Final report on the Nova Scotia Species at Risk Conservation Fund project titled: Detecting and defining genetic structure in the Nova Scotia population of eastern ribbonsnake (*Thamnophis sauritus)*

This project was undertaken to address a specific knowledge gap identified by the Eastern Ribbonsnake Recovery Team: the need to understand any existing genetic structure in the species in Nova Scotia. This information is particularly important in recovery planning as it contributes to the ability to match the scale of recovery actions to the ecological scale of the species. The goals of this project were to identify a suite of microsatellite loci for use with the Eastern Ribbonsnake and to use these loci to examine samples collected from ribbonsnakes at Molega Lake, McGowan Lake and Grafton Lake in Queens County Nova Scotia for genetic structure. While the project had several successes there were several challenges encountered; overcoming these challenges will increase the success of this ongoing project.

Eighty-three samples collected between 2004 and 2007 were used in this study, this included three samples from Massachusetts that were used as an outgroup. Seven microsatellite loci were identified as being potentially useful in this species; these had all been developed for the closely related common garter snake (*Thamnophis sirtalis).* Three of these loci had been previously used in our lab and were known to amplify in *Thamnophis sauritus* (Harwood, 2005). Five of the seven loci amplified in *Thamnophis sauritus* and were optimized for this study.

Two major challenges presented themselves during this study and, while they have subsequently been overcome, affected the outcome of this project. The first was sample storage. A small tissue sample has been collected from the tail of individual snakes and stored in ethanol. These samples have been kept at ambient temperatures during the research period (usually one day but occasionally multiple days) and then stored at -20C until DNA extraction. This storage period has been up to 4 years and in samples from other species has not been a problem. We discovered a strong correlation between storage time and successful DNA extraction with samples stored beyond 1.5 years not yielding sufficient DNA for analysis. This resulted in DNA from 58 of 87 samples. The storage and extraction protocols have now been modified to take this into account.

The second problem relates to marking individual snakes. The generally accepted method of marking snakes is to clip ventral scales in an individually unique pattern. This was

found to work very well within years but less well between years. Over multiple sheddings these scale clips fade and eventually it is no longer obvious that the snake was ever marked. This has consequence for genetic analysis in that the independence of samples requires confidence that each sample is from a different individual. The non-permanence of the markings means that samples can be compared within years but not between years. We are currently exploring the use of Passive Integrated Transponder (PIT) tags as an alternative marking technique; results from the first year of use are promising.

These challenges did not mean that there were not also successes with this project. Five of seven loci were found to amplify well in *Thamnophis sauritus* and have been optimized for use. Genotypes for all samples were obtained at only one locus and, while this limits the conclusions that can be drawn from this project, it does provide a first look at the distribution of genetic variation.

Harwood, B.N. 2005. Microsatellite primers for studying structure of the Eastern Ribbonsnake (*Thamnophis sauritus*) in Nova Scotia. BScH Theses. Acadia University, Nova Scotia, Canada

McLaughlin, C.M. 2008. Microsatellite analysis of population structure in the Eastern Ribbonsnake (*Thamnophis sauritus*). BScH Theses. Acadia University, Nova Scotia, Canada