)	19 (17%, 11–26%)	3.92 (0–113)
Nematodirus sp.	NEC	1 (0.5%, 0.1–2.8%)	1 (0.7%, 0.1–4%)	0.01 (0–1)
	EC	0 (0%, 0–2%)	0 (0%, 0–3%)	0
Trichostrongylus sp.	NEC	42 (22%, 16–28%)	35 (25%, 19–33%)	0.98 (0–48)
	EC	70 (56%, 47–64%)	63 (58%, 48–67%)	7.56 (0–90)
Passalurus ambiguus	NEC	0 (0% 0–2%)	0 (0%, 0–3%)	0
	EC	8 (6.4%, 3–12%)	8 (7%, 4–14%)	1.68 (0–186)
Graphidium strigosum	NEC	0 (0%, 0–2%)	0 (0%, 0–3%)	0
	EC	7 (5.6%, 3–11%)	7 (6%, 3–13%)	1.07 (0–44)
Protozoa				
Eimeria spp.	NEC	172 (88%, 83–92%)	128 (92%, 86–96%)	958 (0–11,138)
	EC	111 (89%, 82–93%)	99 (91%, 84–95%)	1894 (0–41,874)

Based on overall appearance, size, and a comparison with the literature, we were able to categorize some ova to genus or species (Table 1). The species were as follows, with size ranges (length and width in μm) following each: *Obeliscoides cuniculi* (81–88 × 39–47), *Passalurus ambiguus* (105–123 × 44–54), *Graphidium strigosum* (84–92 × 37–43), and a *Nematodirus* species (143 × 96) and *Trichostrongylus* species (73–81 × 38–45). One parasite species (*Nematodirus* sp.) was found exclusively in NEC samples, while two parasite species (*Passalurus ambiguus* and *Graphidium strigosum*) were found exclusively in EC samples (Table 1). The *Trichostrongylus* species, *O. cuniculi*, and *Eimeria* species were shared by both hosts, an in all cases, EC had higher mean counts than NEC (Table 1).

Discussion

In this study, 94% of all rabbits, and 91% of pellet samples, harbored at least 1 species of parasite, a similar percentage to those reported in previous rabbit studies. The 1940 study of NEC in Connecticut found 89% were infected with at least 1 parasite (Clancy et al. 1940), while another study of EC in Kansas showed parasites in 63% of cottontails (Franklin et al. 1966). Parasite communities of NEC in this study were similar to the Clancy et al. (1940) study, although we found a greater number of parasite species. In Connecticut, coccidia were found in only 67% and 62% (west and east Connecticut, respectively) of NEC compared with our 88% for NEC; coccidians were found in 73% and 69% of EC in west and east Connecticut, respectively, compared with our 89% for EC. The Connecticut study found *P. ambiguus* in NEC only (8% and 27%, west and east Connecticut, respectively; Clancy et al. 1940), whereas we found this parasite at a low prevalence in EC only. Prevalence of *Obeliscoides cuniculi* was lower in our study, which may be attributed to seasonal variation, as our samples were collected only from December through February (to facilitate genetic analyses) and it is known that incidence and severity of *O. cuniculi* decrease significantly in the winter (Clancy et al. 1940). Other differences between this and the 1940 study on NEC may stem from differences in host population size and distribution, as the significant decline (nearly 75%) in NEC population occurred by 1960 (Litvaitis and Litvaitis 1996) and it is also possible we are seeing a shifted parasite community in NEC due to altered ecosystems as was seen in eastern cottontails following brush treatments in Oklahoma, USA (Boggs et al. 1990). Habitat fragmentation specifically can lead to both the introduction of new parasites to an area and increased pathogenicity of those parasites (Holmes 1995).

In some cases, parasitism has been shown to limit mammal populations (Pedersen and Greives 2008) but little is known of the diversity of NEC parasites, how the historic environmental context of NEC may have altered parasite diversity or abundance, or how these changes may affect transmission of parasitic diseases. Although most cottontails in our study were parasitized, potential impacts of these parasites are difficult to predict because egg and oocyst counts are not necessarily indicative of burdens within the host (Anderson and Schad 1985). The purpose of identifying parasites in these rabbits was to explore whether the prevalence or parasite species were

of concern and meriting further study; it is worth noting the potential pathogenicity of the identifiable parasites we found. The disease associated with *Eimeria* spp. infections is variable and depends on age of host, exposure, and parasite species involved (Duszynski and Couch 2013). At very high burdens, these parasites cause coccidiosis which can be accompanied by weight loss (Lello et al. 2005), diarrhea, blood in feces, dehydration, decreased breeding in rabbits, and death in extreme cases. Younger rabbits are generally more susceptible to these effects (Duszynski and Couch 2013). Based on morphology of oocysts, *Eimeria* species we believe to have been found include but are not limited to *Eimeria magna*, *Eimeria neoleporis*, *Eimeria perforans*, and *Eimeria irresidua*. Infections by *Trichostrongylus* spp. have been associated with weight loss and mortalities in experimental infections, and some natural infections subclinical despite large worm burdens (Schoeb et al. 2007). With *Obeliscoides cuniculi*, Sollod et al. (1968) noted mild pathogenicity in white rabbits, and another study described poor reproduction in rabbits that were coinfected with both *O. cuniculi* and *P. ambiguus* (Düwel and Brech 1981). In wild snowshoe hares, Murray et al. (1997) reported that predators disproportionately killed hares with higher burdens of *O. cuniculi* relative to hares with lower parasite burdens. Furthermore, under certain conditions, hares treated with ivermectin had better survival than untreated hares (Murray et al. 1997), although this could be due to one or more parasites, and not just *O. cuniculi*.

In this study, we presented the first comprehensive survey of NEC gastrointestinal parasites, in the context of a sympatric invasive congeneric, EC, in New York State. Future work will genetically confirm gastrointestinal parasite IDs and distinguish *Eimeria* spp. to understand potential pathogenicity and resolve further interspecific host overlap.

Acknowledgments Special thanks to A. Cheeseman who helped with collections and coordination of sampling efforts. Thanks to A. Bloomfield, J. Decotis, M. delPuerto, K. Deweese, K. Farrell, J. Jaycox, P. Novak, E. Underwood, and G. Walters, who aided with pellet collection. Thanks to K.A.W Lindsay, C. Michaud, S. Page, and M. Wilson who assisted with lab work. Thanks to A. Bielecki who assisted with data entry.

Funding information The New York Department of Environmental Conservation provided funding and logistical support.

Compliance with ethical standards

Animal care and use Although there was no handling of live animals as a direct component of the research reported here, this work was part of a larger project which was conducted with approval of the SUNY-ESF Institutional Animal Care and Use Committee (protocol #120801).

Conflict of interest The authors declare that there is no conflict of interest.

References

Anderson RM, Schad GA (1985) Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. Trans R Soc Trop Med Hyg 79:812–825

Barry R, Lazell J, Litvaitis J (2008) Sylvilagus transitionalis. In: The IUCN Red List of Threatened Species 2008 http://www.iucnredlist.org/details/21212/0. Accessed September 5, 2018

Bertolino S, Hofmannová L, Girardello M, Modry D (2010) Richness, origin and structure of an *Eimeria* community in a population of eastern cottontail (*Sylvilagus floridanus*) introduced into Italy. Parasitology 137:1179–1186

Boggs JF, McMurray ST, Leslie DMJ, Engle DM, Lochmiller RL (1990) Parasitism of cottontail rabbits (*Sylvilagus floridanus*) by *Obeliscoides cuniculi* in response to habitat modification in the cross timbers of Oklahoma. J Helminthol Soc Wash 57:146–152

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Chapman JA, Morgan RP (1973) Systematic status of the cottontail complex in western Maryland and nearby West Virginia. Wildl Monogr 36:3–54

Cheeseman AE, Cohen JB, Whipps CM, Kovach AI, Ryan SJ (2019) Hierarchical population structure of a rare lagomorph indicates recent fragmentation has disrupted metapopulation function. Conserv Genet (in press)

Clancy CF, Jungherr E, Sime PR (1940) Internal parasites of cottontail rabbits in Connecticut. J Wildl Manag 4:162–168

Duszynski DW, Couch L (2013) The biology and identification of the Coccidia (Apicomplexa) of rabbits of the world. Academic Press, San Diego, CA

Duszynski DW, Wilber PG (1997) A guideline for the preparation of species descriptions in the Eimeriidae. J Parasitol 83:333–336

Düwel D, Brech K (1981) Control of oxyuriasis in rabbits by fenbendazole. Lab Anim 15:101–105

Erickson AB (1947) Helminth parasites of rabbits of the genus *Sylvilagus*. J Wildl Manag 11:255–263

Fenderson LE, Kovach AI, Litvaitis JA, Litvaitis MK (2011) Population genetic structure and history of fragmented remnant populations of the New England cottontail (*Sylvilagus transitionalis*). Conserv Genet 12:943–958

Franklin J, Simmons ML, Cosgrove GE (1966) A pathogen survey in the Kansas cottontail. Bull Wildl Dis Assoc 2:52–53

Frantz A, Pope L, Carpenter P, Roper T, Wilson G, Delahay R, Burke T (2003) Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Mol Ecol 12:1649–1661

Holmes JC (1995) Parasites as threats to biodiversity in shrinking ecosystems. Biodivers Conserv 5:975–983

Jackson SN (1973) Distribution of cottontail rabbits (*Sylvilagus* spp.) in northern New England. M.S. Thesis. University of Connecticut, Storrs, Connecticut, 48 p

Jacobson HA, McGinnes BS, Catts EP (1978) Bot fly myiasis of the cottontail rabbit, *Sylvilagus floridanus* mallurus in Virginia with some biology of the parasite, *Cuterebra buccata*. J Wildl Dis 14:56–66

King TL, Eackles M, Aunins A, McGreevy TJ, Husband TP, Tur A, Kovach AI (2017) Microsatellite marker development from next-generation sequencing in the New England cottontail (*Sylvilagus transitionalis*) and cross-amplification in the eastern cottontail (*S. floridanus*). BMC Research Notes 10:741

Kovach AI, Litvaitis MK, Litvaitis JA (2003) Evaluation of fecal mtDNA analysis as a method to determine the geographic distribution of a rare lagomorph. Wildl Soc Bull 31:1061–1065

Lello J, Boag B, Hudson P (2005) The effect of single and concomitant pathogen infections on condition and fecundity of the wild rabbit. Int J Parasitol 35:1509–1515

Levine ND (1968) Nematode parasites of domestic animals and of man. Burgess, Minneapolis, Minnesota, 600 p

Linkkila TE (1971) Influence of habitat upon changes within interspecific Connecticut cottontail populations. M.S. Thesis. University of Connecticut, Storrs, Connecticut, 21 p

Litvaitis JA (1993) Are pre-Columbian conditions relevant baselines for managed forests in the Northeastern United States? For Ecol Manag 185:113–126

Litvaitis JA (2001) Importance of early successional habitats to mammals in eastern forests. Wildl Soc Bull 29:466–473

Litvaitis MK, Litvaitis JA (1996) Using mitochondrial DNA to inventory the distribution of remnant populations of New England cottontails. Wildl Soc Bull 24:725–730

Litvaitis JA, Barbour MS, Brown AL, Kovach AI, Litvaitis MK, Oehler JD, Probert BL, Smith DF, Tash JP, Villafuerte R (2008) Testing multiple hypotheses to identify causes of the decline of a lagomorph species: the New England cottontail as a case study. In: Alves PC, Ferrand N, Hacklander K (eds) Lagomorph biology. Springer Berlin Heidelberg, Heidelberg, pp 167–185

Murray DL, Cary JR, Keith LB (1997) Interactive effects of sublethal nematodes and nutritional status on snowshoe hare vulnerability to predation. J Anim Ecol 66:250–264

NYDEC (1999) List of endangered, threatened and special concern fish & wildlife species of New York State - NYS Dept. of Environmental Conservation. Available at http://www.dec.ny.gov/animals/7494.html. Accessed 15 September 2017

Pedersen AB, Greives TJ (2008) The interaction of parasites and resources cause crashes in a wild mouse population. J Anim Ecol 77:370–377

Probert BL, Litvaitis JA (1996) Behavioral interactions between invading and endemic lagomorphs: implications for conserving a declining species. Biol Conserv 76:289–295

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/. Accessed 23 July 2017

Schoeb TR, Cartner SC, Baker RA, Gerrity LW (2007) Parasites of rabbits. In: Baker DG (ed) Flynn's parasites of laboratory animals, 2nd edn. Blackwell Publishing, Iowa, pp 451–499

Smith DF, Litvaitis JA (2000) Foraging strategies of sympatric lagomorphs: implications for differential success in fragmented landscapes. Can J Zool 78:2134–2141

Sollod AE, Hayes TJ, Soulsby EJL (1968) Parasitic development of *Obeliscoides cuniculi* in rabbits. J Parasitol 54:129–132

Tizzani P, Catalano S, Rossi L, Duignan PJ, Meneguz PG (2014) Invasive species and their parasites: eastern cottontail rabbit *Sylvilagus floridanus* and *Trichostrongylus affinis* (Graybill, 1924) from northwestern Italy. Parasitol Res 113:1301–1303

Trani MK, Brooks RT, Schmidt TL, Rudis VA, Gabbard CM (2001) Patterns and trends of early successional forests in the Eastern United States - forest service research & development. Wildl Soc Bull 29:413–424

Whittaker RH (1972) Evolution and measurement of species diversity. Taxon 21:213–251

Wiggins JP, Cosgrove M, Rothenbacher H (1980) Gastrointestinal parasites of the eastern cottontail (*Sylvilagus floridanus*) in Central Pennsylvania. J Wildl Dis 16:541–544