

# Biodiversity Survey of Watersnake Populations in Northwest Georgias Using eDNA qPCR Analysis

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With the rapid expansion of human settlement in the Northwest Georgia region, it has become more critical than ever to understand the ecological impact, development has had on our local ecosystems. Often, this would require traditional survey studies that require a high amount of labor and time to complete. The logistical issues of conventional survey methods are even worse with water systems. Environmental DNA (eDNA) presents an attractive alternative to traditional trapping, utilizing genetic analysis of a water sample to determine species in a water system. This extracted sample is then amplified using quantitative PCR (qPCR), which allows the sample to be a high enough quantity for sequencing. Novel primer sets for qPCR amplifications of the genera *Nerodia*, *Thamnophis/Regina*, *Farancia*, and *Etheostoma* were designed using Primer-BLAST software for this survey. Each primer was tested with a positive control and was confirmed to work. The amplified samples show whether the specific genus was present in the water system sampled. Watersnakes were chosen due to their higher trophic position in the aquatic ecosystem (indicating lower trophic level health through their detection), general elusiveness (making them harder to traditionally survey), and the lack of scientific exploration of utilizing eDNA with watersnakes. Furthermore, the endangered Etowah Darter (*Etheostoma etowahae*), was surveyed using this methodology. The results of this show that eDNA can be used with watersnakes and the Etowah Darter with specialized sampling methods; furthermore, eDNA, in addition to traditional surveying, methods can help track small and elusive taxa and reduce logistical costs associated with population surveys of these species.