CATESBEIANA

JOURNAL OF THE VIRGINIA HERPETOLOGICAL SOCIETY

ISBN 0892-0761

Volume 44 **Spring 2024** **Number 1**

JOURNAL INFORMATION

Catesbeiana is published twice a year by the Virginia Herpetological Society. Membership is open to all individuals interested in the study of amphibians and reptiles and includes a subscription to Catesbeiana, two newsletters, and admission to all meetings. Annual dues for regular membership is $15.00. Payments received after September 1 of any given year will apply to membership for the following calendar year.

HERPETOLOGICAL ARTWORK

Herpetological artwork is welcomed for publication in Catesbeiana. If the artwork has been published elsewhere, we will need to obtain copyright before it can be used in an issue. We need drawings and encourage members to send us anything appropriate, especially their own work. Digital submissions are required.

EDITORIAL POLICY

The principal function of Catesbeiana is to publish observations and original research about Virginia herpetology. Rarely will articles be reprinted in Catesbeiana after they have been published elsewhere. All correspondence relative to the suitability of manuscripts or other editorial matters should be directed to: Dr. Paul Sattler, Co-Editor, Catesbeiana, Biology/Chemistry Department, Liberty University, MSC Box 710155, 1971 University Blvd., Lynchburg, VA 24515, (email: psattler@liberty.edu).

Major Papers

Manuscripts for consideration of publication in Catesbeiana should be submitted to the Co-Editors electronically. Consult the style of articles in this issue for additional information, including the appropriate format for literature citations. The metric system should be used for reporting all types of measurement data. Email attachments in Word format is desired for all papers. Submissions concerning the herpetofauna of selected areas, such as a park, city or county, should be prepared in article rather than field note format. Articles will be refereed by the editor and one or more qualified reviewers. All changes must be approved by the author before publication; therefore, manuscripts must be received by the editor before March 1 and August 1 to be considered for publication in the spring and fall issues, respectively, of Catesbeiana. Reprints of articles are not available, but authors may reprint their own articles to meet professional needs.

(Editorial policy continued on inside back cover)

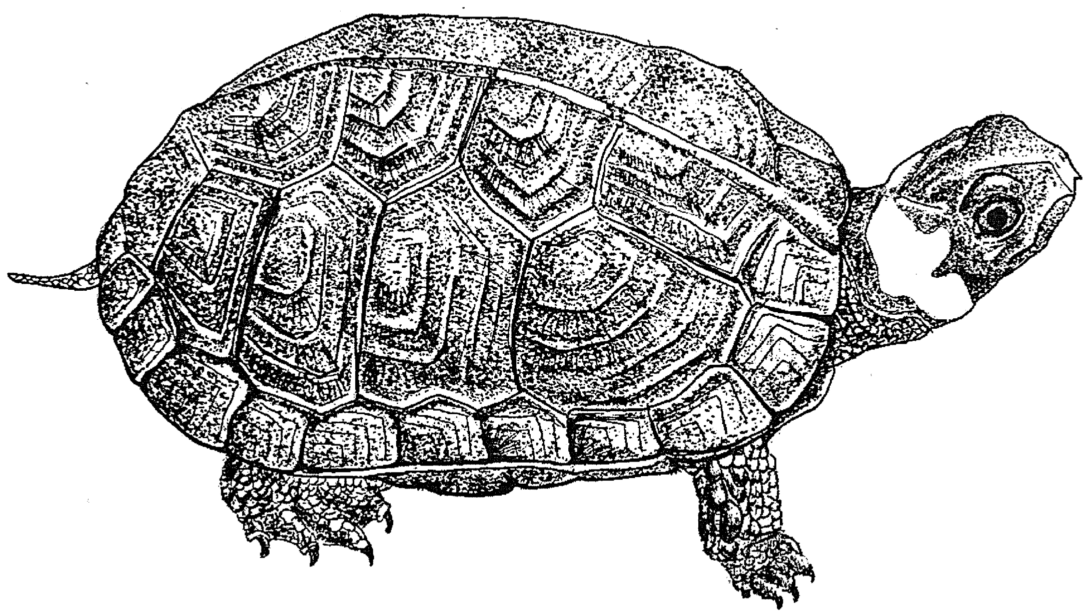
Cover Photo: *Pseudotriton ruber nitidus* (see p 30).

CATESBEIANA

Journal of the Virginia Herpetological Society

Volume 44 Spring 2024 No. 1

Contents



Clemmys muhlenbergii MJP'97

Off-road vehicle trail development as a driver of pool-breeding amphibian mortality in the Virginia coalfields

Walter H. Smith

Department of Natural Sciences, The University of Virginia's College at Wise, Wise, Virginia 24293 (whs2q@uvawise.edu)

Abstract: Pool-breeding amphibians are reliant upon small, isolated, and fishless wetlands as sites for oviposition and larval development. These pools are often susceptible to impacts from anthropogenic disturbances, with off-road vehicle (ORV) trail development becoming an increasingly common form of wetland disturbance in the southwest Virginia coalfields. I performed a before-after control-impact (BACI) comparison of egg mass loss at 15 pools impacted and unimpacted by ORV trail development at a recently-opened public ORV trail system in Russell County, Virginia during the spring of 2024. Pools impacted by ORV use showed a mean 83% egg mass loss rate over just one week of trail use relative to no egg loss in unimpacted pools, with distinct mechanisms associated with ORV use (physical crushing, wheel-thrown eggs, and egg mass relocation) acting in concert to drive egg mass loss. My results provide some of the first empirical data documenting mechanisms of egg mass loss and embryonic mortality in pool-breeding amphibians from ORV disturbance and support several best management practices that land managers in Virginia and elsewhere can use to minimize impacts to resident amphibian populations on public and private lands managed for recreation.

Keywords: wetland; salamander; frog; disturbance; reproduction

Introduction

Pool-breeding amphibians are reliant on mostly shallow, temporary, and fishless wetland habitats to complete their lifecycles (Baldwin & deMaynadier, 2009). Specifically, oviposition sites that are small and ephemeral enough to prevent the establishment of fish populations – yet not so temporary that they dry before larvae can complete metamorphosis – are critical landscape features for a number of Virginia's amphibian taxa, including ambystomatid salamanders (Buhlmann & Mitchell, 2000; Baldwin et al., 2006), Wood Frogs (*Lithobates sylvaticus*) (Skidds et al., 2007; Karraker & Gibbs, 2009), and members of *Pseudacris* (Burne & Griffin, 2005; Ethier et al., 2021).

The aforementioned taxa use naturally-occurring vernal pools and other ephemeral wetlands as breeding habitat, although artificially-created wetlands can often mimic such natural wetland features and provide suitable surrogate breeding habitat (Rothenberger et al., 2019). Adam and Lacki (1991), for example, found eight amphibian species selecting artificial pools associated with flooded ruts along forest roads in Kentucky's Daniel Boone National Forest, while Barry et al. (2008) found similar associations between anurans and road-rut pools along abandoned haul roads in West Virginia. Gibson and Sattler (2020) additionally documented the use of numerous types of artificial wetlands – including flooded roadside ditches, constructed vernal pools, and flooded agricultural fields – for amphibian breeding in a 20-year study of a wildlife management area in the Virginia Piedmont.

Artificial pools can especially represent important supplemental habitat in disturbed landscapes where natural pools have been eliminated by intensive resource extraction activities (Brand & Snodgrass, 2010; Rannap et al., 2020). The southwest Virginia coalfields are dominated by such artificially-created wetlands on former surface mines, with artificial wetlands outnumbering naturally-occurring wetlands by more than threefold at the regional scale (Goodman & Smith, 2023). These wetlands are often colonized by pool-breeding amphibians in the years following disturbance, with one recent study (Smith & Hamed, 2020) highlighting the use of artificial wetlands associated with flooded extractive industry access roads, surface mine benches, and gas well pads by Mountain Chorus Frogs (*Pseudacris brachyphona*), a Tier II (Very High Conservation Need) species in Virginia (Virginia DWR, 2015). However, the long-term viability of these pools as amphibian breeding sites is likely reliant upon their protection from anthropogenic disturbances, such as physical disturbance by vehicles, excavation and draining, or water quality degradation.

Current trends in the Virginia coalfields are prioritizing formerly surface-mined landscapes for industrial and recreational development activities (Zipper et al., 2021). This is particularly the case for off-road vehicle (ORV) trail development, with many postmined landscapes being targeted for ORV trail systems (Sharp et al., 2020) and more than 800 km of state-managed ORV trails developed in the Virginia coalfields since 2012 (Institute for Service Research, 2017). A popular feature of ORV trail development is the conversion of pre-existing artificial pools and natural wetland habitat into play areas for vehicles, in which wetland areas are opened to “mudding,” or in-pool recreational vehicle use (Meyer, 2002; Smith, 2021). One potential impact from these activities is the functional loss of artificial wetlands as amphibian breeding habitat, since extensive in-pool vehicle use may crush amphibian eggs and larvae or degrade water quality below levels supporting amphibian development. To date, however, few empirical data exist to quantify these impacts or guide the design of best management practices aimed at mitigating them.

To address these knowledge gaps, I used a before-after control-impact (BACI) assessment of impacts to pool-breeding amphibians at a recently-opened, state-managed ORV trail system on a Virginia Department of Wildlife Resources public access parcel in Russell County, Virginia. I specifically visited a collection of 15 artificial pools formed on a 40-50 year-old abandoned surface mine that had been colonized by pool-breeding amphibians since the completion of mining activities, monitoring amphibian breeding activity and associated mortality from ORV use following the conversion of the surface mine into a motorized recreation area in late 2023. I discuss the observed impacts on pool-breeding amphibians from this land use change, report mechanisms of egg mass loss from in-pool recreational vehicle use, and discuss potential best management practices stemming from these results.

Survey Sites

I sampled 15 pools created as a result of surface coal extraction on the approximately 110 ha Squirrel Hollow surface mine in Russell County, Virginia, which was mined and abandoned in the late 1970s (36.963911, -82.310675; Figure 1). Pools at the site were formed at the base of highwalls and in areas

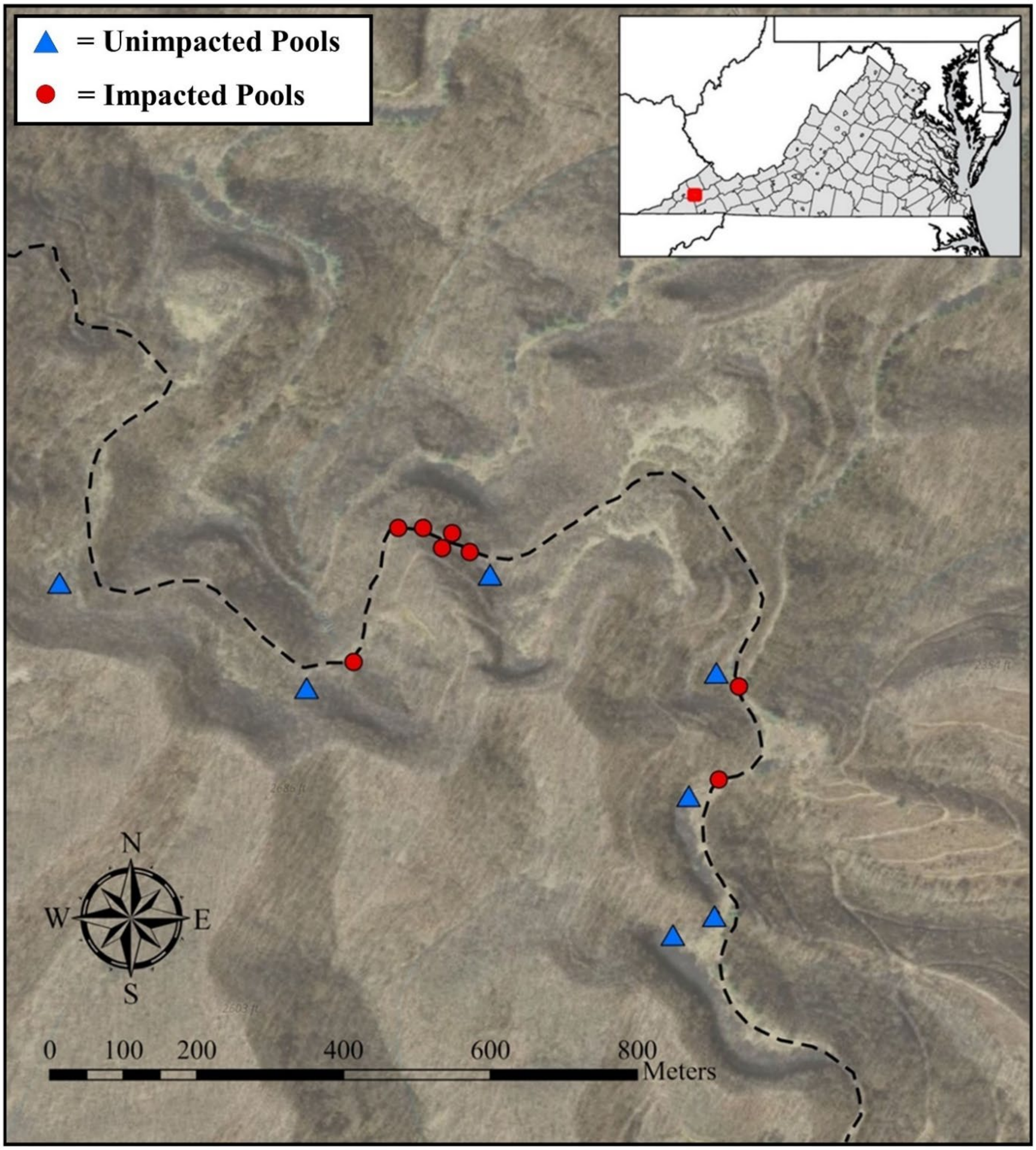


Figure 1: Location of study site and pools impacted (red circles) and unimpacted (blue triangles) by off-road vehicle (ORV) use at an approximately 110 ha former surface mine in Russell County, Virginia opened to ORV use in late 2023. Dashed line represents the location of an official, state-managed ORV trail routed through several pools at the site. Red box in inset denotes location of the study site.

of flattened terrain on mine benches (e.g., Atkinson & Cairns, 1994). Archived aerial imagery with Nationwide Environmental Title Research (http://NETRonline.com) and the Virginia Geographic Information Network's Virginia Base Mapping Program (https://vgin.vdem.virginia.gov/) indicate that these pools and the broader site have remained undisturbed by significant additional anthropogenic alterations for the past 40-50 years.

The majority of land encompassing the Squirrel Hollow surface mine was purchased by The Nature Conservancy (TNC) in 2019 as part of its "Cumberland Forest Project," a 102,385 ha area managed as a timber and carbon offset investment fund across southwest Virginia and adjacent portions of the Kentucky and Tennessee coalfields (Schwartzman, 2022). Portions of the aforementioned property encompassing the Squirrel Hollow surface mine were opened as public access land in April 2023 through an agreement with the Virginia Department of Wildlife Resources' Public Opportunities for Wildlife-Related Recreation (POWRR) program. The property was then opened to ORVs in October 2023 along state-designated trails constructed through a collaborative effort between the Commonwealth of Virginia's Spearhead Trails initiative, TNC, Appalachian Voices, the Virginia Department of Energy, and local government partners. Official ORV trails on the property borrowed abandoned haul roads and reforested mine benches, with trails routed directly through several (n = 8) preexisting pools as recreational water features. Several other (n = 7) larger pools on the property, particularly those associated with abandoned highwalls, were not incorporated into designated ORV routes, with several signed as prohibited to in-pool ORV use.

Materials and Methods

I performed surveys for amphibian egg masses during the first spring breeding season following the Squirrel Hollow surface mine's opening as a public ORV recreation area. I specifically surveyed pools during the first week after the season's main Spotted Salamander (*Ambystoma maculatum*) migration and oviposition were observed in the surrounding area, with initial egg mass surveys taking place on 13 March 2024. Egg mass surveys consisted of visual inspections of each pool, performed by walking the perimeter of each pool and counting the number of egg masses visible in the pool. Although *A. maculatum* egg masses were the primary focus of this study, I also counted egg masses from *L. sylvaticus* and members of *Pseudacris* (locally *P. crucifer* and *P. brachyphona*), which were also actively breeding during this same time period. I photographed and obtained a photo-skinned, ground-based light detection and ranging (LiDAR) scan of each pool using Scaniverse (Askar & Sternberg, 2023; Figure 2) to document the location of egg masses within each pool and aid in relocating each egg mass during follow-up surveys.

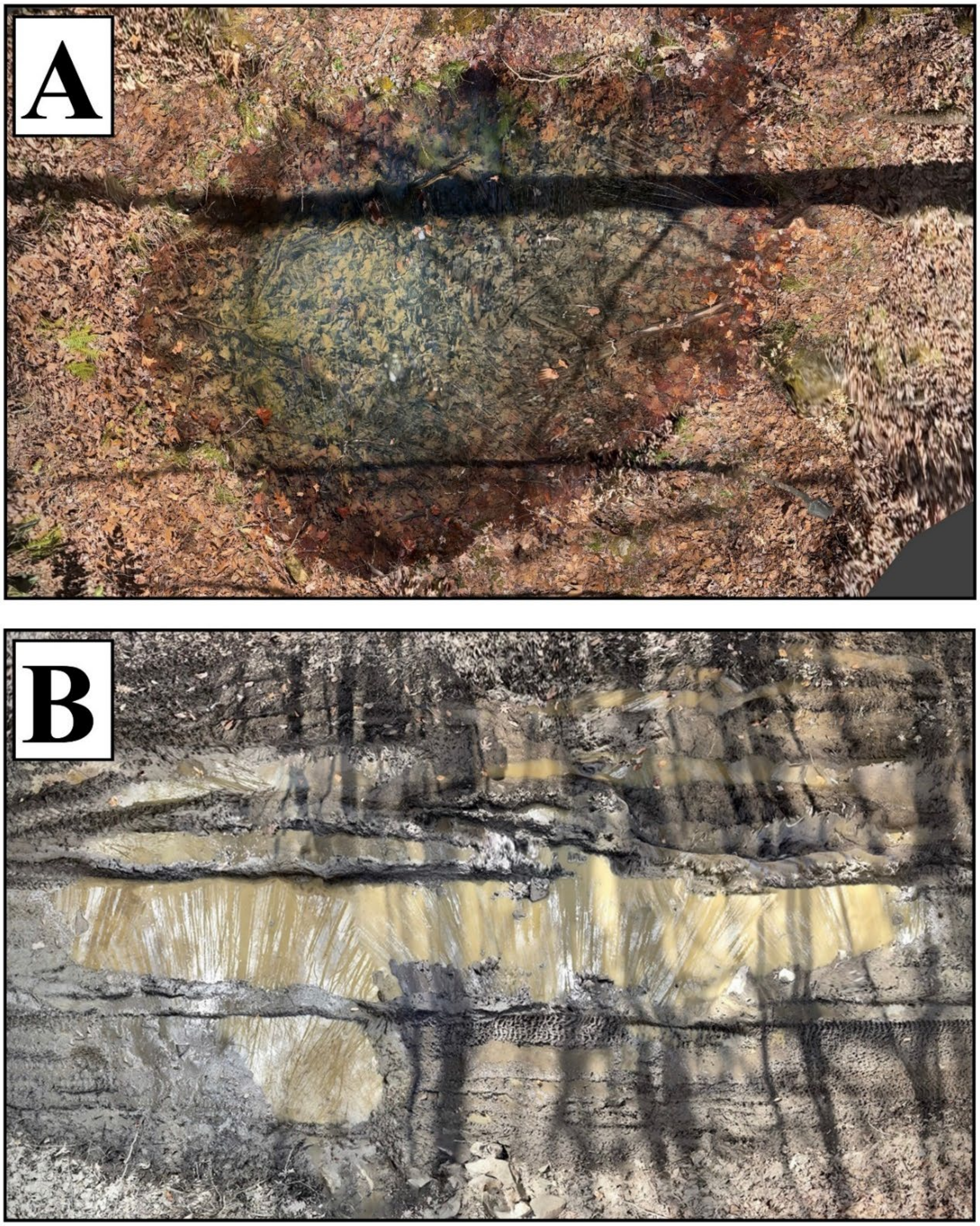


Figure 2: Representative visual examples of pools unimpacted (A) and impacted (B) by recreational off-road vehicle use. Overhead photos were assembled as photo-skinned, ground-based light detection and ranging (LiDAR) scans using Scaniverse (see Askar & Sternberg, 2023).

I returned for follow-up egg mass surveys on 21 March 2024, which allowed for one week of trail use and a warm-weather weekend (peak conditions for trail visitation) to occur between survey visits. I repeated the aforementioned methodology for follow-up surveys, visiting the same pools and again counting the number of visible egg masses within each pool. I took note during these surveys of any evidence of egg mass loss (crushed eggs, eggs thrown from the pool). Lastly, I measured several physicochemical variables for each pool, including pool area (m^2), maximum depth (cm), water temperature (degrees Celsius), specific conductance (µS/cm), and pH. I measured pool area by walking the perimeter of each pool with a Garmin GPSMap 62s GPS unit and measured water quality variables using a ThermoScientific Orion 5-Star multimeter. I also returned for two follow-up site visits in early April to confirm a lack of development in egg masses recorded as lost in initial post-disturbance surveys.

I sought to examine differences in physicochemical attributes between impacted and unimpacted pools, as well as to compare egg loss rates before and after vehicle disturbance at impacted and unimpacted pools. I used Mann-Whitney U-Tests to examine differences in each physicochemical variable between pool types. A nonparametric analysis was chosen for these comparisons due to heteroscedasticity that could not be corrected using data transformations. I also used Chi-Square Goodness-of-Fit Tests to compare observed egg mass frequencies in impacted and unimpacted pools during follow-up surveys against theoretical expectations of no egg mass loss, using data originally obtained during pre-disturbance survey visits as expected frequencies. All statistical tests were performed at a significance level of $\alpha = 0.05$.

Results

I inventoried 49 total egg masses during pre-disturbance pool surveys (Table 1), 30 of which were found in pools open to ORV use (impacted pools; Figure 3A) and 19 of which were found in pools closed to ORV use or not colocated with official vehicle trails (unimpacted pools). Half of impacted and 57% of unimpacted pools showed evidence of amphibian breeding, with at least one egg mass deposited within the pool. With the exception of one small raft of *L. sylvaticus* eggs and two *P. brachyphona* egg masses, all egg masses encountered during surveys were from *A. maculatum*.

Impacted pools showed distinct differences in physicochemical attributes and egg mass loss rates during post-disturbance visits relative to unimpacted pools (Table 2), in addition to exhibiting substantially higher amounts of physical disturbance and wheel-rutting from ORV use (Figure 2). Impacted pools exhibited significantly higher conductivity ($U = 9$, $n = 15$, $p = 0.032$) and pH ($U = 1.5$, $n = 15$, $p = 0.003$) compared to unimpacted pools, while pool area ($U = 12.5$, $n = 15$, $p = 0.082$), maximum depth ($U = 32$, $n = 15$, $p = 0.960$), and water temperature ($U = 21$, $n = 15$, $p = 0.453$) did not differ significantly between pool types.

Observed egg mass counts at post-disturbance surveys did not differ from expected egg mass frequencies within unimpacted pools ($\chi^2 = 0.00$, df = 3, $p = 1.00$), with no observations of egg mass loss between pre- and post-disturbance visits. However, observed egg mass counts at post-disturbance surveys did significantly differ from expected egg mass frequencies at impacted pools ($\chi^2 = 16.78$, df = 3, $p < 0.001$), with a mean 83% egg mass loss rate per pool. All impacted pools with egg masses present during pre-disturbance visits showed evidence of egg mass loss in follow-up surveys, with two pools experiencing a total loss of all egg masses observed during pre-disturbance visits.

Forty-seven percent of egg masses originally inventoried at impacted pools could not be located during post-disturbance visits; it is assumed that these eggs were either crushed or buried under wetland soils during disturbance. I also did not detect evidence of new egg masses deposited at any pools after my initial survey during this visit. Remaining egg masses counted as lost in

Table 1: Amphibian egg mass counts for pre-disturbance ("Eggs Before") and post-disturbance ("Eggs After") visits at pools impacted (n = 8) and unimpacted (n = 7) by off-road vehicle use at a former surface mine in Russell County, Virginia in 2024. "Loss Rate" refers to the percent of eggs initially deposited in pools but later lost to physical crushing, relocation out of the pool, or other mechanisms.

Pool	**Type**	**Eggs Before**	**Eggs After**	**Loss Rate (Percent)**
1	Impacted	0	0	n.a
2	Impacted	0	0	n.a
3	Impacted	11	4	63.7
4	Impacted	2	0	100
5	Impacted	0	0	n.a
6	Impacted	12	4	66.6
7	Impacted	5	0	100
8	Impacted	0	0	n.a
9	Unimpacted	5	5	0
10	Unimpacted	3	3	0
11	Unimpacted	0	0	n.a
12	Unimpacted	5	5	0
13	Unimpacted	0	0	n.a
14	Unimpacted	0	0	n.a
15	Unimpacted	6	6	0

Table 2: Physicochemical parameters of pools impacted (n = 8) and unimpacted (n = 7) by off-road vehicle use at a former surface mine in Russell County, Virginia in 2024. Values are represented as means ± 1 SD. Asterisks denote variables with significant differences (Mann-Whitney U-Test; $\alpha = 0.05$) between impacted and unimpacted pools.

Pool Type	**Pool Area (m^2)**	**Maximum Depth (cm)**	**Temperature (^oC)**	**Conductivity (µS/cm)***	**pH***
Impacted	33.1 ± 18.1	9.4 ± 6.0	12.0 ± 3.6	266.8 ± 135.0	6.63 ± 0.58
Unimpacted	216.0 ± 278.1	14.0 ± 10.4	13.6 ± 3.0	131.8 ± 67.3	5.21 ± 0.39

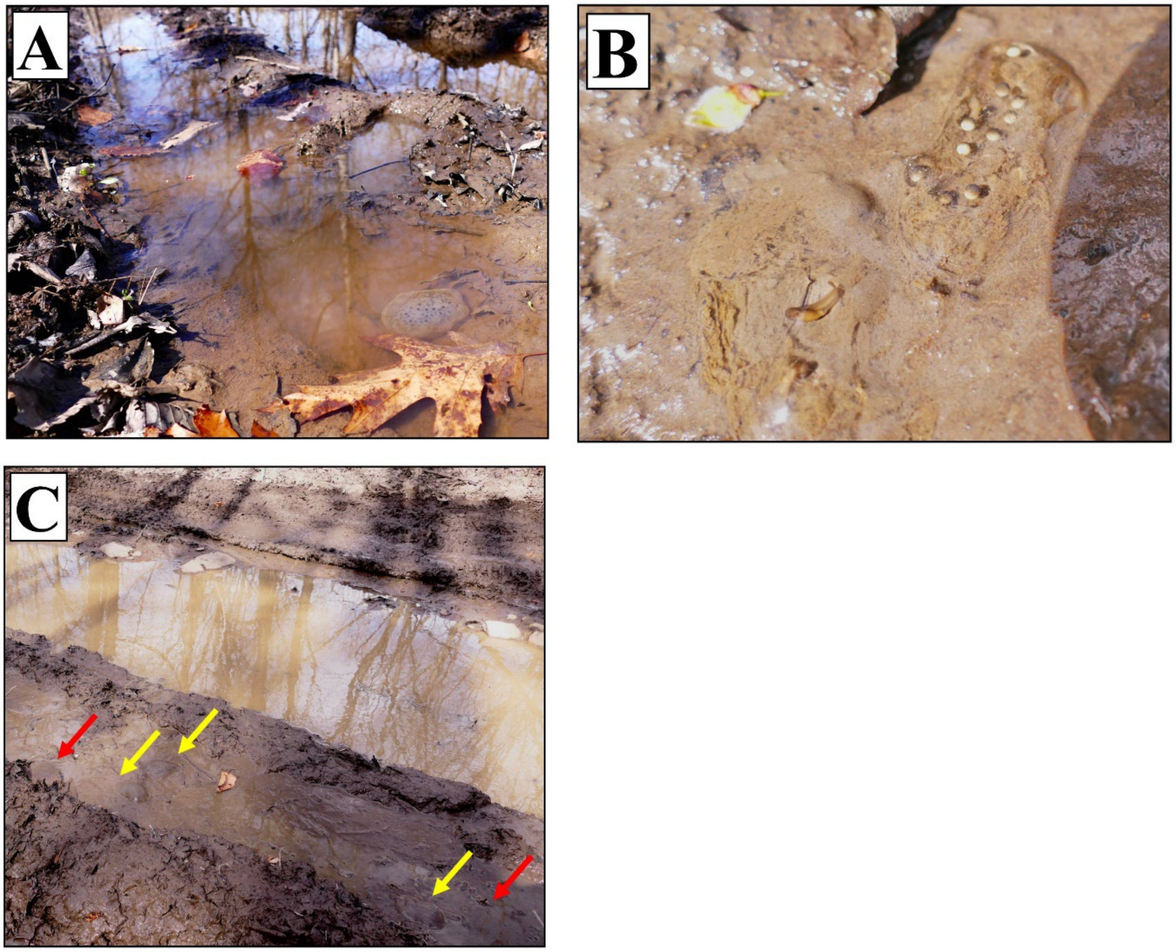


Figure 3: Examples of original egg mass position and mechanisms of egg mortality observed following disturbance at pools opened to off-road vehicle use on a former surface mine in Russell County, Virginia in 2024: (A) a Spotted Salamander (*Ambystoma maculatum*) egg mass deposited in a mine bench pool; (B) Mountain Chorus Frog (*Pseudacris brachyphona*) egg masses and associated embryos thrown 1-2 m from their original site of deposition into terrestrial habitat adjacent to the pool; (C) a group of five *A. maculatum* egg masses (highlighted by arrows) originally deposited near the center of a pool relocated into suboptimal habitat at the pool margin following wetland rutting and redistribution of wetland soils (red arrows indicate relocated egg masses also showing evidence of physical crushing).

post-disturbance visits showed distinct mechanisms of embryonic mortality. Seven *A. maculatum* egg masses and the one raft of *L. sylvaticus* eggs encountered during pre-disturbance surveys were found at or immediately adjacent to their original locations but had been crushed, with egg mass integrity lost and embryos crushed or absent. Three egg masses, including *one A. maculatum* and two *P. brachyphona* egg masses, had been thrown from pools and were left desiccated on dry land 1-2 m from the pool (Figure 3B). Each of these egg masses was found in wetland soils that had also been thrown from the pool immediately behind visible tire tracks, indicating wheel-spin from passing vehicles as the cause of egg mass movement. The remaining six *A.*

maculatum egg masses had been relocated from the center of the pool to suboptimal locations within the pool (e.g., locations in <1 cm of water on the pool margin) when vehicle disturbance redistributed wetland soils via wheel-rutting (Figure 2B), changing pool microtopography in areas of original egg mass deposition. Two of these egg masses also showed evidence of physical crushing, including a loss of egg mass integrity (Figure 3C).

Discussion

Pool-breeding amphibians require intact breeding habitat throughout oviposition, hatching, and larval development periods for individuals to complete their life cycles. Predation and premature pool drying represent natural threats to larval survival and growth during these periods (Figiel & Semlitsch, 1990; Rowe & Dunson, 1995), although the physical disturbance of pools and adjacent terrestrial habitat from anthropogenic activities may also place resident amphibian populations at risk (Semlitsch & Skelly, 2008). I found evidence of significant egg mass loss in ephemeral pools exposed to new anthropogenic land uses, specifically recreational ORV use, on former extractive lands managed as a present-day conservation area in the southwest Virginia coalfields. These results confirm that the conversion of pools into "play areas" for motorized recreational users serve as a source of amphibian mortality, even on properties managed for conservation objectives, and provide documentation of multiple modes of egg mass loss resulting from pool disturbance.

The loss of amphibian eggs due to physical pool disturbance would be expected, since anthropogenic disturbance in wetlands not only impacts water quality but also redistributes and compacts wetland soils (Olson & Doherty, 2012; Herlihy et al., 2019). Recreational use from motorized vehicles within wetlands can be an additional source of these impacts. For example, wetlands in Tazewell County, Virginia opened to ORV use showed a significant decline in wetland condition over a six-year period, with indicators of emergent vegetation loss and soil rutting increasing relative to nearby unimpacted controls (Smith, 2021). Amphibian eggs deposited in similar habitats may also be susceptible to direct or indirect impacts from the same forms of disturbance, as was confirmed in this study.

Physical crushing from passing vehicles was common in this study and is intuitive as a source of egg mass loss. However, I also documented several unexpected mechanisms of egg mass loss, with some pools showing evidence of multiple mechanisms acting in concert. Specifically, wheel-spin from passing vehicles caused several egg masses to be thrown considerable distances beyond the pool along with wetland soils, where eggs subsequently desiccated in exposed terrestrial settings far from water. This was observed for both *A. maculatum* and *P. brachyphona* egg masses, including one egg mass of the latter species containing embryos that had nearly completed pre-hatching developmental stages before being thrown from the pool (Figure 3B). Members of *Pseudacris*, particularly *P. brachyphona*, are especially abundant across the study area in roadside ditches, flooded haul roads, and other pools associated with areas of frequent vehicle use (Smith & Hamed, 2020). The mechanisms of egg mass loss for *P. brachyphona* described in this study may therefore extend beyond recreational ORV use to other pools impacted by frequent vehicle passes. The ongoing expansion of state-managed ORV trail networks on former extractive lands throughout southwest

Virginia may also represent an emerging threat to this Tier II (Very High Conservation Need) species, even on properties otherwise managed for conservation objectives.

I also observed more complex modes of egg mass loss resulting from the redistribution of wetland soils and alteration of wetland microtopography by passing vehicles. Specifically, several *A. maculatum* egg masses were moved from their original positions within deeper, central portions of one pool to the pool margin following one week of repeated vehicle disturbance. While several of these egg masses were also crushed and lost integrity, several others remained intact. However, the long-term survival of embryos contained in these egg masses to hatching was unlikely due to each egg mass being moved to exposed and elevated locations in less than 1 cm of water.

Each of the aforementioned egg masses found relocated following initial site visits was also found coated in sediment during post-disturbance surveys, and pools impacted by ORV use in this study exhibited a more than twofold increase in conductivity compared to nearby pools not experiencing impacts from ORV disturbance. Past work has found evidence of decreased amphibian survival in response to higher levels of pool conductivity and suspended sediment (Brand et al., 2010; Eakin et al., 2019), albeit from different sources than the in-pool ORV use noted in this study. While these impacts may not result in full embryonic mortality in every case, follow-up visits to pools included in this study performed several weeks after post-disturbance surveys failed to detect signs of continued embryonic development in any of the aforementioned sediment-coated egg masses, which also became desiccated as pools underwent slight drying. It is therefore probable that high levels of suspended sediment generated by repeated in-pool ORV use form an additional risk factor and potential source of embryonic and larval mortality.

One limitation of this study was that it was constrained to examining oviposition and the immediate fate of egg masses prior to hatching. Egg mass failure and embryonic mortality are common in pool-breeding amphibians and can be associated with predation, pathogens, and premature pool drying, meaning that egg mass counts often do not equate to actual amphibian recruitment (Stenhouse 1987). Artificially-created pools, in particular, can become “ecological traps” for amphibians when they are abnormally small and shallow (Clevenot et al., 2018), leading to pools drying prior to metamorphosis and an associated loss of reproductive output at the population scale (DiMauro & Hunter, 2002).

While I did not quantify larval survivorship and recruitment as part of this study, archived aerial images of the study area show evidence of pool persistence across various seasons and decades (1980s-present), indicating longer hydroperiods that may be conducive to larval survivorship. In addition, the number of *A. maculatum* egg masses observed in several pools indicates the presence of an established breeding population of this species, which shows strong breeding site philopatry (Whitford & Vinegar, 1966), at pools that remained largely undisturbed for nearly half a century prior to the conversion of the property into a public ORV recreation area. It is impossible to infer recruitment rates from these data, although my results do confirm that anthropogenic disturbance can at minimum form an additional and substantial mortality source for reproducing amphibians in pools well prior to any potential pool drying. The total loss of all egg masses at several impacted pools examined in this study particularly underscores how demographically impactful egg mass and embryo loss from in-pool vehicle use can be at the local scale.

Several management recommendations emerge from my results. Simple pool avoidance during the development of ORV recreation areas is perhaps the most effective first-line best management practice for minimizing impacts on breeding amphibian populations, since a lack of vehicle disturbance would exclude each of the mechanisms of egg mass loss observed in this study. However, it is critical that land managers go beyond cursory assessments of pool structure and also consider pool function when selecting sites for ORV exclusion. Pool avoidance was employed for ORV trails at only the largest pools at the study site, with smaller and shallower pools associated with mine benches left open to permitted ORV use. Despite being smaller in size, these pools were the most heavily-used breeding sites by amphibians during this study, with relatively fewer egg masses deposited in larger pools closed to ORV use (30 versus 19 egg masses, respectively). This trail design strategy therefore resulted in minimal apparent benefit to pool-breeding amphibians at the study site. Routing ORV trails around even small and shallow pools is likely a more effective practice for conserving the ecological function of pools for breeding amphibians in areas open to recreational vehicle use.

Seasonal trail closures (e.g., Turton, 2005) may also be an option for minimizing egg mass loss in resident breeding amphibian populations. However, many pool-breeding amphibian species have larvae that must remain in pools for substantial time periods prior to undergoing metamorphosis (Phillips, 1992). While I did not directly assess larval survivorship as a result of in-pool vehicle use, negative impacts to resident larvae may also be possible through similar mechanisms as observed for egg mass loss in this study. Seasonal pool closures limited to periods during and immediately after breeding migrations and initial oviposition may therefore be insufficient to minimize impacts to pool-breeding amphibian populations.

Recreational managers may also consider constructing new depressions that can fill with water and become “play areas” for ORV users as an alternative to converting existing pools for this purpose. This approach can minimize impacts to existing habitats that may have been colonized as breeding sites by amphibians, although managers should be cognizant of the potential for newly-created pools to become ecological traps for amphibian populations (Clevenot et al., 2018), since these pools would likely be susceptible to the same impacts observed in this study if amphibians subsequently attempted to colonize them as breeding sites. Constructing artificial play areas that have short hydroperiods, are located well outside of forested contexts, and are located far from existing pools or wetland areas may represent approaches that minimize the likelihood of future colonization by breeding amphibians, although it may be impossible to fully exclude impacts on breeding amphibians from such constructed wetlands.

Overall, my results underscore the need to reconsider the value of small, artificially-created wetlands for Virginia’s pool-breeding amphibians, especially in landscapes with a history of intense anthropogenic disturbance. The Virginia coalfields, in particular, are currently receiving heavy focus in terms of the reuse of former minelands as “brownfields” suitable for not only recreational uses like those investigated in this study but also for renewable energy development and other industrial projects (Zipper et al., 2021). While such uses may be preferable on former extractive lands when compared to converting existing, intact forests for anthropogenic uses, it is clear that disturbed brownfields are not immune from impacts to resident wildlife as a result of future land uses and that generalized assumptions about the ecological value of extractive landscapes should not be used to guide management decisions. Improved empirical data are urgently needed to better understand how Virginia's amphibians are using these habitats and how the reuse of brownfields can be better grounded in evidence-based strategies to mitigate impacts.

Acknowledgments
I am grateful to Jeff Marion (United States Geological Survey) for helpful theoretical discussions about the ecological impacts of ORV trail routing that formed the focus of this study. Amphibian surveys were performed under Scientific Collecting Permit Number 2907603 from the Virginia Department of Wildlife Resources. Trail access was performed under Southwest Regional Recreation Authority User Permit Number 5140.

Literature Cited

Adam, M. D., & Lacki, M. J. (1991). Factors affecting amphibian use of road-rut ponds in Daniel Boone National Forest. *Transactions of the Kentucky Academy of Sciences*, *54*, 13-16.

Askar, C., & Sternberg, H. (2023). Use of smartphone lidar technology for low-cost 3D building documentation with iPhone 13 Pro: A comparative analysis of mobile scanning applications. *Geomatics*, *3*(4), 563-579.

Atkinson R. B., & Cairns, J. (1994). Possible use of wetlands in ecological restoration of surface mined lands. *Journal of Aquatic Ecosystem Health*, *3*, 139–144.

Baldwin, R. F., & deMaynadier, P. G. (2009). Assessing threats to pool-breeding amphibian habitat in an urbanizing landscape. *Biological Conservation*, *142*(8), 1628-1638.

Baldwin, R. F., Calhoun, A. J. K., & and deMaynadier, P. G. (2006). The significance of hydroperiod and stand maturity for pool-breeding amphibians in forested landscapes. *Canadian Journal of Zoology*, *84*(11), 1604-1615.

Barry, D. S., Pauley, T. K., & and Maerz, J. C. (2008). Amphibian use of man-made pools on clear-cuts in the Allegheny Mountains of West Virginia, USA. *Applied Herpetology*, *5*, 121-128.

Brand, A. B., & Snodgrass, J. W. (2010). Value of artificial habitats for amphibian reproduction in altered landscapes. *Conservation Biology*, *24*(1), 295-301.

Brand, A. B., Snodgrass, J. W., Gallagher, M. T., Casey, R. E., & Meter, R. V. (2010). Lethal and sublethal effects of embryonic and larval exposure of *Hyla versicolor* to stormwater pond sediments. *Archives of Environmental Contamination and Toxicology*, *58*, 325-331.

Buhlmann, K. A., & Mitchell, J. C. (2000). Age of Eastern Tiger Salamanders (*Ambystoma tigrinum tigrinum*) in a Virginia sinkhole pond complex: implications for conservation. *Journal of the Elisha Mitchell Scientific Society*, *116*(3), 239-244.

Burne, M. R., & Griffin, C. R. (2005). Habitat associations of pool-breeding amphibians in eastern Massachusetts, USA. *Wetlands Ecology and Management*, *13*, 247-259.

Clevenot, L., Carre, C., & Pech, P. (2018). A review of the factors that determine whether stormwater ponds are ecological traps and/or high-quality breeding sites for amphibians. *Frontiers in Ecology and Evolution*, *6*, 40.

DiMauro, D., & Hunter, M. L. (2002). Reproduction of amphibians in natural and anthropogenic temporary pools in managed forests. *Forest Science*, *48*(2), 397-406.

Eakin, C. J., Calhoun, A. J. K., & Hunter, M. L. (2019). Effects of suburbanizing landscapes on reproductive effort of vernal pool-breeding amphibians. *Herpetological Conservation and Biology*, *14*(2), 515-532.

Ethier, J. P., Fayard, A., Soroye, P., Choi, D., Mazerolle, M. J., & Trudeau, V. L. (2021). Life history traits and reproductive ecology of North American chorus frogs of the genus *Pseudacris* (Hylidae). *Frontiers in Zoology*, *18*, 40.

Figiel, C. R., & Semlitsch, R. D. (1990). Population variation in survival and metamorphosis of larval salamanders (*Ambystoma maculatum*) in the presence and absence of fish predation. *Copeia*, *1990*(3), 818-826.

Gibson, J. D., & Sattler, P. W. (2020). Natural history notes on the anurans of White Oak Mountain Wildlife Management Area. *Catesbeiana*, *40*(1), 29-48.

Goodman, D. C., & Smith, W. H. (2023). A landscape-scale comparison of wetlands associated with surface coal extraction and naturally-occurring wetlands in the central Appalachian Mountains, USA. *Wetlands*, *43*, 101.

Herlihy, A. T., Sifneos, J. C., Lomnicky, G. A., Nahlik, A. M., Kentula, M. E., Magee, T. K., Weber, M. H., & Trebitz, A. S. (2019). The response of wetland quality indicators to human disturbance indicators across the United States. *Environmental Monitoring and Assessment*, *19*, 1-21.

Institute for Service Research. (2017). *Economic and Fiscal Impacts*. Spearhead Trails, Coeburn, Virginia.

Karraker, N. E., & Gibbs, J. P. (2009). Amphibian production in forested landscapes in relation to wetland hydroperiod: A case study of vernal pools and beaver ponds. *Biological Conservation*, *142*(10), 2293-2302.

Meyer, K. G. (2002). *Managing Degraded Off-Highway Vehicle Trails in Wet, Unstable, and Sensitive Environments*. United States Department of Agriculture Forest Service, Technology and Development Program, Missoula, Montana.

Olson, E. R., & Doherty, J. M. (2012). The legacy of pipeline installation on the soil and vegetation of southeast Wisconsin wetlands. *Ecological Engineering*, *39*, 53-62.

Phillips, C. A. (1992). Variation in metamorphosis in Spotted Salamanders *Ambystoma maculatum* from eastern Missouri. *The American Midland Naturalist*, *128*(2), 276-280.

Rannap, R., Kaart, M. M., Kaart, T., Kill, K., Uuemaa, E., Mander, U., & Kasak, K. (2020). Constructed wetlands as potential breeding sites for amphibians in agricultural landscapes: A case study. *Ecological Engineering*, *158*, 106077.

Rothenberger, M. B., Vera, M. K., Germanoski, D., & Ramirez, E. (2019). Comparing amphibian habitat quality and functional success among natural, restored, and created vernal pools. *Restoration Ecology*, *27*(4), 881-891.

Rowe, C. L., & Dunson, W. A. (1995). Impacts of hydroperiod on growth and survival of larval amphibians in temporary ponds of Central Pennsylvania, USA. *Oecologica*, *102*, 397-403.

Schwartzman, G. (2022). Climate rentierism after coal: forests, carbon offsets, and post-coal politics in the Appalachian coalfields. *Journal of Peasant Studies*, *49*, 924–944.

Semlitsch, R. D., & Skelly, D. K. (2008). Ecology and conservation of pool-breeding amphibians. Pp. 127-148 in *Science and Conservation of Vernal Pools in Northeastern North America*. CRC Press, Boca Raton, Florida.

Sharp, R., Bradley, M. J., & Maples, J. (2020). Who has the right-of-way? Negotiating environmental and economic impacts of off-highway vehicle use in eastern Kentucky. *Journal of Appalachian Studies*, *26*(2), 191-208.

Skidds, D. E., Golet, F. C., Paton, P. W. C., & Mitchell, J. C. (2007). Habitat correlates of reproductive effort in Wood Frogs and Spotted Salamanders in an urbanizing watershed. *Journal of Herpetology*, *41*(3), 439-450.

Smith, W. H. (2021). Remote detection of disturbance from motorized vehicle use in Appalachian wetlands. *Virginia Journal of Science*, *72*(3-4), 1-20.

Smith, W. H., & Hamed, M. K. (2020). Trade-offs in moving citizen-based anuran call surveys online during the SARS-CoV-2 pandemic: lessons from rural Appalachia, USA. *Ecology and Evolution*, *10*(22), 12465-12471.

Stenhouse, S. L. (1987). Embryo mortality and recruitment of juveniles of *Ambystoma maculatum* and *Ambystoma opacum* in North Carolina. *Herpetologica*, *43*(4), 496-501.

Turton, S. M. (2005). Managing environmental impacts of recreation and tourism in rainforests of the wet tropics of Queensland World Heritage Area. *Geographical Research*, *43*(2), 140-151.

Whitford W. G., Vinegar, A. (1966). Homing, survivorship, and overwintering of larvae of spotted salamanders, *Ambystoma maculatum*. *Copeia*, *1966*, 515–519.

Virginia Department of Wildlife Resources. (2015). *Virginia's 2015 Wildlife Action Plan*. Richmond, Virginia.

Zipper, C. E., Agouridis, C. T., Barton, C. D., & Skousen, J. (2021). Conversion options for mining-affected lands and waters in Appalachia. Pp. 167-192 in *Appalachia's Coal-Mined Landscapes: Resources and Communities in a New Energy Era*. Springer, New York.

Observations on the Development of the Spotted Salamander (*Ambystoma maculatum*)

Kurt E. Johnson, PhD

Professor of Anatomy and Cell Biology, The George Washington School of Medicine and Health Sciences, 2300 I Street NW, Washington, DC

Abstract

The Spotted Salamander (*Ambystoma maculatum*) is a common salamander throughout Virginia. Its embryos and larvae can withstand both low and high temperatures, but because the time from fertilization to complete metamorphosis is about 5.5 months, depending on latitude and ambient temperatures, they require long-lived vernal ponds for their development. Adults home year after year to their natal ponds by olfactory imprinting. Various stages in their development, through metamorphosis, are documented here. Some clutches of eggs contained mutualistic algae.

Key Words: Spotted Salamander, *Ambystoma maculatum*, Temperature Tolerance, Metamorphosis, Mutualistic Algae

Introduction

Spotted Salamanders (*Ambystoma maculatum*) come to vernal ponds to mate on cool, humid, rainy, evenings in Northern Virginia in late February to early April, soon after the spring thaw. Although rarely seen, due to its usual habit of hiding under decaying logs or the burrows of small mammals, this species is one of the most common salamanders in the eastern United Sates. It ranges from southeastern Canda, New England, the Middle Atlantic states, and the southeastern United States (Powell, et al., 2016). Males deposit pale, gelatinous sacs called spermatophores in long-lived vernal ponds. Then females, after brief courtship with males (Arnold, 1976; Maex, et. al., 2016), collect the spermatophores, mobilize spermatozoa for fertilization, and deposit masses of 40-200 fertilized eggs surrounded by a vitelline membrane, two egg capsules, and a stiff jelly, usually attached to submerged sticks or vegetation. During a subsequent period of development, often extending for many months, the eggs form embryos, and then feeding larvae, which eventually metamorphose into terrestrial subadults. It is essential that adults deposit eggs in long-lived vernal ponds, to accommodate the extended period of development from fertilization to metamorphosis.

Materials and Methods

Fertilized eggs were collected in Lorton, Virginia from a short-lived vernal pond; and, in Manassas, Virginia, from temporary drainage ditches. In both locations, the ponds had once been long-lived, allowing development through metamorphosis, but now, due to human intervention, dried up long before metamorphosis in late summer. These rescued embryos were studied and then introduced into a man-made pond in Alexandria, Virginia.

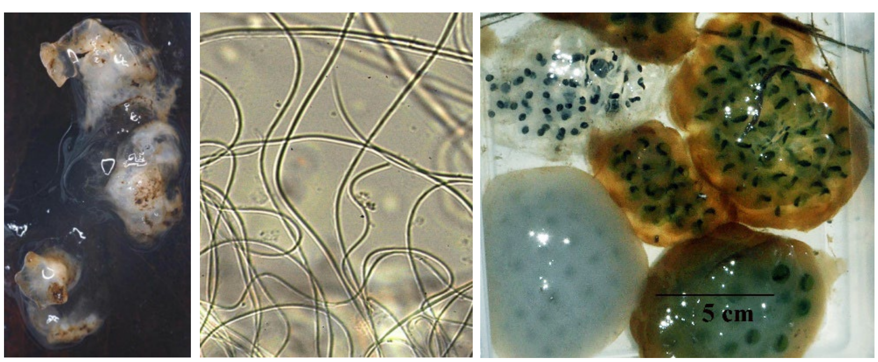
Development was observed using a Wild dissecting microscope, and photography was with a digital Nikon D90 camera. Images were processed using Adobe Photoshop, attempting to make the captured images as true to life as possible. Embryos, with their vitelline membranes intact, were carefully removed from their gelatinous masses with forceps. Groups of six were placed in Falcon plastic petri dishes (100X15 mm, #351029), half full of purified water for observation of further development. Some petri dishes were incubated at 18-20º C. (warm, room temperature) and others were kept at 8-10º C. (cold, in refrigerator). Developmental stages were according to Harrison (1969). Some embryos contain green algae between the vitelline membrane and the embryo itself. These algae were collected in a micropipette, a slide was prepared, and the algae were photographed in a Zeiss light microscope. The larvae were fed brine shrimp and eventually released into my permanent pond to fend for themselves.

Results

An adult salamander was found under a decaying log in Lorton, Virginia, photographed, and returned to the log (Figure 1). This adult was 19 cm long from snout to the tip of the tail. Most of the dorsum was dark gray and covered with bright yellow

Figure 1. Adult Spotted Salamander, 19 cm

spots except that there were a few orange spots on the dorsum of the head. The ventrum was a lighter gray. Males deposit spermatophores randomly in breeding ponds, often firmly attached to submerged vegetation. These 8-mm-tall spermatophores (Figure 2, left) have a milky, gelatinous capsule, surrounding a myriad of 200-µm-long, filamentous spermatozoa with a streamlined head at one end and a long, motile, flagellum extending from the head (Figure 2, middle). Soon, females collect these spermatophores in their cloacae, releasing the spermatozoa to fertilize eggs, and then deposit masses of eggs, often attached to submerged vegetation or branches fallen from trees. Different egg masses may have clear jelly, milky, cloudy jelly; and, either type may or may not have a greenish tint between the vitelline membrane and the embryo itself, due to the presence of mutualistic algae.



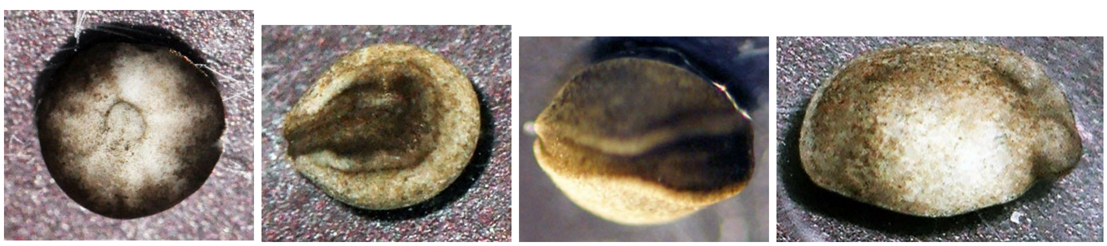
Spermatophores 8 mm **Spermatozoa 200 µm** **Fertilized Eggs 5-15 cm**

Figure 2. Spotted Salamander Sperm and Eggs

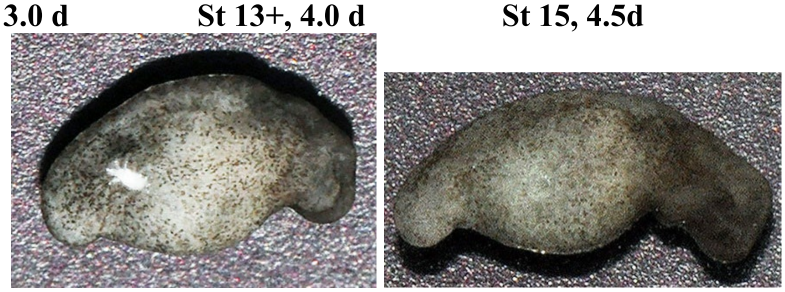
During this study, seventeen egg masses were observed. Eleven had clear jelly with a greenish tint, two had milky jelly with a greenish tint, one had milky jelly with no greenish tint, and three had clear jelly with no greenish tint. For example, on March 3, 2024, a small milky egg mass without green algae was collected in Lorton. It was 6.2 cm X 5.3 cm and contained 49 uncleaved eggs. The estimated time of oviposition was 10 PM, March 2, 2024. These eggs were used for monitoring development at 18-20º and 8-10º C. During March 2024, the average day temperature was 16.2º C. +/- 4.6 s.d. (range 10.0-25.0º C.) and the average night temperature was 5.9º C. +/-3.4 s.d. (range 0.0-13.9º C.)

The normal development of the Spotted Salamander is well documented (Harrison, 1969). The embryos and larvae developed synchronously as expected at essentially the same rate as published by Harrison (1969). Once they reached feeding Stage (St) 45, they were released to my home pond to fend for themselves (Figure 3, Table I).

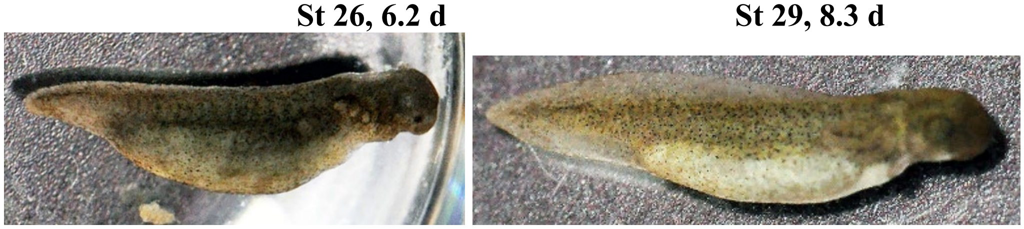
St 1, O d St 2, 0.8d St 4, 0.96 d St 5, 1.2 d St 10, 2.5d



St 11+, 3.0 d St 13+, 4.0 d St 15, 4.5d St 20, 5 d



St 26, 6.2 d St 29, 8.3 d



St 36, 10 d St 38, 12 d

St 40, 14 d

St 44, 23 d

Figure 3. Normal Development of Spotted Salamander, 18-20º C., St 1-29 X 10, St 36-44 X 7.5

Table 1. Development Spotted Salamander (*Ambystoma maculatum)* at two temperatures

	8-10º C.		18-20º C.		
Date	**Age (days)**	**Stage***	**Age (days)**	**Stage***	**Comments****
3/3/24	0.6	1	0.6	1	Uncleaved
3/3/24	0.9	Early 2	0.8	2	Two cells
3/3/24	1	2	1	4+	Six cells
3/4/24	2	4+	1.5	7+	Blastula
3/4/24			1.7	8	Early Blastula
3/4/24			1.9	8+	Late Blastula
3/5/24			2.5	10	Early gastrula
3/5/24			3	11+	Middle gastrula
3/6/24			3.5	12	Late gastrula
3/6/24			4	12+	Yolk plug
3/7/24			4.5	14	Neural plate
3/7/24			5	20	Neural folds partially fused
3/8/24	5.5	7+	5.5	22	Five somites
3/9/24			6.2 d	26	Body lengthens, head prominent
3/11/24			8.3 d	29	Eye forming, tail elongated
3/12/24	10	9	10	36	Rudimentary gills
3/14/24	11.5	9+	11.5	38	Long branched gills
3/14/24	12	10	12	38	Forelimb bud simple
3/16/24	14	11	14	40	Forelimb bud longer

3/17/24	**15**	**12**			
3/20/24			**17.5**	**43**	**Forelimb bud bifurcated**
3/25/24			**23**	**44**	**Gut tube complete**
3/29/24	**27**	**16**	**27**	**45**	**Feeding, released to home pond**

***Stages according to Harrison (1969)**
**** Comments apply only to embryos and larvae raised at 18-20º C.**

In 2017, embryos, feeding larvae and metamorphosing subadults were captured from a previous home pond, photographed, and released (Figure 4). For eggs laid in March, metamorphosis was completed in September.

April 1.0 cm | **July 3.9 cm** | **August 3.8 cm** | **September 4.2 cm**

Figure 4. Development of the Spotted Salamander through Metamorphosis

Most urodeles undergo metamorphosis, albeit much less dramatically than that seen in anurans. Salamander eggs are typically deposited in water, hatch into feeding larvae, then metamorphose into terrestrial, miniature subadults. I have documented this process in photographs for the Spotted Salamander (Figure 3). This common adult salamander, 19-22-cm long, is rarely seen because it spends most of its life hidden under decaying logs or in small mammalian burrows. In early spring, males and females gather in vernal ponds to breed. Females deposit their 2.5 mm eggs in semisolid masses of jelly. Cleavge, blastulation, and gastrulation occur during the first 4 days. The formation of a rudimentary central nervous system, called neurulation, is complete a day later. Somites and innervated muscle tissues form, so that the embryos twitch sluggishly inside their vitelline membranes. After hatching, gills sprout by ten days, quickly branching extensively, with circulating red blood cells propelled by a beating heart. The forelimbs now sprout, grow and branch. The larvae have a functional muscular system by 14 days so they can swim rapidly. Once the gut has matured, they begin to feed. The larvae grow slowly throughout the spring and summer. They have large, extensively branched gills on both sides of their heads and sprout hindlimbs long before metamorphosis. But later in the summer, the brown larvae transform into small, black, unspotted and finally yellow-spotted miniature subadults and crawl onto the land (Figure 4).

Amphibian development is generally temperature sensitive. I collected embryos from one clutch on March 3, 2024 and incubated some in warmer indoors (18-20º C.), leaving the others in the cold refrigerator (8-10º C). The warmer embryos developed more rapidly than the colder embryos. Both embryos were from the same clutch and were fertilized at the same time. After three days of warmer indoor incubation, the embryos were significantly advanced compared with those left at colder temperatures (Figure 3, Table I).

Most embryos incubated in the cold developed normally, albeit more slowly than those incubated at warmer temperatures (Table I). Some embryos were incubated at 8-10º C. and then transferred to 18-20º C., where most of them showed acceleration of normal development (Figure 5).

Figure 5. Normal development stage 50, 10 d cold, 5 d warm (10X)

However, a few cold-incubated embryos showed abnormalities of gastrulation (Figure 6), where migration of the chorda-

Figure 6. Abnormal gastrulation, 15 d cold (10X).

mesoderm was defective, ultimately resulting in abnormal neurulation (Figure 7) with developmental

Figure 7. Abnormal neurulation, 20 d cold, 1 d warm (10X)

defects resulting in aberrant head structures (Figure 8).

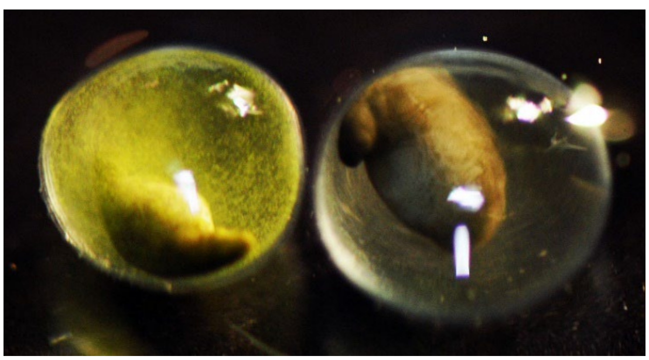
20d cold, 5 d warm

Figure 8. Two Normal Stage 31, One Abnormal development. (10X)

Embryos incubated at 8-10º C (cold). had apparently normal development for two-three weeks, albeit more slowly than companion embryos raised at 18-20º C (warm). A few embryos were incubated in cold for 10 days or 20 days and then transferred for warm incubation. Their sub-sequent development was apparently normal (only St 30, Figure 7), although lagging substantially behind siblings (St 41) raised continuously in the warmth. On day 15, a few continuous cold embryos showed an abnormality of development. On the ventral surface, they had formed a circular blastopore but had not completed gastrulation. There was no rotation of the embryo so that the yolk plug was now visible from the dorsal aspect. In addition, they had a conspicuous round bulge on the dorsal side, consisting of thinned out and lightly pigmented dorsal presumptive ectoderm (Figure 5).

Once cold treated embryos reached 17.5 d of development, the animal pole hemispheric blister was reduced and because rotation had occurred, it seems that gastrulation was complete. Even after 20 days of cold incubation, followed by 5 days in the warm, normal development proceeded apace except for the rare abnormal embryo with reduced and distorted head structures. (Figure 8).

In many egg masses, the space between the embryo and the vitelline membrane was filled with green algae (*Oophila ambystomatis*) (Figure 9, left). Other egg masses, laid at the same time, didn't acquire these algae (Figure 9, middle). The greener specimen on the left had a plethora of tiny algae within the vitelline membrane, inside the space surrounding embryos and had reached Stage 28 (more advanced). The specimen on the right lacks these algae and is at Stage 26 (less



Embryos with (left) and without (right) algae, **Alga, X 200**

Figure 9. Spotted Salamander Embryos and Algae

advanced). Experimental studies have shown that embryos with these algae grow larger and faster than those without. In the light microscope, the algae are streamlined, ovoid, with a fatter and a narrower end (Figure 9, right).

These algae swim with the narrower end forward and are about 15-25 µm long. They swim rapidly using flagella on their outer surface. Their peripheral cytoplasm is full of green chloroplasts, the organelles that they use for photosynthesis. I am not sure what the darker structures (perhaps stored starch) are in Figure 9, right. Surprisingly, when I prepared a slide to look at these algae in a light microscope, I also saw one 1-mm-long, nearly transparent, round worm squirming about. Its gut was filled with numerous dark granules resembling those in the algae. Perhaps the worm was eating the algae.

Discussion

The collecting sites in Lorton and Manassas, Virginia had once been long-lived; but, due to human drainage intervention, now dry completely in May or June. Nevertheless, Spotted Salamanders faithfully deposited their eggs in these doomed locations, where they had matured and became imprinted (Stenhouse, 1985). I rescued few of these eggs and embryos for this study, and placed the rest in my home pond, aiming to establish a breeding population in Alexandria, Virginia. My lot is wooded, has appropriate refugia under decaying logs and small mammal burrows, and now contains a small population of Spotted Salamanders. So far, I have not observed breeding adults in my home pond. Perhaps imprinted adults will eventually come home to and breed in my pond in a few years.

Spotted Salamanders in northern Virginia typically breed from late February to mid-April, depending on temperature and rainfall. Since their metamorphosis isn’t completed until early September; and, since larvae are obligated to develop in water, they must choose semipermanent bodies of water for breeding. By some mechanism, perhaps the odor of the pond where they matured and became imprinted, adult salamanders return year after year to the same ponds (Twitty, 1966; Stenhouse, 1985; McGregor and Taska, 1989, Madison, 1997). In the late 1960s, when I was a graduate student at Yale University, there were two, small, apparently similar ponds in the woods at Chatfield Hollow State Park in Killingworth, Connecticut. In March, one pond had many clutches of Spotted Salamander eggs while a seemingly similar, nearby pond had none. By late summer, the first pond (perhaps spring fed) had many mature larvae, ready for metamorphosis. In contrast, the second pond was dry (Johnson, personal observations). Perhaps breeding adults sensed subtle differences in the water quality in the two ponds, homing on the first but not the second.

The rate of development of Spotted Salamanders at 18-20º C. was nearly identical to the that published by Harrison (1969). Embryos from New Hampshire, Connecticut, Virginia, and North Carolina all develop at this predictable rate (Johnson, personal observations). Embryologists invariably establish a standard table of developmental stages, so that other scientists can know the stage of development of any embryo used for different experiments. At Yale, Harrison and his colleagues published a normal table of stages of development for *Ambystoma maculatum*. This table has black-and-white photographs of an exquisite collection of hand-painted images of each stage (Harrison, 1969). This group of scientists formed a center for studies of Spotted Salamander development. They did early lineage tracing experiments perfecting techniques of labeling embryonic structures with blue dye, performing reciprocal transplants between labeled and unlabeled embryos, allowing subsequent development; and, tracing the subsequent development of labeled grafts in graft recipients (Figure 10).

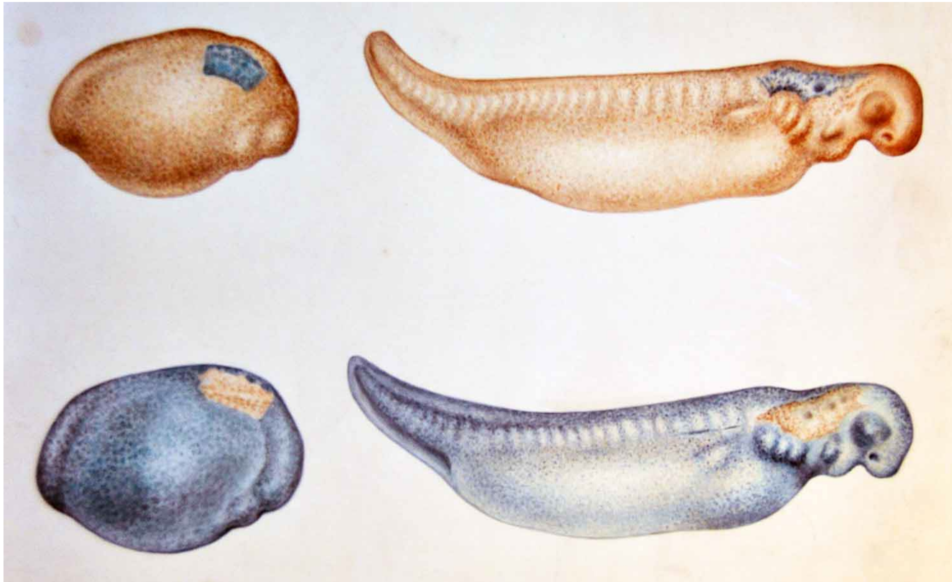


Figure 10. Transplantation of Embryonic Tissues Between Labeled (Blue) and Unlabeled (Tan) Embryos. Hand Painted Drawing from Harrison's Laboratory at Yale University, c1920

Spotted Salamander embryos are impressively cold tolerant. It rained on March 2, 2024 (day high 13.0º, night low 5.6º). One clutch of eggs, laid on March 2 around 10 PM, was used to monitor development at warm and cold temperatures (Table I). The March 2024 monthly average was 16.2º C. +/- 4.6 s.d. (range 10.0-25º C.) high; 5.9º C. +/- 3.4 s.d. (range 0-13.9º C.) low. Most embryos raised at 8-10º C. developed normally, although much slower than those incubated at 18-20º C. In a few cold-treated embryos, gastrulation was disrupted, resulting in abnormal development. During gastrulation, migrating superficial cells move inside the embryo, crawl across the roof of the blastocoel, and induce formation of the central nervous system in the overlying surface ectoderm. Apparently, this inductive interaction is disrupted in a few cold-treated embryos, leading to the formation of abnormal cephalic structures.

The cold tolerance of Spotted Salamander embryos means that it is possible to adjust incubation temperatures within certain limits to obtain embryos of some desired stage for experimental studies. Even after extended cold treatment, most embryos develop normally. The embryos are quite large, so they are amenable to various microsurgical manipulations. Furthermore, by obtaining embryos along the north-south axis of its range, it is possible to have this excellent embryonic material available for experimental studies from February through May of any given year.

The photosynthetic algae provide oxygen needed for embryonic function and the embryos perhaps provide carbon dioxide, nitrogenous wastes, and protection for the algae—another example of mutualistic cooperation between different species. Experimental studies have shown that embryos with algae develop more rapidly than those without (Hutchison and Hammer, 1958; Tattersall and Spiegelaar, 2008). My observations confirm these earlier results.

The round worm, a rare player here, probably lives off the algae. But could it grow enough to attack the salamander embryo, like a much larger lamprey attacking fish? I don't know, but I would not be surprised to find, with further, more detailed investigations, that the salamander's environment harbors not only beneficial algae, but perhaps a predator of the algae and the embryo itself. Whenever you look closely at any system in Nature, it turns out to be much more complicated than first expected.

Many amphibian species undergo a complex transformation called metamorphosis. In the spring and summer, salamander eggs develop from embryos to larvae, sprout external gills and four legs, all the while feeding on small invertebrates and growing larger and stronger. Later in the summer, they crawl out of the natal pond, and transform into terrestrial subadults, losing their external gills while they develop lungs to become air-breathers. Salamanders keep their larval tail and typically have four legs approximately equal in size. In contrast, anuran larvae usually resorb the tail into their bodies at metamorphosis and form much larger hindlimbs when compared with the forelimbs. These enlarged, webbed hindlimbs of anurans enable substantial jumping and powerful swimming to avoid predators.

Aquatic amphibian larvae are subject to intense predation (Stenhouse, et al., 1983), especially from fish and the nymphs of dragonflies. There were six species of dragonflies mating near and depositing eggs in my current pond but no fish. To increase survival of larvae, I raised many in captivity through feeding stages before releasing them into my pond.

In the early 1990s, there was a large, woodland pond just inside the Washington Beltway in Fairfax County Virginia, near where US Route 50 intersects the Beltway (Johnson, K.E., personal observations). For millennia, Spotted Salamanders and Wood Frogs (*Lithobates sylvatica*) bred there. A few of their eggs were collected and brought to my laboratory at George Washington University for research purposes each spring. But one year, suddenly a village of condominiums had replaced the pond and woods. The native oak trees had been replaced by paved streets. The pond was drained. Habitat loss, especially loss of wetlands, is a major driver in the disappearance of formerly abundant amphibians. This was just one particularly sudden and striking example of habitat loss.

Literature Cited

Arnold, S.J. (1976) Sexual behavior, sexual interference and sexual defense in the salamanders. Ethology 42:247-300.

Harrison, R.G. (1969) *Organization and Development of the Embryo.* Yale University Press, New Haven. , pp. 60-66.

Hutchison, VH and CS Hammer (1958) Oxygen utilization in the symbiosis of the embryos of the salamander, *Ambystoma maculatum* and the alga *Oophila ambystomatis*. Biol. Bull. 115:483-489.

Johnson, K.E., personal observations

Madison, D.M. (1997) The emigration of radio-implanted spotted salamanders, *Ambystoma maculatum*. J. Herpetology 31:542-551

Maex, M., et. al., (2016) Courtship pheromone use in a model urodele, the Mexican axolotl (*Ambystoma mexicanum*). Scientific Reports 6:281-284

McGregor, J.H. and W.R. Taska (1989) Olfaction as an orientation mechanism in migrating *Ambystoma maculatum*. Copeia 1989:779-781.

Powell, R., Conant, R., and J.T. Collins (2016). *Peterson Field Guide to Reptiles and Amphibians: Eastern & Central North America, ed. 4*. Houghton Mifflin, New York.

Stenhouse, S.L. (1985) Migratory orientation and homing in *Ambystoma maculatum* and *Ambystoma opacum*. Copeia 1985:631-637.

Stenhouse, S.L., Hairston, N.G. and A.E. Cobey (1983) Predation and competition in *Ambystoma* larvae: field and laboratory experiments. J. Herpetol. 17:210-220

Tattersall, G.J. and N. Spiegelaar (2008). Embryonic motility and hatching success of *Ambystoma maculatum* are influenced by a symbiotic alga. Can. J. Zool. 86:1289-1298.

Twitty, V.V. (1966) *Of Scientists and Salamanders*. Freeman, San Francisco.

Field Notes

***Hyla chrysoscelis* (Cope's Gray Treefrog)**: VA. Frederick County, 334 Ronner Ln, Winchester. 26 May 32024. Danielle Brooks.

County Record: Cope's Gray Treefrog is one of two related species of treefrogs. Hyla chrysoscelis (Cope's Gray Treefrog) is a diploid and has two sets of chromosomes like most vertebrates) and Hyla versicolor (Gray Treefrog) is a tetraploid with four sets of chromosomes. Other than chromosome number, the two can be differentiated only by the mating call. The overall morphology is identical, making identification outside the breeding season impossible. On 26 May 2024 I recorded the treefrogs calling from a pool in the home we moved into the previous summer. The surrounding area is wooded and frogs were calling from trees around the pool. I sent the recording to the Virginia Herpetological Society for confirmation of the identity of the treefrog. Cope's Gray Treefrog has a state-wide distribution, although there are few records from the western tier of counties (https://www.virginiaherpetologicalsociety.com/amphibians/frogsandtoads/copes-gray-treefrog/index.php). This is the first record for Frederick County and helps fill a distribution gap. It has not been reported from any of the surrounding counties in Virginia. The sound recording was submitted to the VHS Digital Archive (#816) as a voucher for this record.

Danielle Brooks
Winchester, VA

***Hyla squirella* (Squirrel Treefrog)** VA: Goochland County, 3500 Woods Way, State Farm, State Farm Correctional Complex, Building 62. 5 December 2023. Carmen Crater.

County Record: Squirrel Treefrogs are a small treefrog inhabiting southeastern Virginia, where they reach the most northern extent of their range. They appear to be habitat generalists, occupying various types of forests, but also occur in and around buildings (https://www.virginiaherpetologicalsociety.com/amphibians/frogsandtoads/squirrel-treefrog/index.php). On 5 December 2023 I found a juvenile Squirrel Treefrog on the floor of my office at the State Farm Correctional Complex. I posted a picture of the frog on my Virginia Wildlife Facebook page and other members tentatively identified it as a Squirrel Treefrog and mentioned it had not previously been reported from Goochland County. I was encouraged to send the photo to the Virginia Herpetological Society's identification page to confirm the identification and report this new county record. It has been previously recorded in Hanover, Henrico and Chesterfield Counties to the east. The digital photo was submitted as a voucher for this observation (VHS Archive # 802).

Carmen Crater
Goochland Co., VA

Lithobates clamitans **(Green Frog)** VA: Lunenburg County, Keysville, VA 23947 (37.00899 N, 78.45910 W), May 24 2024. Kristin E. Duty

County Record: The Green Frog (*Lithobates clamitans*) is commonly found in all regions of Virginia. These frogs occupy temporary and permanent freshwater bodies with mixed vegetation for refuge. Having been verified in 90 counties and 19 cities (https://www.virginaherpetologicalsociety.co m/ amphibians/frogsandtoads/green- frog/ index_php) here I report the first county record for Lunenburg County

On 24 May 2024 a Green Frog was observed and photographed after nightfall at a private pond in Keysville, Virginia. The recognizable "banjo" call was heard across the pond from several individuals of the same species keeping protective cover in the grasses, sedges, and underbrush of the pond's edges. Along with this calling, the two dorsolateral folds extending from behind the eye down the back., greenish brown color, and distinct tympanum rule out other true frogs found in its range. This report confirms inhabitants for Lunenburg and serves as a county record filling in one of the two county gaps for Southside Virginia. A photograph and this report was submitted to the VHS (Archive #820) as verification.

Kristin Duty
Keysville, VA

Notophthalmus viridescens **(Red-spotted Newt):** VA. Loudoun County, Dulles Greenway mitigation wetlands (39.033040, -77.610481). 10 April 2024. Jenny Erickson, Nicola Jones and Erin Brunner.

County Record: The Red-spotted Newt has a statewide distribution in Virginia, found in 88 of the 95 counties. Loudoun is the only county in northern Virginia without a record (https://www.virginiaherpetologicalsociety.c om/amphibians/salamanders/red-spotted-newt/index.php). Here, we report an observation of adult Red-spotted Newts for the county. On 10 April 2024 a team from the Loudoun Wildlife Conservancy wase looking for herps at the Dulles Greenway Mitigation Wetlands outside of Leesburg, Va off Rt 15 south. Loudoun Wildlife Conservancy are stewards of the property, which is not open to the public, and we regularly walk the property, check trail cams and look for herps. The newt(s) have been seen a couple of years in a row, specifically in a canal-type waterway (it was part of a small stream until a beaver created a dam and blocked this section of the stream from the rest. Now the rest of this small stream is fed by run off over the beaver dam. Red-spotted Newts were seen in this wetland and photographed. The digital photo was submitted to the VHS Archive (#809) as a voucher for this observation. Loudoun County now completes the list of counties in northern Virgina with records for the Red-spotted Newt.

Jenny Erickson
Lovettsville, VA

***Pseudotriton ruber nitidus* (Blue Ridge Red Salamander)**: VA, Campbell Co, Candler Mountain, Camp Hydaway (37º 20' 19.9"; 79º 08' 57.5"). 14 April 2024. Cheyenne Brooks and Jordan Tennis.

County Record: The Red Salamander is named for its typically bright red coloration. It has small black spots along its dorsum. The Blue Ridge Red Salamander lacks black spots on the distal half of its tail, and any spotting on its ventral surface. Mitchell and Reay (1999. Atlas of amphibians and reptiles in Virginia. Virginia Department of Game and Inland Fisheries Special Publication Number 1. Richmond, VA 122 pp.) limit its distribution to Grayson, Carroll and Floyd Counties in the southern Blue Ridge Mountains. Since then, reports have been added for five additional counties in the southern mountains, as well as Amherst and Page Counties in the mountains of central Virginia. Although Martof (1975. *Pseudotriton ruber*. Catalogue of American Amphibians and Reptiles, pp. 167.1-167.3) recommended against recognizing subspecies until a comprehensive genetic study had been performed, most authors (Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C. 587 pp.) recognize four, two of which (*Pseudotriton ruber ruber* and *P. r. nitidus*) occur in Virginia. Subspecies are typically used to identify "geographically and/or ecologically defined subdivisions of a species with distinctive characteristics." (Lillywhite, H.B. 2008. Dictionary of Herpetology. Krieger Publ. Co., Malabar, FL. 376 pp.). Thus, there are two criteria, morphological distinctives and geographic or ecological separation needed to recognize subspecies. The first genetic study of Red Salamanders was conducted by Bonett et al. (2013. Evolution of paedomorphosis in Plethodontid salamanders: Ecological correlates and re-evolution of metamorphosis. Evolution 68: 466-482.). Their species tree (Figure 4) shows that *Pseudotriton ruber ruber* and *P. r. nitidus* do not group together but are dispersed among each other. A larger study by Folt et al. (2016. Phylogeography and evolution of the Red Salamander (*Pseudotriton ruber*). Molecular Phylogenetics and Evolution 98: 97-110) shows a similar pattern where the "four subspecific taxa of *P. ruber* did not form monophyletic groups to the exclusion of one another".

Here, we present evidence of a morphological *Pseudotriton ruber nitidus* from Campbell County. However, we also found morphological *P. ruber ruber* from the same locality. This brings into question how robust and useful the subspecific status is for specimens from Virginia. The genetic basis and geographic separation needed for subspecific status, at least in this location, is questionable. It may simply reflect genetic variability found within many populations. Alternatively, the outer Piedmont of central Virginia could represent an area of overlap and integration of the two "subspecies". Digital photos of the two specimens, both from Candler's Mountain, were presented to the VHS Archives as vouchers (Archive # 809 and 810).

Paul Sattler, Cheyenne Brooks and Jordan Tennis
Lynchburg, VA

Figure 1. *Pseudotriton ruber nitidus*.

Figure 2. *Pseudotriton ruber ruber*.

***Pseudotriton ruber nitidus* (Blue Ridge Red Salamander)**: VA. Bedford County, Black Horse Gap north of the Blue Ridge Parkway, Jefferson National Forest, (N 37º 25' 15.5"; W 79º 45' 43.9"). 4 June 2024. Jordan Whitt and Paul Sattler.

County Record: The Red Salamander has a statewide distribution in Virginia. There are four subspecies recognized by most authors (Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington D.C. 587 pp.), two occurring in Virginia (Mitchell, J.C. and K.K. Reay. 1999. Atlas of Amphibians and Reptiles in Virginia. Special Publication Number 1, Virginia Department of Game and Inland Fisheries. Richmond, VA. 122 pp.). Most records of the Blue Ridge Red Salamander are from the Blue Ridge Mountains in southern counties, although records now exist for two counties in the outer Piedmont of central Virginia (Amherst and Campbell). Here we report finding a Red Salamander from Bedford County with the morphology of the Blue Ridge Red Salamander.

On 4 June 2024 while on a Herpetology class Field Trip to Black Horse Gap on the north side of the Blue Ridge Parkway in the Jefferson National Forest, one of the authors (JW) found a Red Salamander under a log. It had the morphology of the Blue Ridge Red Salamander, with no spotting on the distal half of the tail and no spotting on the chin. This makes the third county (Amherst, Campbell and now Bedford) in the outer Piedmont where this form has been found. While Sattler et al. (2024. Pseudotriton ruber nitidus. Catesbeiana. 41(1): 30-31) questions the validity of subspecies recognition in Virginia, the morphology of Red Salamanders in central Virgina often resembles that of the Blue Ridge Red Salamander. A digital photo of the specimen was deposited with the VHS Digital Archive (#819) as a voucher.

Paul Sattler
Lynchburg, VA

Jordan Whitt
Danville, VA

***Lampropeltis Triangulum* (Eastern Milksnake)**: VA. Tazwell County, Cedar Bluff (37.1131286, -81.6439305) 13 April 2024. Renée Wienecke.

County Record: The Eastern Milksnake is a large snake, up to a meter in Virginia (https://www.virginiaherpetologicalsociety.com/reptiles/snakes/eastern-milksnake/index.php) often prized as pets because of their colorful appearance and docile nature. Their range includes the western montane counties from northern Virginia to the southwestern tip. There are a few counties (Tazwell, Buchanan and Wise) from the southwest without records. Here I provide the new record for Tazwell County. On 13 April 2024 I found a large snake in my yard which I photographed. I used the app Seek and the Virginia Wildlife Facebook page to obtain an identification. Someone on the Facebook page informed me there was no record for Tazwell County for the Eastern Milksnake so I sent the photo to the VHS as a voucher (VHS Archive #807). As a Virginia Master Naturalist I find this record very exciting.

Renée K. Wienecke
Cedar Bluff, VA

***Nerodia erythrogaster* (Plain-bellied Watersnake)**: VA. Charles City County, 5420 Old Union Road, Charles City. 6 May 2024. Lynn Legg.

County Record: The Plain-bellied Watersnake is a large Watersnake inhabiting all sorts of aquatic habitats in southeastern Virginia (https://www.virginiaherpetologicalsociety.com/reptiles/snakes/plain-bellied-watersnake/index.php). On 6 May 2024 I was working in my hay barn on our farm when I saw a large snake. I photographed the snake and sent the picture to the VHS herp identification page. I was informed the snake was a Plain-bellied Watersnake and there was no previous record of this species from Charles City County. The barn is located approximately 200 m from a half hectare pond, which is the most likely habitat normally frequented by this particular snake. This represents the first record of the Plain-bellied Watersnake in Charles City County, although it is found in most of the counties to the south and east. The photograph was submitted to the VHS Digital Archive (#814) as a voucher for this observation.

Lynn Legg,
Charles City, VA

Nerodia taxispilota **(Brown Watersnake)**: VA. Hanover County, 71a49 Autumn Ridge Lane, Mechanicsville (37.5971181, -77.3340719) 7 May 2024. Jacquelyn Crain.

County Record: The Brown Watersnake is a large, aquatic snake associated with large bodies of water. It is often found in areas with branches overhanging open water. Its diet consists mostly of fish. The distribution in Virginia is the southeastern counties and cities. This is the first report of Brown Watersnakes from Hanover County. On 7 May 2024 a friend called me to come remove a snake from a tree in her yard. The snake had been present the previous two days. I removed the approximately 100 cm snake to Dabney Lake, the nearest large body of water. Curiously, there was no water on the property where the snake was originally found. I sent a photo of the snake to the VHS for positive identification and submitted it to the VHS Archive (#816) as a voucher for this record.

Jacquelyn Crain
Mechanicsville, VA

Pantherophis alleghaniensis **(Eastern Ratsnake):** VA. Northumberland County,. near the intersection of Eagle Point Rd.(Co. Rt, 602) and Swampson's Wharf Road (Co. Rt. 610) (37.855719, -76.394332 approximate).15 May 2020. Christian "Alec" Jarboe.

County Record: The Eastern Ratsnake has a statewide distribution in Virginia (https://www.virginiaherpetologicalsociety.com/reptiles/snakes/eastern-ratsnake/index.php) being verified in 91 of the 95 counties. The few for which there are not verified records is most likely due to a lack of surveys or reporting. Northumberland County is one of those for which there is no voucher. Here I provide a photo voucher (VHS Archive #804) for Northumberland County for an animal I photographed on a road back in May of 2020. It was a beautiful animal, recently having shed, and on the road at about 8:45 am. I moved the snake off the road and shot a photograph before releasing it in the direction it was heading. This record leaves only Lunenburg, Dickenson and Russell Counties without a record.

Christian "Alec" Jarboe

Regina septemvittata **(Queensnake)** VA: Henrico County, Nature Trail in Eco-corridor at the University of Richmond (37.572041, -77.535944.). 27 January 2024. Baker Wright.

Early Record: The Queensnake is a diurnally active medium sized snake, known for its diet of consuming recently molted crayfish. As such it inhabits shallow, rocky streams where its prey abounds. They typically divide their time between searching under rocks for crayfish and basking on the shore, or more typically in branches overhanging the stream. According to Mitchell (J. M. 1994. The Reptiles of Virginia. Smithsonian Institution Press. Washington DC. 352pp.) activity records from museum specimens indicate the snake is active from 21 February to 10 October. Here, I report the observation of a Queensnake from the University of Richmond Campus on 27 January 2024, moving the recorded activity forward by about a month. The weather this spring has been quite warm. On this date, the weather was overcast and about 17ºC. A photo of the snake was submitted as a voucher for this observation (VHS Archive # 803). Mitchell (op. cit.) also mentions that Queensnakes were extirpated from Westham Creek on the University of Richmond campus sometime in the early 1980s due to siltation. This observation verifies the species has repopulated Westham Creek at this site.

Wally Wright
Henrico, VA

Storeria occipitomaculata **(Red-bellied Snake).** VA: Petersburg City, Petersburg Battlefield Park (37.22240° N, 77.36557° W). 23 February 2024. John Allen.

City Record: The Red-bellied Snake is a small secretive snake found in a variety of habitats (https://www.virginiaherpetologicalsociety.com/reptiles/snakes/northern-red-bellied-snake/index.php). While it is a habitat generalist, it is a predatory specialist, feeding almost exclusively on slugs (Op. Cit.). It has a statewide distribution although there are few records from southwestern Virginia. The secretive nature and small size of the snake does not lend itself to common observations. Here, I report the first record of a Red-bellied Snake from the City of Petersburg. I was walking in the Petersburg Battlefield Park on 23 February 2024 when it was overcast but warm for late February (about 15ºC) when it began to rain. I looked down and saw a small snake in the leaf litter. I shot a photograph as it began to rain harder, and sent the photo (VHS Archive #805) to the VHS to verify my identification, which they did. This record is the first voucher for the City of Petersburg, although it is verified in the adjacent counties of Chesterfield and Prince George, but not Dinwiddie.

John Allen
Fort Gregg-Adams
Natural Resources Manager

***Storeria occipitomaculata* (Red-bellied Snake):** VA. Franklin County, Moneta, 2170 Bluewater Drive by Scruggs Fire Station 10. 1 April 2024. Heather Pritchard.

County Record: The Red-bellied Snake is a small and secretive snake found throughout Virginia, although records from the far southwestern portion of the state are scarce. Most records are from eastern and central Virginia (https://www.virginiaherpetological society.com/reptiles/snakes/northern-red-bellied-snake/index.php). Activity records from museum specimens are given as 18 March to 19 October (Mitchell, J.C. 1994. The Reptiles of Virginia. Smithsonian Institution Press, Washington D.C. 352pp.). They are said to be habitat generalists (Op. Cit.). Here I report the occurrence of a road-killed specimen in the City of Moneta in Franklin County. I frequently walk and run along Bluewater Drive and on 1 April 2024 I saw a dead snake near the centerline of the road near the Scruggs Fire Station (2170 Bluewater Drive). I photographed the snake to see if I could identify it. The photo (VHS Archive #806) was sent to the VHS Identification Page where the snake was identified as a Red-bellied Snake, and I was informed there was no previous record of the species for Franklin County, although there are for all surrounding counties except Floyd. This record helps fill a gap in the distribution in central Virginia.

Heather Pritchard
Moneta, VA

***Tantilla coronata* (Southeastern Crowned Snake):** VA. Buckingham County, Holliday Lake State Park (37.391808,-78.635350). 24 April 2024. Henry Nase and Evan Spears.

County Record: The Southeastern Crowned Snake is a small secretive snake rarely seen. They spend most of their time underground and only observed when overturning cover objects (https://www.virginiaherpetological society.com/reptiles/snakes/southeastern-crowned-snake/index.php). They are associated with dry pine forests. In Virginia they are only reported from eight counties in the southcentral portion of the state. On 24 April 2024 Evan Spears and I were looking for *Tantilla coronata* at Holliday Lake State Park since one had been found there previously by Evan. Flipping quartz and schist rocks around noon on a southwestern facing slope overlooking the dam of Holliday Lake within the borders of the State Park, we found four in total, three on the slope around noon and one found in a rock pile a half mile away at the edge of a small field. This extends the known range in Virginia to the east, and adds Buckingham County to the known locations. A digital photo was submitted to the VHS Archive (#808) as a voucher for the observation.

Henry Nase
Dillwyn, VA

***Virginia valeriae* (Smooth Earthsnake)**: VA. Warren County, 168 Forest Manors Drive, Front Royal. 10 May 2024. Georgia and Robert Walsh.

County Record: The Smooth Earthsnake is a small, secretive snake found in a variety of habitats, usually under some type of cover object. It is nearly statewide in distribution, but with few verified records in far southwestern Virginia (https://www.virginia herpetologicalsociety.com/reptiles/snakes/eastern-smooth-earthsnake/index.php). On 10 May 2024 we were working in the yard when we found a small snake under a pile of leaves and straw. A photo was sent to the VHS for positive identification. We were informed the snake was a Smooth Earthsnake and there was no previous record of them from Warren County. This is thus the first report of the Smooth Earthsnake from Warren County and helps fill a distribution gap in northern Virginia. The photo was submitted to the VHS Digital Archive (#812) as a voucher for this observation.

Georgia and Robert Walsh
Front Royal, VA

***Clemmys guttata* (Spotted Turtle)** VA: Culpepper County, Thoms Road (38°29'52.7"N 77°51'15.8"W). 5 May 2024. Erin C. Anthony and Matthew Anthony.

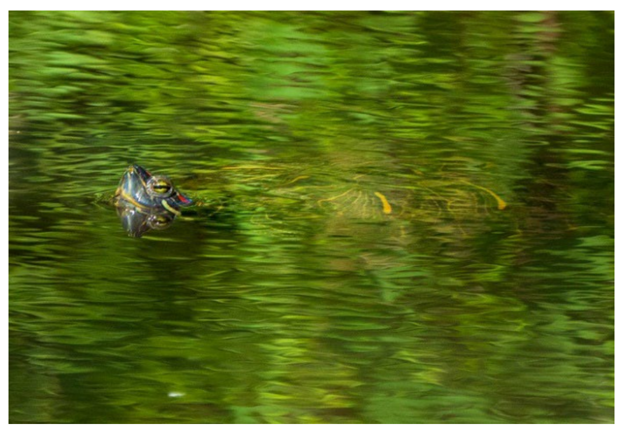
County Record: Erin and Matt drove Thomas Road while birding around noon. The spotted turtle was crossing the road. Wet depressions were on each side of the road as Flat Run Creek was flooding due to persistent rain. The adjacent fields were not actively being used for agriculture, and the road was wet with large puddles. A picture was submitted to VHS archives (#817) as a voucher. Spotted Turtles are reported from four of the six surrounding counties, Madison Co., Spotsylvania Co., Fauquier Co., and Stafford Co. This record fills in a range gap, but it does not change our assumptions of their range and habitat preference in the state of Virginia.

Erin C. Anthony
Post Oak Middle School
Spotsylvania County, VA

***Trachemys scripta scripta* (Red-eared Slider)**: VA. City of Fredericksburg, Gayles Pond (38.3088706, -77.4744339). 18 April 2024. Allen Melson.

City Record: The Red-eared Slider is not native to Virginia, but has been widely introduced throughout the state, as pets which have been released when they grew too large for most home tanks. The species is now considered to be "naturalized" with breeding populations found in many localities. Here I report the first instance of a Red-eared Slider from the City of Fredericksburg. On 18 April 2024 I was walking along Gayles Pond, which is adjacent to a canal used for flood control, when I saw and photographed a Red-eared Slider in the pond. The slider has been reported from Spotsylvania County to the south of Fredericksburg, but not Stafford to the north. The digital photograph was submitted to the VHS Digital Archive (#811) as a voucher for this observation.

Allen Melson,
Fredericksburg, VA



***Aspidoscelis sexlineata* (Six-lined Racerunner)**: VA. Buckingham County, approximately 670 m southwest of pipeline clearing crossing Hidden Valley Drive (37.460419,-78.462953). 2 June 2024. Henry Nase.

County Record: The Six-lined Racerunner has a spotty distribution in the eastern two-thirds of Virginia (https://www.virginia herpetologicalsociety.com/reptiles/lizards/eastern-six-lined-racerunner/index.php). It is a lizard of open spaces where it's speed is the major advantage over possible predators. Here I report the fist record of Six-lined Racerunners in Buckingham County. On 2 June 2024 I was walking a pipeline clearing when I saw a Six-lined Racerunner run from a clump of grass and pause under a bush, where I was able to obtain a photograph. The habitat was reminiscent of native prairie with sparse clearings in the grass and very little rock cover. The weather was about 22ºC with clouds moving in. Racerunners have been reported from Albemarle County to the north and Appomattox to the south. The photograph was submitted to the VHS Archive (#813) as a voucher for this record.

Henry Nase
Dillwyn, VA

***Plestiodon laticeps* (Broad-headed Skink)**: Scott County, 36.61N; 082.75W. 17 May 2024. Aaron Mazuelos

County Record: At 4:45pm on an overcast but warm evening, about 25ºC, a skink fell into a watering can. Upon closer investigation, this skink was larger than the average five- lined skinks normally found in this location. The skink measured 23cm total body length. Its head was orange and its body a brassy brown color. Based on its five labial scales, it was determined to be a broad-headed skink. The photos were cross-referenced with herpetologist Erin Anthony, who confirmed identification.

Broad-headed skinks are not listed as existing in Scott County, according to the Virginia Herpetological Society webpage, therefore this would be a county record and extend the current range westward. The habitat was an edge habitat, between a hay field and a wooded ridge. The digital photo was submitted to the VHS Archive (# 818) as a voucher for this observation.

Aaron Mazuelos

6756 Upper Possum Creek Road

Gate City, Virginia

***Scincella lateralis* (Little Brown Skink)**: VA. City of Lynchburg, Liberty University Campus (N 37º 21'08.6"; W 79º 10' 55.0"). 10 June 2024. Paul Sattler.

City Record: The Little Brown Skink is a small terrestrial lizard with a statewide distribution in Virginia, although there are few records from the western tier of counties. They are particularly prevalent in the Coastal Plain but occur in smaller numbers in the Piedmont. Here I report the first record from the City of Lynchburg, although they are found in all surrounding counties. On 10 June 2024 I was walking near the academic parking garage of Liberty University in the afternoon when I found a small lizard on the road where it had apparently been run over. It was a Little Brown Skink, which I photographed. The Little Brown Skink is diurnal and had not been there that morning, so had apparently been killed that day. The area adjacent to the lizard was a flower bed with a thick growth of Saint John's Wort, which would have provided ample habitat for an insectivorous lizard. The photo was deposited in the VHS Archive (#815) as a voucher.

Paul Sattler
Lynchburg, VA

President’s Corner

Throughout our history, we have strived to deepen our understanding of Virginia's herpetofauna and to share that knowledge with the broader community. From educational outreach programs in schools to holding surveys across the state, our collective efforts have made a lasting impact on the preservation of our native species.

I encourage each of you to actively participate in our society's events. Your passion and expertise are invaluable assets in our mission to conserve Virginia's herpetofauna for generations to come. We’ve already held three well-attended herp surveys this year. I was encouraged to see a large turnout for a very wet Saturday at Sweet Run State Park.

Throughout the coming year, we will continue to expand our initiatives in education and research. There are a lot of outreach opportunities coming up. Thank you to our education committee volunteers for all their time and effort. Be on the lookout for a hold-the-date announcement for our Fall meeting. After an excellent meeting in Southwestern Virginia last year, we plan to return to Virginia Department of Wildlife Resources Headquarters in Richmond this Fall.

I want to acknowledge VHS’s leadership team. They do a lot of work behind-the-scenes to make things run smoothly. I especially want to mention our Vice President, Arianna Kuhn, for going above and beyond to help out.

Thank you and Happy Herping,
John Orr, VHS President

Minutes of the Spring VHS Business Meeting
Location: Picnic Shelter of Sweet Run State Park
Date/Time: May 17th, 2024; 6-7:30pm

President Opens Meeting
Welcome

Committee Updates
Bylaws Committee - **Erin C. Anthony:** *Update*: To be discussed at fall meeting (ECA)

Grants Committee - **Kory Steele:** *Update*: Two proposals were submitted, one was awarded. *Agenda item*: I would like for an advertisement plan to get more competitive applicants to future grants (ALK)
Update: **Grant application to DWR** outreach initiative was submitted By Arianna K. & M. Boyd, but not funded. Ideas or contacts for applying next year? (ALK)

Permits - **Susan Watson:** *Update:* I sent the latest version of the **Scientific Collection Permit** out last week, based on survey events I know about. Everyone should contact me if there are any additions or edits needed on either the Scientific Collection or the Exhibitor's Permits.

Newsletter - Clinton Markwell: No report.

Outreach Committee - **Kelly Geer:** *Agenda item*: I would like for a definite plan for the **Fall meeting (date and location)** to be decided on at the Spring meeting (or at least potential sites generated and voted on electronically). If we need to reserve a place, we need this information ASAP (MC)
Update: The Board Room at DWR headquarters is open for Saturdays in most of October and all of November (when we usually look to have the VHS Fall Mtg). Only the first Saturday, October 5th, is booked right now (SW, ALK)
Agenda item: We have been in touch with the **North Carolina Herpetological Society**, and there is interest in a joint spring meeting– they are discussing the possibility at their next committee meeting in July (one day of talks, one day of survey) (ALK for more details).
Agenda item: We need someone to be the primary contact for the **facebook page.** We also need to discuss duties/frequency expectations. I propose a "Media" role (ALK)
Agenda item: Are there any zoos or museums that still offer a discount to VHS members? If so, can we include that information on our website and in our membership emails? More importantly, if this is no longer a **VHS membership perk**, we should get rid of it.

Merchandise - Erin C. Anthony

Legislation - Larry Mendoza

Conservation Committee - Yohn Sutton: *Agenda item*: Official recognition with **PARC** on their website and alongside their initiatives to engage more actively, particularly around our conservation mission. Report on concerns with clear rationale with

whatever obligations we would have succinctly spelled out. (ECA/ALK)
Voting item: Motion to consider, with rationale, on the above agenda item for the ExCom to vote on at the Fall Meeting after receiving more information (email has been sent by ALK 05/16/24).

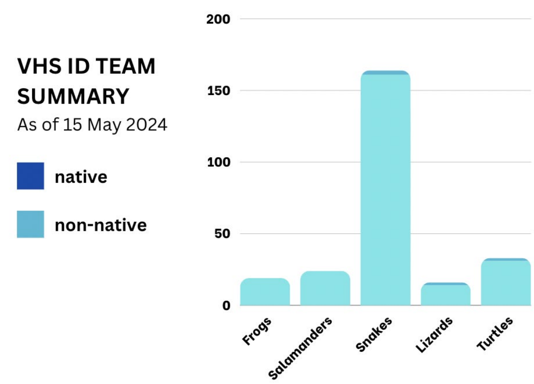
Survey Committee - Jason Gibson: The HerpBlitz for 2024 is scheduled for 1-2 June at Powhatan Wildlife Management Area.

Catesbeiana - Paul Sattler: Paul expressed concern that not all VHS surveys seem to be written up for publication in Catesbeiana. If survey results are not published it is as if the survey never happened. If people are sitting on survey results, please get them written up. The data is important in establishing distribution and abundance information for future trends.

Education Committee - Caroline Seitz:
Update: Summary of events performed by leadership
Update: Goals accomplished
Update: Goals moving forward

Treasurer's Report - Matt Close: *Agenda Item*: I am going to try to send you a draft **treasurer's report** and draft **budget** for the next fiscal year (the plan discussed last year was that a draft would be provided annually at Spring meeting and then discussed/amended/finalized and voted on at Fall Meeting) by Thursday. <u>We won't need to vote on this but everyone should see it</u> (MC). *Voting Item:* whether or not to open a new 6- or 12- month Certificate of Deposit when the current Certificate of Deposit matures. The current one will mature I believe on June 1st (I will have to check) and the plan was to open a new one at the best current rate the day that this one matures or, if the rates are terrible, to just put the principle and interest all back into the account (MC)

Website Report - John White: *Update* review of herps identified
Update: review of models made/disseminated/painted



Bylaws - Discussions on updates to the Bylaws led by E. C. will take place at the 2024 Fall Meeting

Grants - This year the VHS Grant in Herpetology was awarded to Lauren Fuchs, a PHD candidate at George Mason University

Permits - Susan Watson let us know the latest version of the Scientific Collection Permit was shared with everyone, and in the future if there are any additions or edits needed on either the Scientific Collection or the Exhibitor's Permits.

Outreach Committee -Fall Meeting - MC requested that the planning for the Fall meeting & dates be organized as soon as possible. ALK confirmed availability for the location to the DWR headquarters with SW, and plans are underway to finalize that date and location.
Spring Meeting - We have been in touch with the North Carolina Herpetological Society, and there is interest in a joint spring meeting– they are discussing the possibility at their next committee meeting in July (one day of talks, one day of survey). ALK is currently corresponding with the NCHS VP to discuss this further, and it will be brought up again for discussion at the midsummer council meeting and the Fall VHS meeting.

Facebook - KG requested that we have someone be the primary contact for the facebook page. EC noted that she would be happy to contribute to the role. ALK would like more discussion on the exact parameters of this as a formal role at the Fall 2024 Meeting.
Membership perks -The website currently states that our membership offers zoo/museum discounts. If so, can we include that information on our website and in our membership emails? More importantly, if this is no longer a **VHS membership perk**, we should get rid of it. JW has since taken information about membership discounts off the website until we decide to confirm these memberships or reinstate them with partners. Discussion around a "VHS" membership card progressed, but did not reach a conclusion on whether this would be useful, or feasible given variation in membership enrollment. Further discussion on this topic will be revisited at the Fall 2024 Meeting.

Conservation - PARC affiliation - Discussion around Official recognition with **PARC** on their website and alongside their initiatives to engage more actively, particularly around our conservation mission. Report on concerns with clear rationale with whatever obligations we would have succinctly spelled out. (ECA/ALK). ALK has since reached out (05/16/24) to PARC and received communication about their interests in meeting with us to discuss this opportunity, and notes that there are no obligations, rather, they hope to discuss how both parties can benefit from the affiliation. ALK will forward email correspondence, and council members interested in chatting to the PARC representatives will be invited to join. A motion to consider with rationale will be visited at the Fall 2024 Meeting.
Education -

CS shared a report on Education activities including summary of events performed by leadership, goals accomplished, and goals moving forward.
Live Animals in Education - A discussion on the continued use of live animals in educational activities was mentioned but postponed to Fall Meeting 2024 for greater participation.
CS mentioned potential need for a credit card for education. **Voting was in favor of getting an additional credit card (linked to same account) for CS.**

Treasurer - MC circulated the treasurer's report and draft budget for the next fiscal year

(the plan discussed last year was that a draft would be provided annually at Spring meeting and then discussed/amended/finalize and vote at Fall Meeting) We didn't need to vote on this but MC wanted to make sure everyone had the chance to look it over
Voting was request on whether or not to open a new 6- or 12- month Certificate of Deposit when the current Certificate of Deposit matures. The current one matured on June 1st and the plan was to open a new one at the best current rate the day that this one matures or, if the rates are terrible, to just put the principle and interest all back into the account (MC). **Voting was in favor of opening a new CD and following MC's guidance and recommendations.**

Website Report -

John White shared the report of herps identified and models made/disseminated/painted. At this meeting, JW brought several new models for various committee members to take home and paint. After this, we won't be sending out more models until we receive more painted models back as many are still at large.

Submitted by Arianna Kuhn
VHS Vice-President

Virginia Herpetological Society
Treasurer's Report
June 28, 2024

Previous Balance- December 4, 2023	$	12,489.46
*Certificate of Deposit-Truist Bank (matured 6/14/24)	$	10,000.00
Receipts		
Dues	$	4265.03
Donations (One-time and recurring)	$	1801.50
*CD interest	$	288.26
Expenses (06/15/2023-12/04/2023)		
Operational Expenses		
VA SCC Filing Fee	$	25.00
Liability Insurance	$	701.20
Educational Materials	$	1257.60
Promotional and Outreach Materials	$	507.00
PARC Rattlesnake Books	$	320.00
Awards and Recognition	$	201.65
2024 VHS Grant-in-Herpetology (L. Fuchs)	$	1000.00
Postage	$	42.84
Service Fees (Paypal, Stripe, etc.)	$	204.06
Current Gross Balance (06/28/2024)	$	24,584.90
*Certificate of Deposit-Truist Bank (matures 11/14/24)	$	10,288.26
Current Available Balance	**$**	**14,296.64**

VHS Memberships (dues current)

Regular:	343
Student:	35
Lifetime:	101
Total	**479**

Matthew Close
VHS Treasurer

Field Notes

The Field Notes section of *Catesbeiana* provides a means for publishing natural history information on Virginia's amphibians and reptiles that does not lend itself to full-length articles. Observations on geographic distribution, ecology, reproduction, phenology, behavior, and other topics are welcomed. Field Notes will usually concern a single species. The format of the reports is: scientific name (followed by common name in parentheses), state abbreviation (VA), county and location, date(s) of observation, observer(s), data and observations. The name(s) and address(es) of the author(s) should appear one line below the report. Consult the editor if your information does not readily fit this format. **All Field Notes must include a brief statement explaining the significance of the record** (e.g., new county record) **or observation** (e.g., unusual or rarely observed behavior, extremely early or late seasonal record, abnormal coloration, etc.). Submissions that fail to include this information are subject to rejection. Relevant literature should be cited in the body of the text (see Field Notes in this issue for proper format). All submissions will be reviewed by the editor (and one other person if deemed necessary) and revised as needed pending consultation with the author(s).

If the Field Notecontains information on a **new county (or state) record**, **verification is required in the form of a voucher specimen** deposited in a permanent museum (e.g., Virginia Museum of Natural History) or a **photograph** (print, slide, or digital image) **or recording** (cassette tape or digital recording of anuran calls) deposited in the archives of the Virginia Herpetological Society. Photographs and recordings should be sent to the editor for verification and archiving purposes; the identity of voucher specimens must be confirmed by a museum curator or other qualified person. Include the specimen number if it has been catalogued. Prospective authors of distribution reports should consult Mitchell and Reay (1999. *Atlas of Amphibians and Reptiles in Virginia*), Mitchell (1994. *The Reptiles of Virginia*), and Tobey (1985. *Virginia's Amphibians and Reptiles: A Distributional Survey*) [**both atlases are available on-line on the VHS website**] as well as other recent literature to determine if they may have a new county record. New distribution records from large cities that formerly constituted counties (Chesapeake, Hampton, Newport News, Suffolk, and Virginia Beach) are acceptable, but records from smaller cities located within the boundaries of an adjoining county will only be published if the species has not been recorded from that county. Species identification for observational records (e.g., behavior) should be verified by a second person whenever possible.

PHOTOGRAPHS

High contrast photographs (digital images) of amphibians and reptiles will be considered for publication if they are of good quality and are relevant to an accompanying article or field note. Published photographs will be deposited in the Virginia Herpetological Society archives.

Paul Sattler, Editor
Department of Biology
Liberty University
MSC Box 710155
1971 University Blvd.
Lynchburg, Virginia 24515