**Report on Research Conducted in Partial Support by the Nuttal Ornithological Club – November 2010**

**Grant issued to: New England Institute for Landscape Ecology, 266 Prospect Hill Road, Canaan, New Hampshire 03741, Principal: Dr. Len Reitsma**

**Project: Breeding ecology and mating system of the Canada Warbler in New Hampshire**

The New England Institute for Landscape Ecology completed its eighth consecutive year studying Canada Warbler breeding ecology in north-central New Hampshire (Canaan). This work has yielded the most detailed information about this species to date. The species is in 4.1% decline per year in the state and 2.1% regionally. We have quantified the species' habitat selection, measured age-specific reproductive performance, compared performance along a habitat gradient, and described their breeding biology which has been incorporated into the Birds of North America updated species account. We have provided information to forest managers to enhance this species' breeding success through pamphlets, publications, and presentations. We continued this study in the summer of 2010 with a focus upon quantifying patterns of the mating system. We are currently analyzing DNA in the blood to determine parentage of each nestling, and we are comparing individual fitness (number of young produced per parent) with morphological and physiological parameters such as plumage and feather carotenoid concentrations. With many returning marked birds of ages three, four, five and even six years old, we have the unique opportunity to analyze the cumulative advantage of age and experience in breeding. This is now a long-term ecological study of a long-distance migrant. The findings will have much relevance to all North American migratory songbirds, especially those that migrate long distances with shorter breeding season duration.

The primary objective of the past field season with support from Nuttal was to explain the mechanisms responsible for Canada warbler reproductive success, and the pattern of that success over years, among age classes, and at several spatial scales. We are measuring reproductive success at the level of individuals in the context of populations. We consider the habitat features and behavioral milieu that best promote successful fledging. We characterize the behaviors most associated with success, such as neighborhood dynamics, male and female mating opportunism, individual attributes – including age, plumage and physiological condition measured by carotenoid concentrations and reflectance values in feathers - that increase success. We examine whether "hot" areas occur within neighborhoods based upon asymmetries in paternity among males in each neighborhood, and whether males and females shift territories or choices for extra-pair copulations, respectively, recognizing these hot spots.

Over the last eight years (2003-2010) in Canaan, New Hampshire (Figure 1), we have color-banded Canada warblers in an area of approximately 100 ha. This in-depth population level study expands our knowledge of Canada warbler breeding habitat requirements (Hallworth et al. 2008a and b), pairing and fledgling success rates (Reitsma et al. 2008), return rates, and the complex array of social interactions that influence individual fitness and dispersion. Beginning in 2005, we compared reproductive output within two structurally distinct habitats (Hallworth et al. 2008b). We quantified pairing and fledging success, return rates and site fidelity, age class ratios, and habitat preferences. We individually marked > 90% of territorial males (100% in 2009 and 2010 seasons) each year allowing us to quantify territory sizes/configurations, and measure breeding success for all males (Figures 2 and 3).

Canada warblers have high pairing and fledging success in both habitat types. Both habitats are inhabited predominantly by males greater than two years of age (ASYs) and individuals exhibit high site fidelity. Overall, 56% of marked male individuals in this eight year period returned the following breeding seasons. The rate increased from earlier years at around 50% to over 60% in more recent years. Performance to date does not differ significantly between the two sites, despite a greater proportion of first-year males in the younger forest (Reitsma et al. 2008). But the average age has shifted in more recent years to slightly older birds in the younger forest. This suggests the early succession stand may provide equally high-quality breeding habitat and offers potential for forestry practices to increase suitable habitat for the species in this region (more details in Reitsma et al. 2008 and Hallworth et al. 2008b).

With the support from Nuttal this past season, we were able to sustain the standardized baseline data collection through its eighth year, and slightly expand the area covered. We built upon our recent success in finding nests and growing identification of microsatellite loci markers for parentage analyses in measuring fitness and mate selection. In so doing, we link our successfully implemented habitat metrics, new measures of phenotypes and body condition, and reproductive performance across social and structural gradients within contiguous habitats.

## Methods for season for which funding was received (2010)

Paternity analyses: We have screened five microsatellite loci from approximately 200 individuals for use in genotyping Canada Warblers for parentage analysis. Blood is sampled during the field season by brachial venous puncture and collected in lysis buffer and stored at -20 degrees Celsius. DNA is isolated from blood samples. Initially, we test PCR primer sets that have successfully amplified microsatellite loci in closely related avian species on DNA isolated from Canada Warblers. If these amplify the correct band size in multiple individuals, we order fluorescently labeled PCR primers for each microsatellite locus and amplify them from the DNA of each individual in the population. PCR products are then diluted and mixed with the proper fluorescent size standards and sent to the Dartmouth Molecular Biology Core Facility for fragment analysis on their ABI sequencing machines. For paternity testing we will use the Cervus 3.0 field genetics software to compare microsatellite genotype profiles of each offspring to potential parents. We will continue to screen microsatellite loci until we have enough variable alleles to determine parentage in our populations at a greater than 99% certainty (pending funding). This analysis is an advanced state but is still incomplete. We hope to have paternity data within the next three months.

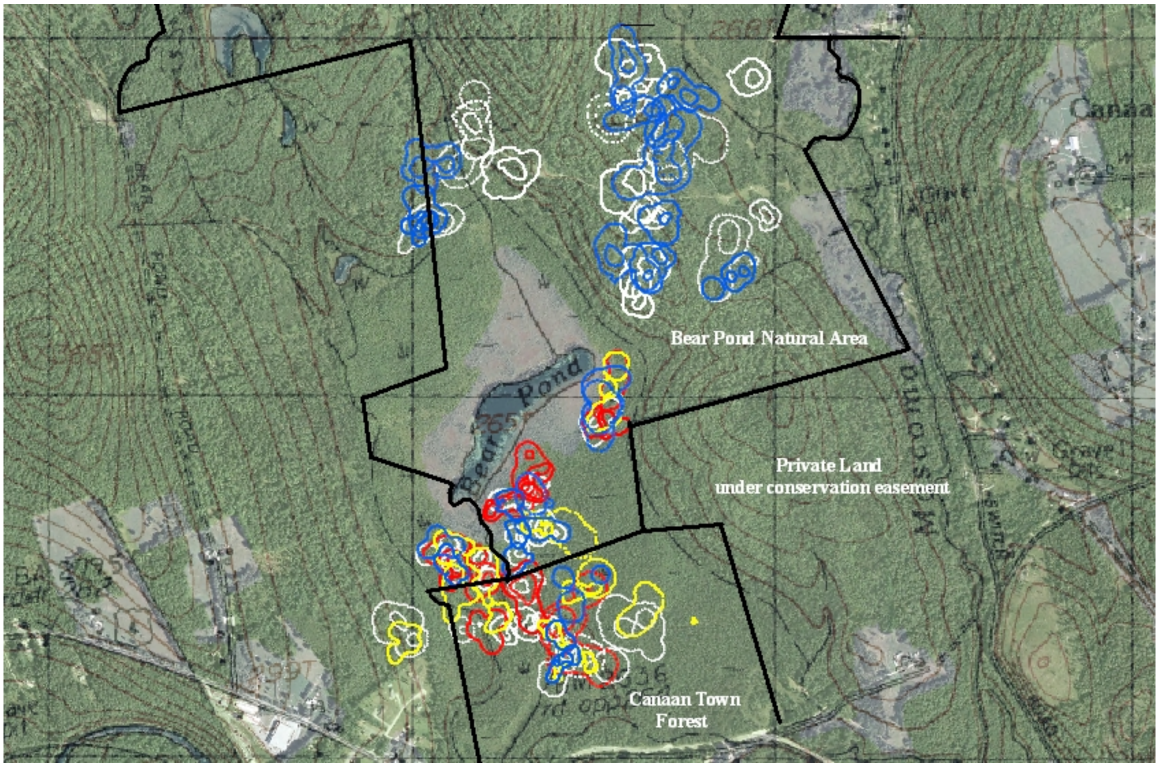


Figure 1. Aerial photograph, with topographic map overlaid showing the study site. The Bear Pond Natural Area is 415 ha (Northwest section not shown) and the adjacent Canaan Town Forest is 36.5 ha. Bear Pond is in the middle of the study area separating the upper (post-harvest, early-succession, relatively poorly drained forest intermixed with deciduous/coniferous upland forest) and the lower (red maple forested wetland intermixed with predominantly coniferous upland forest) habitats. Male territories for each of the four earliest years are shown (2003-red, 2004-yellow, 2005-blue, 2006-white). Territories for 2007-2009 are excluded to maintain clarity.

Analysis of carotenoids: Carotenoids are pigments that are acquired through food and assimilated into the integument of many birds, resulting in bright red, yellow and orange feathers, bills or skin (McGraw et al. 2003). Carotenoids are anti-oxidants and have been shown to act as immunoenhancers (Helfenstein et al. 2008) as well as detoxifying agents (Kristiansen et al. 2008). Thus carotenoid concentrations in feathers and plasma have been used as indicators ofindividual condition in many birds (McGraw et al. 2003). Pigmented barbules are trimmed off of each feather and weighed. We will add 1 mL of acidified pyridine to each tube of barbules and fill the head-space with argon. Samples will be placed in a hot bath then cooled before adding 2 mL of distilled water. Two mL of tert-butyl methyl ether will be added. Then the solution will be thoroughly mixed before centrifuging at 5000g for 5 minutes. The supernatant will be removed and evaporated under a stream of nitrogen. The pigmented residue will be resuspended in 200 μL HPLC mobile phase (methanol: acetonitrile, 50:50, v/v) and 50 μL injected into a Waters 717plus autosampler HPLC fitted with a Develosil RPAqueous RP-30 column and an Eppendorph TC-50 column heater. An isocratic system will be run for 25 min at a flow-rate of 1.2 mL/min$^{-1}$. Pigments will be quantified using an internal standard of known concentration (McGraw and Gregory 2004).

Table 1. Overall return rates for six years of data on territorial males is 56% (166 of 296 individuals).

| Year | Percent returning males (sample size) | Red maple wetland | Early succession forest |
|---|---|---|---|
| 2003-2004 | 0.57 (12 of 21) | 0.57 (12 of 21) | NA |
| 2004-2005 | 0.50 (10 of 20) | 0.50 (10 of 20) | NA |
| 2005-2006 | 0.47 (27 of 57) | 0.52 (13 of 25) | 0.44 (14 of 32) |
| 2006-2007 | 0.49 (28 of 58) | 0.52 (12 of 23) | 0.46 (16 of 35) |
| 2007-2008 | 0.63 (30 of 47) | 0.53 (10 of 19) | 0.71 (20 of 28) |
| 2008-2009 | 0.64 (23 of 36) | 0.57 (4 of 7) | 0.79 (19 of 29) |
| 2009-2010 | 0.63 (36 of 57) | 0.40 (8 of 20) | 0.76 (28 of 37) |

## Preliminary Results

Table 1 indicates the significantly increased sample size of marked males in part due to the funding received from Nuttal. As mentioned above, the paternity analyses are ongoing. We collected blood from 73 nestlings, all male parents (N=29), and 7 female parents in 2010 to add to the sample size from the previous three seasons of intensified nest searching. The reflectance data for the feathers of the males has been obtained but requires further analysis and will be examined in concert with the paternity analysis to determine any correlations between feather brightness and reproductive performance. The carotenoid concentrations still need to be obtained and the approach will be identical to the reflectance data. The ultimate objective is to determine if the feather qualities correlate with overall reproductive performance including extra-pair paternity. We will also analyze spatial aspects of male performance to determine if territory location within the study area, or demography of neighborhoods influences the success of a male at securing extra-pair paternity, but this is not our immediate priority. The funding from Nuttal was instrumental in having a successful field season in 2010.

**Literature Cited**


Chace, J.F., S.D. Faccio and A. Chacko. In manuscript. Canada Warbler habitat use in Vermont: Influence of forest community type, canopy structure and understory density. .

Hallworth, M., P. M. Benham, J. D. Lambert, L. Reitsma. 2008a. Canada Warbler (*Wilsonia canadensis*) breeding ecology in young forest stands compared to a red maple (*Acer rubrum*) swamp. Forest Ecology and Management: 255: 1353-1358.

Hallworth, M., A. Ueland, E. Anderson, J. D. Lambert, L. Reitsma. 2008b. Habitat selection and site fidelity of the Canada Warbler in central New Hampshire. Auk 125:1-9.

Helfenstein, F., S. Losdat, V. Saladin, H. Richner. 2008. Females of carotenoid-supplemented males are more faithful and produce higher quality offspring. *Behavioral Ecology* 19: 1165-1172

Hensler, G.L., and J.D. Nichols. 1981. The Mayfield method of estimating nesting success: A model, estimators, and simulation results. Wilson Bulletin 93(1): 42-53.

McGraw, K.J. and A.J. Gregory. 2004. Carotenoid pigments in male American goldfinches: what is the optimal biochemical strategy for becoming colorful? *Biological Journal of the Linnean Society* 83: 273-280.

McGraw, K.J., A.J.Gregory, R.S. Parker, E. Adkins-Regan. 2003. Diet, plasma carotenoids and sexual coloration in the zebra finch (*Taeniopygia guttta*). *The Auk* 120: 400-410.

Peters, K. A., R. A. Lanica, and J. A. Gerwin. 2005. Swainson’s warbler habitat selection in a managed bottomland hardwood forest. J. Wildl. Manage. 69(1): 409-417.

Reitsma, L., M. T. Hallworth, P. M. Benham. 2008. Does age influence territory size, habitat selection and reproductive success of male Canada Warblers in central New Hampshire? Wilson Journal of Ornithology: 120: 446-459.