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RESEARCH ARTICLE

HERPETOLOGICAL INVENTORY AT SWEET'S FARM IN HIGHLAND COUNTY, VIRGINIA

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ABSTRACT

Sweet's Farm is a 157-hectare site located in Highland County, VA. From April 2017–May 2021 efforts were made to document the herpetofaunal biodiversity at this site using a variety of survey methods, including visual encounter surveys, bioacoustics surveys, road surveys, cover boards, and aquatic turtle traps. A total of 20 species, including 13 amphibians and 7 reptiles, were recorded over the survey period. Based on survey results, two species listed by the Virginia Department of Wildlife Resources as Species of Greatest Conservation Need in their 2015 Wildlife Action Plan were identified, including the Woodland Box Turtle (*Terrapene carolina carolina* [Linnaeus, 1758]) and Snapping Turtle (*Chelydra serpentina* [Linnaeus, 1758]). Of these, *T. c. carolina* was identified from shell fragments only. The presence of the Gray Treefrog (*Hyla versicolor* LeConte, 1825) was verified as a new county record. DNA barcoding was used to confirm the identities of three species, including the Northern Dusky Salamander (*Desmognathus fuscus* [Green, 1818]), Northern Two-lined Salamander (*Eurycea bislineata* [Green, 1818]), and Green Frog (*Lithobates clamitans* [Latreille, 1801]). These initial surveys serve to inform conservation management, facilitate research and educational opportunities, and provide a baseline for monitoring changes in the populations of amphibians and reptiles at Sweet's Farm.

Keywords: amphibians**,** biodiversity, *Desmognathus fuscus*, *Eurycea bislineata*, *Lithobates clamitans*, reptiles, *Terrapene carolina carolina*.

INTRODUCTION

In 2018, John Sweet donated a parcel of 157 hectares in Highland County (Sweet's Farm hereafter) to the Virginia-based Valley Conservation Council (VCC). Sweet's Farm is in the town of Mustoe, situated along the Jackson River in the Monterey Valley of Highland County. The property represents a predominately forested area with an assigned biodiversity rank of B1 (outstanding significance) by the Virginia Department of Conservation and Recreation (DCR). A portion of the property lies within the DCR Natural Heritage Program's Mustoe Seeps Conservation Site (Wilson, 2018). The property is not currently publicly accessible.

Highland County is in the Valley and Ridge physiographic province of Virginia and is the second-highest elevation county in the state, at an average elevation of around 850 meters above sea level (asl). Due to its elevation, the area has a relatively cool and wet climate with long winters. Between 2000 and 2020 at Sweet's Farm, the mean temperatures were -2.2°C in January and 19.8°C in July, mean annual precipitation was 120.9 cm, and the mean time between last and first freeze was 142 days (NWS, 2022). The county is exclusively rural and has the lowest population in Virginia at 2,226 people, and a population density of approximately 2 people per square kilometer (US Census Bureau, 2021). Protected areas cover approximately 28.7% of the county area and include George Washington National Forest and Highland Wildlife Management Area (Boyd, 2021).

To date a total of 40 species and subspecies of amphibians and reptiles have been documented in Highland County (VHS, 2022). Surveys of amphibians and reptiles were conducted several times in Highland County during the 1980s and 1990s. Young (1993) documented 31 species from multiple sites across Highland County from 1984–1990. The Laurel Fork Recreation Area in Highland County (Approximately 25 km NNW of Sweet's Farm) was considered in two studies. Adams et al. (1996) identified nine species of salamanders across eight sites in the area. Roble (1999) identified nine different amphibian and reptile species in beaver ponds at Laurel Fork, including one salamander species previously identified by Adams et al. (1996). Except for a few recent county records (e.g., Sattler & Sattler, 2014; Stinson, 2020), the amphibians and reptiles of Highland County have received relatively little attention in the 21st century.

The goal of this study is to update the herpetology of Highland County by documenting the diversity and ecology of the amphibians and reptiles at Sweet's Farm. A herpetological inventory at Sweet's Farm also helps to inform management, create a baseline for future research, and pave the way for educational opportunities at the property.

MATERIALS AND METHODS

Site Description

Sweet's Farm includes several different habitat types (Fig. 1). Three wetland areas are designated as Habitat Protected Areas (HPAs) by the property's conservation management plan and afforded special protections (Central Appalachian Pitch Pine Bog, Seeps and Springs, and Calcareous Fen). An unofficially named stream, referred to herein as Spring Run, flows through the center of the property and through the bog. Just below the Seeps and Springs HPA a man-made dam creates a 0.3-hectare pond. In the southern portion of the property, a stream running out of the calcareous fen and a manmade canal combine into Spring Run before merging with Vinegar

Run off the property. Vinegar Run then drains into the Jackson River approximately 100 meters from the property.

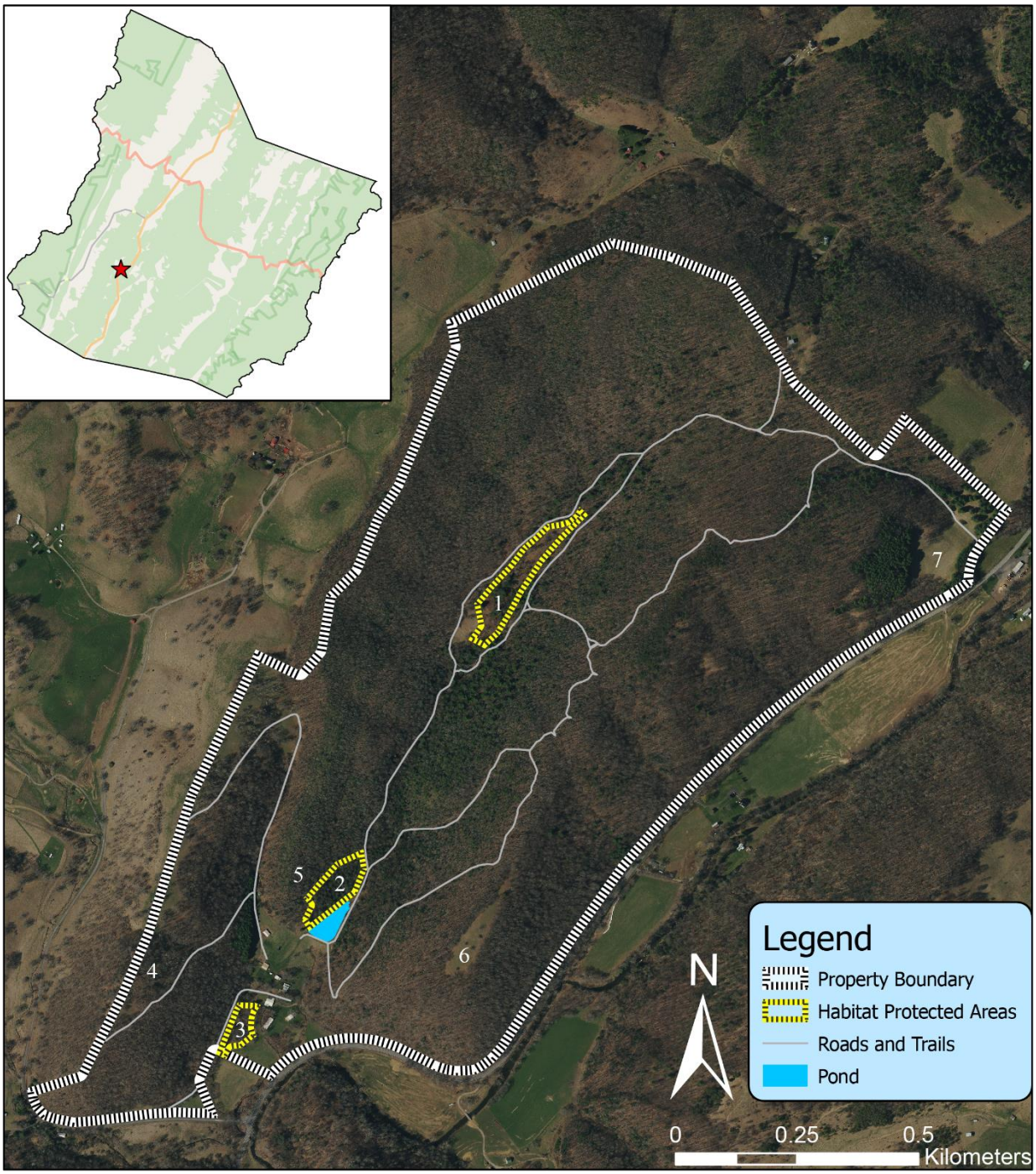

Figure 1. Map of Sweet's Farm including the property boundary (white hatched) and trails (grey), with important features numbered. Three Habitat Protected Areas (yellow hatched) include the Central Appalachian Pitch Pine Bog (1), Seeps and Springs (2), and Calcareous Fen (3). The three ridges on the property are Stark Ridge (4), Cemetery Ridge (5), and Cedar Ridge (6). Other areas of interest include the pond and the northeastern hay meadow (7). Red star in inset indicates the location of the property in Highland County. Basemaps by VGIN (2018) and OpenStreetMaps Contributors et al. (2022).

Elevation at Sweet's Farm ranges from 710m to 870m asl. Three ridges exist on the property (Stark Ridge, Cemetery Ridge, and Cedar Ridge) characterized by rock outcrops and talus slopes. The upland area comprises primarily mixed hardwood forest. Several areas are mowed annually to create meadows totaling approximately 2.4 hectares. These areas include the tops of Stark Ridge and Cedar Ridge, on the southern slope of Stark Ridge, an area near the southern end of the bog, and a hay meadow in the northeastern portion of the property. Two small stands of planted pine woods covering approximately 2.2 hectares occur at the eastern foot of Stark Ridge and adjacent to the northeast hay meadow. The southern end of the property is developed, being occupied by several permanent structures and consistently mowed. This area totals approximately 0.9 hectares. Pastures covering approximately 4.6 hectares around the developed area and on much of Stark Ridge are maintained for the grazing of three cows.

Survey Techniques

Between April 2019 and May 2021, surveys for amphibians and reptiles were conducted at Sweet's Farm by undergraduate researchers at James Madison University. Survey effort totaled 236.5 person-hours over the two-year study period (Table 1). Multiple methods, including visual encounter surveys, dip netting, road surveys, auditory surveys, artificial cover boards, and aquatic hoop traps, were used to maximize the number of species encountered.

Table 1. Summary of survey effort. Survey time (recorded in person hours) is the total time spent surveying using various methods (e.g., visual encounter, dip-netting) multiplied by the number of surveyors.

Month	Survey Time (person hours)
January	11.5
February	0
March	13
April	7.5
May	63
June	41
July	35.5
August	33.5
September	11.5
October	17
November	2
December	1
Total	**236.5**

Most sampling was done via a combination of visual encounter and auditory surveys. Sections of the property were walked at differing times of day (both day and night) and cover (e.g., rocks, logs, and artificial cover) was flipped. Any amphibians and reptiles encountered were collected for identification either by hand or net. Dip nets were employed in water bodies to capture aquatic amphibians. Road surveys were employed opportunistically on rainy nights during the study to identify amphibians and reptiles found crossing public roads adjacent to the property.

In each survey method, identified animals were either released shortly after capture or retained as voucher specimens.

Auditory surveys occurred during visual encounter and road surveys. Frog calls were identified and recorded using a Tascam DR-100 MkII recorder with a Sennheiser ME 64 microphone. Audio recordings are accessioned in the Macaulay Library of Sounds, and accession numbers can be found in the annotated checklist.

Seven artificial cover boards were placed along the property throughout the study. Of these, two corrugated metal sheets (60x70cm) were placed on the southern edge of Stark Ridge, three rubber stall mats (60x90x1.5cm) were placed around the pond, and a rubber stall mat was placed around the northeast hay meadow.

A single aquatic trap (0.75 m diameter hoop trap with 5 cm cotton mesh) was placed in the pond on two visits in June 2019 to identify aquatic turtles. Traps were placed perpendicular to the shore, with the mouth completely submerged and a portion of the trap above water. Traps were baited with either dry dog food or sardines and checked every 24 hours.

Specimen Preservation

For most species, at least one individual was collected and preserved to serve as a voucher specimen. Specimens were euthanized using tricaine methanesulfonate (MS-222; Simmons, 2015). Samples of liver tissue were collected for future molecular analysis and stored in RNAlater (Invitrogen). Specimens were fixed in 10% neutral buffered formalin and later transferred to 70% ethanol. Specimens and tissue samples are accessioned in the collections at the North Carolina Museum of Natural Science. Accession numbers for each species can be found in the annotated checklist.

DNA Barcoding

DNA barcoding is a rapid assessment method for identifying and validating the taxonomic identity of a particular specimen by using a short fragment of DNA. For amphibians and reptiles, two mitochondrial gene regions (COI and 16S rRNA) are commonly used (Vences et al., 2005). At present, however, more comparative data are frequently available for the 16S rRNA gene in repositories such as GenBank and the Barcode of Life Data System (BOLD). DNA barcoding is particularly useful in aiding in the identification of morphologically challenging organisms, such as amphibian larvae (Grosjean et al., 2015) and members of cryptic species complexes (Vietes et al., 2009). In this study, DNA barcoding was used to confirm the identities of five specimens for which morphology alone could not provide positive identification. These included three adult *Desmognathus* sp. (one from each HPA), a single adult *Eurycea* sp., and an egg mass belonging to *Lithobates* sp. DNA barcode sequences were compared with existing sequences in Genbank to confirm the identities of these specimens. All DNA barcode sequences are accessioned in Genbank.

Total genomic DNA was extracted from the tissue samples of five specimens using Dneasy blood and tissue kit (QIAGEN, Inc.). A fragment of the mitochondrial 16S rRNA gene was amplified via the forward primer 16Sar (CGCCTGTTTATCAAAAACAT) and reverse primer 16Sbr (CCGGTCTGAACTCAGATCACGT; Palumbi et al., 1991). PCR conditions are as follows: 94°C, 30 s; 52°C, 30 s; 72°C 1.1 min for 35 cycles. PCR clean-up and sequencing was

performed by Eurofins Genomics. Sequences were edited and assembled in Geneious Prime v2019.2.

Conservation Status

The conservation status of amphibians and reptiles reported in this study follows the criteria of the Virginia Department of Wildlife Resources (DWR) 2015 Wildlife Action Plan (http://www.bewildvirginia.org/wildlife-action-plan/). Under these criteria, Species of Greatest Conservation Need (SGCN) are assigned an alphanumeric designation to identify their conservation status and management strategies. Roman numerals represent conservation need, with "I" being species of critical conservation need and "IV" those of moderate conservation concern. Letters represent the level to which management strategies have been identified that could benefit the species. An "a" indicates that on-the-ground management practices have been identified and can be reasonably implemented to benefit the species' conservation, "b" indicates that management practices have been identified but cannot currently be implemented, and "c" indicates that management practices to benefit the species have not been identified.

RESULTS

A total of 13 amphibian species (5 frogs and 8 salamanders) and 7 reptile species (2 turtles and 5 snakes) were found at Sweet's Farm (Table 3). One species (*Terrapene carolina carolina* [Linnaeus, 1758]; Woodland Box Turtle) was only represented by a shell fragment. The Gray Treefrog (*Hyla versicolor* LeConte, 1825) was not found on the property but was documented during a road survey approximately 300 meters east of Sweet's Farm. DNA barcode sequences all had high sequence identity matches (>99.7%) that confirmed the identities of five specimens, including three *Desmognathus fuscus* (Green, 1818), one *Eurycea bislineata* (Green, 1818), and one *Lithobates clamitans* (Latreille, 1801) (Table 2).

Table 2. Summary of Genbank sequences for all species barcoded from Sweet's Farm. Specimen number indicates the catalog number assigned to the full-body voucher specimen from which tissue samples were sequenced. Four specimens are catalogued in the North Carolina Museum of Natural Science, indicated by an NCSM accession. The fifth specimen is in the collection of Dr. Christopher Rose at James Madison University. Genbank accession indicates the identifier associated with the sequence deposited in Genbank (https://www.ncbi.nlm.nih.gov/genbank/). The highest BLAST match for each sequence is indicated by its accession, percent identity (similarity between the two sequences) and species.

Specimen No.	Genbank	Match Species	Percent	Match
NCSM 105537	MT936357	*Desmognathus fuscus*	99.80%	MK029509.1
NCSM 105538	MT936356	*Eurycea bislineata*	99.79%	AY728217.1
NCSM 105567	MT936355	*Desmognathus fuscus*	100.00%	MK029509.1
NCSM 105568	MT936354	*Desmognathus fuscus*	100.00%	MK029509.1
DW3-1	MZ298986	*Lithobates clamitans*	99.77%	KY677802.1

Table 3. Checklist of amphibians and reptiles found at Sweet's Farm. Those species present in HPAs are indicated by an x. SGCN status is indicated for species listed in Virginia's 2015 Wildlife Action Plan. 'NR' indicates that a species is not represented in the wildlife action plan.

Taxon	Common Name	Bog	Seeps and Springs	Fen	SGCN Status
Amphibians					
Anaxyrus americanus americanus	Eastern American Toad		x		NR
Desmognathus fuscus	Northern Dusky Salamander	x	x	x	NR
Hyla versicolor	Gray Treefrog				NR
Eurycea bislineata	Northern Two-lined Salamander		x	x	NR
Eurycea longicauda longicauda	Long-tailed Salamander				NR
Gyrinophilus p. porphyriticus	Northern Spring Salamander		x		NR
Hemidactylium scutatum	Four-toed Salamander				NR
Lithobates clamitans	Green Frog	x	x	x	NR
Lithobates sylvaticus	Wood Frog				NR
Notophthalmus viridescens viridescens	Red-spotted Newt	x	x	x	NR
Plethodon cinereus	Eastern Red-backed Salamander	x	x	x	NR
Plethodon cylindraceus	White-spotted Slimy Salamander		x		NR
Pseudacris crucifer	Spring Peeper	x		x	NR
Reptiles					
Chelydra serpentina	Common Snapping Turtle				IVb
Diadophis punctatus edwardsii	Northern Ring-necked Snake				NR

Table 3 cont.

Lampropeltis triangulum	Eastern Milksnake	NR
Nerodia sipedon sipedon	Northern Water snake	NR
Pantherophis alleghaniensis	Eastern Ratsnake	NR
Terrapene carolina carolina	Woodland Box Turtle	IIIa
Thamnophis sirtalis sirtalis	Eastern Gartersnake	NR

Annotated Checklist

Frogs

Eastern American Toad (*Anaxyrus americanus americanus* [Holbrook, 1836]; Fig. 2A)

Anaxyrus americanus americanus were found in upland areas at Stark Ridge and Cedar Ridge, and along Jackson River Road. No *A. a. americanus* were heard calling during the survey and no breeding habitats were identified. This species was found in April, June, and August.

Specimens: NCSM 105498

Gray Treefrog (*Hyla versicolor* LeConte, 1825; Fig. 2B)

Hyla versicolor were not encountered on the property, but a single juvenile was found in October 2020 approximately 300 meters east of the property on Big Valley Road. *Hyla versicolor* and its sister species, *Hyla chrysoscelis* (Cope, 1880), are impossible to distinguish morphologically, and can only be distinguished genetically or by call (Mitchell & Pague, 2011). The single specimen was not collected for genetic analysis and no calls were identified. However, with a few exceptions (e.g., Tekin, 2012), *H. chrysoscelis* is not known from the mountains of Virginia (Mitchell & Pague, 2011) and we therefore assign this specimen to *H. versicolor.*

Green Frog (*Lithobates clamitans* [Latreille, 1801]; Fig. 2C)

Lithobates clamitans can be found in nearly all permanent water bodies on the property, including those in the bog and other portions of Spring Run, the pond, the fen, and the canal on the southern end of the property. *Lithobates clamitans* were also frequently encountered on Jackson River Road. This frog was active from March–November. It was heard calling around the pond, and to a lesser extent the bog, from May–July. Larvae of this species were found in the pond. Eggs were found on 22 June 2019, 29 May 2020, and 5 June 2020. Eggs from 2019 were verified as *L. clamitans* using DNA barcoding data.

Specimens: NCSM 105572; DW3-1

Wood Frog (*Lithobates sylvaticus* [LeConte, 1825]; Fig. 2D)

Lithobates sylvaticus were encountered along Spring Run upstream and downstream from the bog and along Jackson River Road from May–September. No *L. sylvaticus* were heard calling during the survey and no breeding habitats were identified.

Specimens: NCSM 105573; NCSM 105595

Figure 2. Frogs from Sweet's Farm. **A**: *Anaxyrus americanus americanus* (Eastern American Toad); **B**: *Hyla versicolor* (Gray Treefrog); **C**: *Lithobates clamitans* (Green Frog); **D**: *Lithobates* sylvaticus (Wood Frog); **E**: *Pseudacris crucifer* (Spring Peeper).

Spring Peeper (*Pseudacris crucifer* [Wied-Neuwied, 1838]; Fig. 2E)

Pseudacris crucifer were found calling around the pond and calcareous fen from March–June. A single male was heard calling in the bog on 17 May 2019. Individuals were found around the pond and Seeps and Springs HPA in August and October.

Specimens: NCSM 105496
Recording: ML506567731

Salamanders

Northern Dusky Salamander (*Desmognathus fuscus* [Green, 1818]; Fig. 3A)

Desmognathus fuscus were found throughout Spring Run and in all three HPAs from March–October. The identities of a single individual from each HPA were verified using DNA barcoding data. All three barcoded individuals had a high match with mitochondrial sequences from *D. fuscus*. Recent analyses have revealed potential undescribed cryptic diversity in *D. fuscus* (Beamer & Lambs, 2020; Pyron et al., 2020). Whereas barcodes of candidate species are not currently available on Genbank, the *D. fuscus* from Sweet's Farm likely belong to the clade *D. fuscus* B, which is the most widespread clade in the mountains of Virginia (Beamer & Lambs, 2020). Voucher specimens and DNA barcodes of the *D. fuscus* found at Sweet's Farm will help to update the species list as the systematics of this species complex are worked out.

Specimens: NCSM 105527; NCSM 105536; NCSM 105526; NCSM 105534; NCSM 105535; NCSM 105537; NCSM 105528; NCSM 105566; NCSM 105567; NCSM 105568; NCSM 105569; NCSM 105570; NCSM 105571

Northern Two-lined Salamander (*Eurycea bislineata* [Green, 1818]; Fig. 3B)

Eurycea bislineata were found around the pond, calcareous fen, and the western slope of Cedar Ridge from April–September. Larvae belonging to this species were found in Spring Run and the Seeps and Springs HPA. Highland County is just North of the contact zone between *E. bislineata* and *Eurycea cirrigera* (Green, 1831) identified by Sattler & Brophy (2019). A single individual barcoded from the property had a high match with mitochondrial sequences from *E. bislineata*.

Specimens: NCSM 105525; NCSM 105538; NCSM 105521; NCSM 105562

Long-tailed Salamander (*Eurycea longicauda longicauda* [Green, 1818]; Fig. 3C)

Eurycea longicauda longicauda were found under rocks and logs in upland areas throughout the property, and commonly around Stark Ridge. They were also found active on rainy nights along Cedar Ridge and on Jackson River Road. This species was found from May–July.

Specimens: NCSM 105503; NCSM 105563; NCSM 105564

Four-toed Salamander (*Hemidactylium scutatum* [Temminck, 1838]; Fig. 3D)

A single male was found in the pine woods on the northern portion of the property in September 2020. This salamander mates in upland habitats during the fall and females migrate to wetlands in the spring to lay eggs within sphagnum moss (Blanchard, 1934; Wahl et al, 2008). Although no *H. scutatum* were found during surveys in the bog, it is presumed to be an important habitat for this species.

Specimens: NCSM 105490

Northern Spring Salamander (*Gyrinophilus porphyriticus porphyriticus* [Green, 1827]; Fig. 4A)

Larval *G. p. porphyriticus* were found in Spring Run and the streams in the Seeps and Springs HPA in May and June. Adults of this species were found in the seeps and spring HPA in October 2020.

Specimens: NCSM 105491; NCSM 105581; NCSM 105582

Figure 3. Salamanders encountered at Sweet's Farm. **A**: *Desmognathus fuscus* (Northern Dusky Salamander); **B**: *Eurycea bislineata* (Northern Two-lined Salamander); **C**: *Eurycea longicauda longicauda* (Eastern Long-tailed Salamander); **D**: *Hemidactylium scutatum* (Four-toed Salamander).

Red-spotted Newt (*Notophthalmus viridescens viridescens* [Rafinesque, 1820]; Fig. 4B)

Adult *N. v. viridescens* were common in the pond and occasionally found in Spring Run and portions of the bog. Efts, the terrestrial phase for this species, were found throughout much of the property, including in upland areas and along Spring Run. Efts were found from March–September while adults could be found year-round in the pond.

Specimens: NCSM 105531; NCSM 105532; NCSM 105533

Eastern Red-backed Salamander (*Plethodon cinereus* [Green, 1818]; Fig. 4D)

Plethodon cinereus was one of the most frequently encountered species on the property and was found in most upland areas throughout the property and all three HPAs. Both striped and unstriped morphs were found on the property. This species was found throughout most of the year, including on warm days in January.

Specimens: NCSM 105541; NCSM 105542; NCSM 105544; NCSM 105546; NCSM 105543; NCSM 105545

White-spotted Slimy Salamander (*Plethodon cylindraceus* [Harlan, 1825]; Fig. 4C)

Plethodon cylindraceus were common on all three ridges, in upland areas throughout the property, and in an upland portion of the Seeps and Springs HPA. The species was frequently found under cover during the daytime and could be found active and in abundance on talus slopes during rainy nights. *Plethodon cylindraceus* was found from May–October.

Specimens: NCSM 105551; NCSM 105548; NCSM 105547; NCSM 105561

Figure 4. Salamanders encountered at Sweet's Farm. **A**: *Gyrinophilus porphyriticus porphyriticus* (Northern Spring Salamander); **B**: *Notophthalmus viridescens viridescens* (Red-spotted Newt); **C**: *Plethodon cylindraceus* (White-spotted Slimy Salamander). **D**: *Plethodon cinereus* (Eastern Red-backed Salamander). Image A courtesy of Isabella Bukovich.

Turtles

Snapping Turtle (*Chelydra serpentina* [Linnaeus, 1758]; Fig. 5F)

On 22 June 2019, a single *C. serpentina* was captured in a turtle trap placed in the pond. On 6 June 2019 John Sweet noted a pair of *C. serpentina* mating in the pond. A nest of eggs likely belonging to this species was discovered in the gravel surrounding the pond dock following a predation event in October 2019. Multiple individuals of this species were released into the pond several years ago, and at least three individuals have been observed in the pond since then (J.

Sweet, pers. comm.). This species is identified by Virginia's Wildlife Action Plan (2015) as a species of moderate conservation need (Tier IVa).

Woodland Box Turtle (*Terrapene carolina carolina* [Linnaeus, 1758])

A shell fragment from *T. c. carolina* was found on the top of Stark Ridge. No live *T. c. carolina* were encountered during the survey. *Terrapene carolina carolina* have been observed on the property in the past, and individuals are known to have been intentionally released on the property from nearby roads. No live individuals have been observed in recent years (J. Sweet, pers. comm.). *Terrapene carolina carolina* are identified by Virginia's Wildlife Action Plan (2015) as a species of high conservation need (Tier IIIa).

Snakes

Northern Ring-necked Snake (*Diadophis punctatus edwardsii* [Merrem, 1820]; Fig. 5D)

Diadophis punctatus edwardsii were found under rocks and logs on the slopes and tops of Stark Ridge and Cedar Ridge and under a well cover in the northeast hay meadow. They were encountered in May and June.

Specimens: NCSM 105494

Eastern Milksnake (*Lampropeltis triangulum* [Lancépède, 1789]; Fig. 5E)

Lampropeltis triangulum were found under cover by the warehouse on the southern end of the property, along the edge of the pond, and in the northeast hay meadow. This species was encountered from May–August.

Specimens: NCSM 105599; NCSM 105598

Northern Watersnake (*Nerodia sipedon sipedon* [Linnaeus, 1758]; Fig. 5C)

Nerodia sipedon sipedon were not found during the 2019 surveys, but several individuals were found under coverboards along the southern edge of the pond from May–August of 2020 and 2021.

Specimens: NCSM 105597

Eastern Ratsnake (*Pantherophis alleghaniensis* [Holbrook, 1836]; Fig. 5A)

Several *P. alleghaniensis* were found on Stark Ridge and an individual was found dead on Jackson River Road. This species was found in May and June. Burbrink et al. (2020) found that Western Virginian populations were represented by the central ratsnake clade, which they referred to as *Pantherophis spiloides* (Duméril, Bibron, & Duméril, 1854). Hillis and Wüster (2021) and Burbrink et al. (2021) corrected the taxonomy and placed ratsnakes referred to as *P. spiloides* by Burbrink et al. (2020) as *P. alleghaniensis.* Although there is debate about the placement of members of the *P. obsoletus* complex as a distinct species, those from Sweet's Farm likely belong to the clade called *P. alleghaniensis* following Burbrink et al. (2021) or *P. obsoletus alleghaniensis* following Hillis and Wüster (2021) and Hillis (2022). Burbrink (2021) included a limited number of samples from Virginia, and further sampling is required to understand taxonomic placement and ranges of ratsnakes in Virginia.

Eastern Gartersnake (*Thamnophis sirtalis sirtalis* [Linnaeus, 1758]; Fig. 5B)

Thamnophis sirtalis sirtalis were found under cover near the pond and in the Northeastern hay meadow. This species was found from May–August.

Specimens: NCSM 105600

DISCUSSION

This study documented 20 amphibian and reptile species on or near Sweet's Farm in Highland County Virginia. These results represent a significant proportion of the 31 species reported by Young (1993) from across Highland County. Several species were found during this study that were not identified by Young (1993), including amphibians *A. a. americanus*, *H. scutatum*, and *N. v. viridescens* and reptiles *P. alleghaniensis* and *T. c. carolina*. Three salamander species were found at Sweet's Farm that are not reported in Adams (1996), including *P. cylindraceus*, *H. scutatum*, and *E. l. longicauda*.

Highland County sits at the contact zone of *P. cylindraceus* and *Plethodon glutinosus* (Green, 1818). Adams (1996) found only *P. glutinosus* at the Laurel Fork Area, while this study found only *P. cylindraceus*. The Laurel Fork area is farther West and has a higher average elevation than Sweet's Farm. This is consistent with the observations of Young (1993) that *P. glutinosus* is only found in the western portion of the county, whereas *P. cylindraceus* was found at lower elevations in eastern and central portions of the county.

Hyla versicolor is not recorded in Highland County in either the VHS database (VHS, 2022) or the VaFWIS database (VDWR, 2020a), but is noted to likely occur in the county in VAFWIS and is documented in all surrounding Virginia counties. The species also does not appear in the county in Virginia amphibian and reptile atlases by Mitchell and Reay (1999) or Tobey (1985). A search of VertNet (http://vertnet.org/) returns no results for *H. versicolor* in the county. The species does not appear in Roble (1999), but Young (1993) reported the species from the county. Thus, the individual found during this survey confirms the county record for Highland County.

Both the Virginia Herpetological Society (VHS) database (VHS, 2022) and VaFWIS database (VDWR, 2016) report *Eurycea cirrigera* in Highland County. However, no other publications report the species in the county, a search of VertNet (http://vertnet.org/) returns no results for *E. cirrigera* in the county, and only *E. bislineata* were found in this survey. The narrow contact zone between *E. cirrigera* and *E. bislineata* identified by Sattler & Brophy (2019) is just South of Highland County. Thus, the placement of *E. cirrigera* in Highland County's species list is dubious. Excluding *E. cirrigera* and including species from this survey, a total 41 species and distinct subspecies of amphibians and reptiles have been documented in Highland County.

John Sweet stated that he has seen *T. c. carolina* in the past but has not seen them in recent years. He also stated that he has relocated box turtles from nearby roads to his property. During these surveys, no live *T. c. carolina* were found, but a single shell fragment of a deceased turtle was found. The lack of *T. c. carolina* observations is interesting, as they are a relatively common species throughout the state but are identified by Virginia's Wildlife Action Plan (VDWR, 2015) as a species of high conservation need (Tier IIIa). The species is also not documented in the county in the VHS database (VHS, 2022), VaFWIS (VDWR, 2020b), Mitchell & Reay (1999), Tobey (1985), or the two previous Highland County reptile surveys (Young, 1993; Roble, 1999). While it is possible that the species occurs on the property but was simply not encountered on these

surveys, it warrants further exploration to determine whether the species still occurs on the property.

Figure 5. Reptiles encountered at Sweet's Farm. **A**: *Pantherophis alleghaniensis* (Eastern Ratsnake); **B**: *Thamnophis sirtalis sirtalis* (Eastern Gartersnake); **C**: *Nerodia sipedon sipedon* (Northern Watersnake); **D**: *Diadophis punctatus edwardsii* (Northern Ring-necked Snake); **E**: *Lampropeltis triangulum* (Eastern Milksnake); **F**: *Chelydra serpentina* (Snapping Turtle).

Several amphibian species are within range but did not appear in the surveys. Many species of amphibian, particularly salamanders in the genus *Ambystoma* and multiple frog species, require fish-free ephemeral pools to breed. The only major wetlands on the property are all permanently filled, and there are no known ephemeral pools on the property. Thus, the absence of several amphibian species may be attributed to lack of breeding habitat.

Additionally, three frog species were found on or near the property, but breeding habitats were never found. Of these, *A. a. americanus* and *L. sylvaticus* typically require fish-free waters for breeding (Dorcas & Gibbons, 2008). No breeding habitats were identified for either of these species on the property, indicating that they may breed in ephemeral pools off the property and use habitats on the property as adults. This underscores the importance of maintaining habitat corridors between this property and surrounding habitats to maintain diversity on the property.

Despite an investment of more than 230 person-hours of surveying over two years, it is likely that there are still amphibian and reptile species on the property that have yet to be documented. Future studies at the property could identify species that were missed in this survey and confirm the presence of species such as *T. c. carolina* and *H. versicolor*.

A species list produced by an inventory such as this is often limited in its immediate scope, but it is important to recognize that this list provides a baseline dataset necessary for longer-term research and monitoring programs (Buhlmann, 2013). Future monitoring efforts would help to better understand the diversity at the site as well as trends within populations of species of interest. Long-term monitoring, or at the very least repeated inventorying efforts, should be done to understand the effects of future anthropogenic, landscape, or climate-related changes on the site. Amphibian and reptile diseases, such as *Batrachochytrium dendrobatidis*, ranaviruses, snake fungal disease, or turtle respiratory illnesses were not investigated in this study. Due to their ability to cause declines in amphibians and reptiles, future studies should survey for these diseases.

Understanding the species composition of a site is essential both to management of that site and creation of environmental educational programs and materials (Rossman et al., 1998; Nichols & Langdon, 2007). To this end, surveys of other taxonomic groups should be undertaken to develop a more complete picture of the flora and fauna at Sweet's Farm. The development of an all-taxa biodiversity inventory program at the site would generate valuable data on the biodiversity the property. This type of program would also create educational opportunities if, like this study and other biodiversity inventories, it involves student and public survey participation and student-led research projects (Nichols & Langdon, 2007).

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