

Surveying Isolated Wetlands for Presence of Three Imperiled Amphibian Species Using Environmental DNA at Fort Stewart Military Base, GA

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Abstract. The loss of longleaf pine forests and associated wetlands has led to the decline in fauna dependent on these habitats. However, Fort Stewart contains numerous isolated wetlands that may provide habitat for three imperiled amphibian species: the frosted flatwoods salamander (*Ambystoma cingulatum*), gopher frog (*Lithobates capito*), and striped newt (*Notophthalmus perstriatus*). Because of the time-intensive nature of conducting dipnet surveys at all wetlands, only a fraction of these wetlands have been surveyed for amphibians. We recently developed environmental DNA (eDNA) assays to detect presence of the aforementioned amphibian species and used these assays to assess 287 water samples collected from 95 wetlands across the military base. No evidence of presence of frosted flatwoods salamanders was found at any of the wetlands. Due to evidence of gopher frog genomic DNA contamination in numerous water samples, there is limited confidence in the gopher frog results. Weak evidence of presence of striped newts was found in 11 samples from 7 wetlands. However, all positive samples were at extremely low concentrations, ranging from 2.7×10^{-8} to 9.3×10^{-10} ng/ μ L. These concentrations were well below the level at which eDNA quantity can be reliably estimated, and are near the lower limits of expected detection. While eDNA results for striped newts were not definitive, the seven wetlands that tested positive should be given higher priority among all wetlands on the base for physical surveys to verify presence.