Improving Amphibian Roadway Mitigation to Decrease Mortality and Increase Connectivity by Experimenting with Ecopassage Design

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16. Abstract

The Nelsonville bypass, completed in 2013, runs through the largest remaining tract of continuous forest in Ohio, including wetlands and amphibian migration routes. Such habitat fragmentation affects wildlife populations by increasing mortality and reducing connectivity. Thus, the Ohio Department of Transportation (ODOT) installed wildlife mitigation measures, including two amphibian ecopassages with plastic barrier fencing along the new stretch of State Route 78 (SR 78). Barriers limit access to the roadway and direct animals toward ecopassages, which are corridors that channel animals safely over or under the roadway. Researchers at Ohio University have been studying the effectiveness of the amphibian barrier-ecopassage system in place along SR 78 since March 2015. Using cameras, it was detected that 25 individual amphibians were in the ecopassages, and only eight successfully traversed the length of the tunnel. Meanwhile, about 14,000 amphibians were found dead on the road. The low level of ecopassage use implies that these structures are not adequately reducing roadway mortality.

The goal of this research is to improve upon the barrier-ecopassage system in place along SR 78. The Principal Investigator (PI) will test alternative ecopassage designs to identify the most effective design. The PI will also assess which design provides the most connectivity. A matrix of recommendations will be provided within the first 6 months of the project at which point ODOT will chose a method and install the roadway mitigation structure. Once installed, the PI will monitor activity and effectiveness.

ODOT strives to maintain a world class transportation system. This is an innovative project designed to address the impacts of roads on amphibians, including the effectiveness of mitigation measures. This project will provide guidance on the preservation of native biodiversity, make mitigation work more effective, improve the safety of people and wildlife, and enhance mitigation capacity.

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Executive Summary

The Nelsonville bypass (Figure 1) is a 4-lane divided highway with two interchanges that runs through the United States (US) Forest Service-Wayne National Forest around the city of Nelsonville in Athens and Hocking Counties, Ohio. The State Route 78 (SR 78) interchange of the bypass divides wetland habitats from upland habitats (Figure 2) and compromises the integrity of amphibian migration corridors.

In 2013 the Ohio Department of Transportation (ODOT) installed a commercially available barrier-ecopassage mitigation system specifically manufactured for amphibians (Figure 3, Figure 4). The intent of the 2013 barrier-ecopassage system was to minimize potential amphibian roadway mortality and provide habitat connectivity at a location where the new alignment of SR 78 bisected a known high-quality wetland with documented occurrences of breeding amphibians. We studied the 2013 mitigation system in place along SR 78 in 2015 and 2016. Using cameras, we detected 25 amphibians in the ecopassages, with 8 successfully traversing the length of a passage. During the same time period, pitfall traps placed at the barrier fence ends captured 256 amphibians. Given the number of amphibians captured at the fence ends compared to the number detected in the passages, we determined that the low level of ecopassage use indicated that the 2013 barrier-ecopassage structure was not functioning as well as planned.

Post-construction roadway mortality surveys encountered a total of 13,251 amphibians of 14 species dead along the 1.2 mile stretch of newly constructed SR 78 (Table 1). These surveys found that the greatest density of amphibian mortality was located approximately 1,200 feet south of the installed barrier-ecopassage system, where the new road separated upland habitat from a complex of constructed and preserved wetlands. Greater than 62% of the amphibian mortality was observed as this location (Figure 5).

We hypothesized that the effectiveness of the 2013 barrier-ecopassage system could be greatly improved on by designing passages that provided greater connectivity and by implementing a new mitigation system where we detected the most amphibian movement in 2015-2016 (Figure 5). In 2017 and 2018, we tested alternative ecopassages of varying entrance size, sky exposure, and line-of-sight (i.e., visibility through the ecopassage) to determine amphibian ecopassage preferences. In July 2017, we recommended that a new barrier-ecopassage structure with wider passages, more sky exposure, full line-of-sight, and soil substrate be implemented along SR 78 based on our findings. A new barrier-ecopassage mitigation system following those recommendations was installed in 2018 (Figure 3, Figure 4) along the road section where 62% of carcasses were detected in 2015-2016 (Figure 5). The 2018 barrier-ecopassage system had ecopassages with 48 in. wide by 28-32 in. tall entrances, grated tops, a maintained line-of-sight across the ecopassages, and a natural soil bottom (Figure 3). The 2018 passages also had taller fencing with a folded top lip to prevent amphibians from climbing over the fence (Figure 3). Finally, we monitored the 2018 barrier-ecopassage system to test our hypothesis that the effectiveness of the 2013 mitigation structure could be improved upon.

The objectives of our study included: (1) assessing various ecopassage designs using choice experiments and a pseudoecopassage experiment, (2) quantifying and comparing amphibian use of the 2013 and 2018 barrier-ecopassage systems, (3) assessing and comparing roadway mortality rates before this project (2015-early spring 2017), while the pseudoecopassages were in place, and while the 2018 barrier-ecopassage system was in place, (4) estimating amphibian population sizes to provide context for ecopassage use rates and roadway mortality rates, and (5) assessing barrier fence effectiveness and fence-end treatments that could improve barrier fencing.

Based on the behaviors of 1,280 animals across 20 species (Table 1, Table 2) in our choice experiments we determined that amphibians and reptiles (hereafter herpetofauna) preferred wider passages over narrower passages. We also determined that herpetofauna preferred full sky exposure in passages and full line-of-sight through passages. Full sky exposure and full line-of-sight were also preferred by herpetofauna in the pseudoecopassage experiment.

Through our vertebrate roadkill surveys, we detected a total of 21,302 carcasses, of which 94.62% were amphibian carcasses. From 2015 to 2019 we detected 14 amphibian species dead on the road (Table 1), with the largest peaks in mortality in the spring, and smaller peaks in the fall. We found a decrease in the amount of mortality over time for several species, which is likely tied to decreases in amphibian population sizes over time.

Before the pseudoecopassage experiment, 62% of the mortality was found in road section S4 (Figure 5). While the pseudoecopassage experiment was in place, we still found 34-20% of mortality in S4, but once the 2018 barrier-ecopassage system was in place we saw mortality shift to road sections S3 and S5. The 2018 mitigation structure reduced mortality from 62% of total mortality down to 4% in the area it was located, though we saw evidence of concentrated mortality at the barrier fence ends, (i.e., fence-end effect), with mortality shifted to the road sections on either side of the barrier fence.

Using camera traps, we detected 4,529 more amphibians in the 2018 ecopassages than in the 2013 ecopassages. We also found that amphibians detected in the 2018 ecopassages were 20.95% more likely to travel through an ecopassage than those detected in the 2013 ecopassages. Twelve amphibian species were detected in the 2018 ecopassages (Table 1), with eleven species traveling through a passage.

We calculated capture-recapture population estimates for eight amphibian species and area-density abundance estimates for one species. All species, except the American Toad, have declined in population size since 2015, likely due to high levels of additive mortality from the introduction of SR 78. Ideally, the 2018 barrier-ecopassage system will encourage population sizes to rebound in the future.

Regarding the barrier fencing, we did not encounter any amphibians that could climb over the downward-facing lip of the 2018 barrier fencing, but we did detect amphibians that could climb the 2018 ecopassage walls and go up through the grated top. We also captured 56 individual amphibians across five species in the pseudofence experiment funnel traps. During the pseudofence experiment, significantly more amphibians were captured in control traps (n=43) compared to treatment traps (n=13), indicating that a 45-degree angle bend in the fence turned amphibians around.

We conclude that the 2018 barrier-ecopassage system was more effective than the 2013 system. The 2018 mitigation system facilitated the movement of many more amphibians under the road because it was designed using studies of amphibian preferences. In addition, the 2018 mitigation greatly reduced amphibian roadway mortality in the location it was implemented because mitigation placement was informed by SR 78 post-construction roadway mortality surveys. Given time, the 2018 barrier-ecopassage system should support the rebound of amphibian populations, though this hypothesis warrants further study.

We recommend that the 2018 mitigation structure be well maintained to provide reductions in amphibian roadway mortality and increases in habitat connectivity. As the 2018 system greatly improved upon the 2013 system, any future amphibian barrier-ecopassage mitigation projects should have ecopassages that are as wide as possible, provide as much sky exposure as possible, have a maintained line of sight, and a natural soil substrate on the bottom. Fencing between passages needs to be durable and long lasting, with a downward-projecting top lip facing away from the road, and less than 150 ft. of fencing between passages. In addition, we recommend that the barrier fence ends be bent inward at a 45-degree angle to direct animals back toward the ecopassages. Experimental investigations of the fence-end effect are ongoing.

Project Background

The Nelsonville bypass, completed in 2013, is a stretch of United States Highway 33 that circumvents Nelsonville, Ohio (Figure 1). The bypass is a 4-lane divided highway that includes two interchanges, the Dorr Run Interchange and the SR 78 Interchange. The Nelsonville bypass runs through the largest remaining tract of continuous forest in Ohio, including wetlands and associated amphibian migration routes (Figure 1, Figure 2). Such habitat fragmentation affects wildlife populations by increasing mortality and reducing habitat connectivity (Forman et al. 2003, Jochimsen 2006, Andrews et al. 2015). Thus, ODOT installed wildlife mitigation measures, including an amphibian barrier-ecopassage mitigation system along the new stretch of SR 78 (Figure 3, Figure 4). The mitigation structure installed in 2013 was placed along SR 78, where the new roadway divided an established high-quality wetland. Plastic barrier fences were used to limit small animal access to the road, and direct animals to ecopassages, or corridors that channel animals safely under the roadway.

In 2015 and 2016, we studied the effectiveness of the installed amphibian barrier-ecopassage system implemented along SR 78 in 2013. Using cameras, we detected 25 amphibians in the ecopassages, with 8 successfully traversing the length of a passage. During the same time period, pitfall traps placed at the barrier fence ends captured 256 amphibians. Given the number of amphibians captured at the fence ends compared to the number detected in the passages, we determined that the low level of ecopassage use indicated that the 2013 barrier-ecopassage structure was not functioning as well as planned.

In addition, post-construction roadkill surveys revealed that the new roadway intercepted amphibian migration routes. A total of 13,251 amphibian carcasses of 14 species were found along the 1.2 mile stretch of newly constructed SR 78 (Table 1). These surveys found that amphibian mortality was greatest at a location approximately 1,200 feet south of the installed barrier-ecopassage system where the new road separated upland habitat from a complex of constructed and preserved wetlands. Greater than 62% of the amphibian mortality was observed as this location (Figure 5). Early analyses suggested that the observed levels of mortality were not sustainable for amphibians (Gibbs & Shriver 2005, Hopkins et al. 2018). This data on ecopassage usage and mortality by amphibians is important because there is limited knowledge of amphibian ecopassage use and effectiveness (Beebee 2013, Andrews et al. 2015).

We hypothesized that the effectiveness of the 2013 barrier-ecopassage system could be greatly improved for several reasons. First, the 2013 ecopassages were crowned with the roadway, eliminating the line-of-sight to the other end of the passages, potentially discouraging use if individuals perceived the ecopassages as blind tunnels. Second, the ecopassages had a series of two 1 in. x 1.5 in. perforations along the top, which allowed only about 5% sky exposure into the ecopassages, which may not be sufficient for effective amphibian navigation (Adler 1982, Sinsch 1990, Phillips & Borland 1994, Phillips et al. 1995, Wilson 2001, Sinsch 2006). Third, the ecopassages were 20 in. wide by 16 in. tall, while previous research found that larger tunnels were more effective (Woltz et al. 2008, Patrick et al. 2010). Finally, the ecopassages had concrete bottoms, whereas previous research suggested that ecopassages with natural substrates were preferred by amphibians (Lesbarrères et al. 2004, Woltz et al. 2008, Patrick et al. 2010).

We tested the impacts of these possible limitations by quantifying the use of alternative ecopassage designs by herpetofauna. Using choice experiments and “pseudoecopassages”, we tested alternative ecopassages of varying entrance size, sky exposure, and line-of-sight (see Appendix 3: Choice Experiments, Appendix 4: Pseudoecopassage Experiment, and Appendix 5: Comparison of Choice Experiments & Pseudoecopassage Experiment). We then made recommendations for a new mitigation structure to be implemented along SR 78 (Hopkins & Kuchta 2017, see Appendix 8: Recommendations for 2018 Barrier-ecopassage System).

A new barrier-ecopassage mitigation structure was installed in 2018 (Figure 3, Figure 4, see Appendix 9: Implementation of 2018 Barrier-ecopassage System). The 2018 barrier-ecopassage system had ecopassages with larger entrances, greater sky exposure, maintained line-of-sight across the passages, and a natural soil bottom (Figure 3). The 2018 passages also had taller fencing with an inverted top lip to prevent climbing herpetofauna from going over the fence (Figure 3). In addition, the last 3-8 ft. of the barrier fence ends were curved perpendicular to the road to prevent amphibian trespass around the fence ends. The 2018 barrier-ecopassage system was also in the area with the highest documented occurrences of post-construction roadway mortality (Figure 4, Figure 5).

We monitored the 2018 barrier-ecopassage system to test our hypothesis that the effectiveness of the 2013 mitigation structure could be improved upon. If the 2018 system facilitated the safe movement of more animals and prevented more animal-vehicle collisions than the 2013 system, then roadway impacts to amphibian populations along SR 78 would be reduced. Our findings concerning the 2018 barrier-ecopassage system should be used by future mitigation structure project managers in Ohio and elsewhere.

Research Context

Amphibian Road Ecology Research

Roads bisect habitats, reducing connectivity and creating opportunities for animal-vehicle collisions (Dodd et al. 2004, Langen et al. 2009, Beebee 2013, Andrews et al. 2015). The ecological impacts of roads are increasing as new roads are constructed each year. For many herpetofauna, high adult survival is essential for population persistence, because recruitment rates into the adult class are low (Vonesh & De La Cruz 2002, Price et al. 2012). Consequently, additive mortality imposed by roadways during migrations can lead to population decline or extinction (Gibbs & Shriver 2005). Migratory amphibian species are the most highly impacted by roadway mortality and reductions in habitat connectivity (Beckmann & Shine 2015).

Amphibian Mitigation Systems Research

To lessen roadway impacts, mitigation measures are increasingly being employed throughout the world (Bush et al. 1991, Clevenger et al. 2002, Dodd et al. 2004, Helldin & Petrovan 2019). However, mitigation effectiveness is not commonly evaluated (Aresco 2005b, Patrick et al. 2010, Andrews et al. 2015), and there are no studies that report on amphibian mortality and movement patterns before and after mitigation installation (Aresco 2005b, Baxter-Gilbert et al. 2015).

ECOPASSAGES - Few studies have analyzed herpetofaunal behavior in relation to roads (Mazerolle et al. 2005, Bouchard et al. 2009) or quantified ecopassage effectiveness (Woltz et al. 2008, Patrick et al. 2010, Pagnucco et al. 2011, Helldin & Petrovan 2019). Previous studies of ecopassage design assessed entrance size, length, substrate, and presence of light in the passage (Lesbarrères et al. 2004, Woltz et al. 2008, Patrick et al. 2010); none have assessed line-of-sight nor the amount of sky exposure. Little is known about impacts of barrier-ecopassage systems on herpetofauna roadway mortality, habitat connectivity, and population persistence, as most previous studies lack data prior to mitigation installation (Aresco 2005b, Beebee 2013). The only other Ohio study assessing ecopassages was along State Route 88 in Trumbull County, but that study did not focus on herpetofauna, compare ecopassage designs, or collect quantitative data before and after mitigation installation (Lininger & Perlik 2014).

FENCING - Previous studies have demonstrated that fences may increase the barrier effect of roads if they are not connected to ecopassages (Jaeger & Fahrig 2004). Studies have also documented concentrated animal mortality at barrier fence ends, known as the fence-end effect (Clevenger et al. 2001, Dodd et al. 2004, Cserkész et al. 2013), because animals often move along the barrier and disperse around the fence end (Huijser et al. 2016). Two fence-end treatments have been recommended to combat the fence-end effect: (1) bend the fence ends perpendicular to the road to guide animals away from the road, or (2) bend the fence ends inward to guide animals back to associated ecopassages (Kruidering et al. 2005, Huijser et al. 2015, Gunson et al. 2016). To date, no studies have evaluated fence-end treatment effectiveness for amphibians.

Nelsonville Bypass Study, 2015-2016

We studied amphibian roadway mortality, population sizes, and use of the 2013 barrier-ecopassage system along SR 78 as part of the "Effectiveness of Wildlife Mitigation Treatment on the Nelsonville Bypass" project (Hopkins et al. 2018). During 2015-2016, the 2013 ecopassages (Figure 3, Figure 4) were entered by 25 amphibians, with 8 of those traveling through a passage. At the same time, pitfall traps placed at the barrier fence ends captured 256 amphibians. This suggests that the barriers were functioning, but that individuals chose not to enter the passages. Post-construction roadkill surveys revealed that the new roadway intercepted amphibian migration routes with 13,251 carcasses from 14 amphibian species found along the 1.2 mile stretch of the new SR 78 (Table 1). Based on population size estimates and road mortality data, at least four species may be undergoing population declines due to high roadway mortality (Hopkins et al. 2018). Along SR 78, 62% of detected carcasses were found in a 700 ft. length of road southwest of the present mitigation structure, where the new road divided a wetland complex from forested upland habitat (Figure 5).

Research Goal and Objectives

The goals of this study were to identify the most effective ecopassage design for amphibians through behavioral testing and to assess the effectiveness of a newly implemented amphibian roadway mitigation structure designed based on our recommendations (Hopkins & Kuchta 2017). To complete our first goal, we used choice experiments and a pseudoecopassage experiment to assess different ecopassage designs. We included reptiles in our ecopassage design experiments as they are also commonly impacted by roads, but amphibians are the focal group of this project. Then we monitored roadway mortality, population sizes, and use of the 2018 barrier-ecopassage system along SR 78 to assess effectiveness of the mitigation system.

The following objectives were completed:

i. Assessment of various ecopassage designs using choice experiments and a pseudoecopassage experiment.
ii. Quantification and comparison of amphibian use of the 2013 and 2018 barrier-ecopassage systems.
iii. Assessment and comparison of roadway mortality rates before this project (2015-early spring 2017), while the pseudoecopassages were in place, and while the 2018 barrier-ecopassage system was in place.
iv. Estimation of amphibian population sizes to provide context for rates of ecopassage use and roadway mortality.
v. Assessment of barrier fence effectiveness and fence-end treatments that could improve barrier fencing.

Research Approach

Choice Experiments

We used choice experiments to assess herpetofauna preference of ecopassage entrance size, sky exposure, and line-of-sight. Our choice experiments were behavioral tests in which an animal was placed in a central chamber under a bucket and given one minute to acclimate. Then we lifted the bucket from outside of the chamber and gave the animal 10 minutes to choose an exit passage. Our first experiment tested for preference in passage entrance size. The setup of this experiment included a center chamber and four exits that varied in size: 20 in. x 16 in. (same entrance size as the 2013 ecopassages installed along SR 78), 24 in. x 24 in., 40 in. x 24 in., and 40 in. x 44in. Our second experiment tested for the influence of sky exposure on passage preference. The experimental setup included a center chamber and three exits varying in the amount of sky exposure: no sky exposure, partial sky exposure, and full sky exposure. The third experiment tested for the influence of line-of-sight, or the ability to see across an ecopassage, on passage preference. The setup of the experiments included a center chamber and three exits that varied in line-of-sight to the end of a passage: eliminated line-of-sight, reduced line-of-sight, and fully maintained line-of-sight. To evaluate preference of passage parameters we used two contingency table analyses: a chi-square test (Patrick et al. 2010) and a G-test (Woltz et al. 2008). Our null hypotheses were that all exit passages for each choice experiment would be used equally, independent of the ecopassage parameter being manipulated in each choice experiment. See Appendix 2: Choice Experiments for greater detail.

Pseudoecopassage Experiment

We created a pseudoecopassage experiment to test alternative passage designs along SR 78. Silt fence was installed on both sides of the road to prevent herpetofauna from accessing the road and guide them to pseudoecopassage replicates. Each pseudoecopassage was a 4 ft. long tunnel placed perpendicular to the silt fence and ending in a pitfall trap. Animals found in the trap were transported across the road by hand. Thus, our pseudoecopassages simulated the connectivity provided by ecopassages and allowed us to test alternative ecopassage designs. Replicates include four pseudoecopassage designs: (1) full sky exposure and full line-of-sight, (2) partial sky exposure and full line-of-sight, (3) full sky exposure and no line-of-sight, and (4) partial sky exposure and no line-of-sight (Figure A3-1). These were placed 100 ft. apart along the silt fence barrier (Figure A3-2, Figure A3-3). In 2017 eight pseudoecopassage replicates were in place (covering 500 ft. along SR 78) and in 2018 14 replicates were in place (covering 800 ft. along SR 78). The order of the four pseudoecopassage designs was random among the experimental replicates (Figure A3-1, Figure A3-2). The pseudoecopassage experiment was conducted in the area along SR 78 where 62% of recorded mortality occurred in 2015 and 2016 (Figure 5). Pitfall traps were also installed at both ends of the silt fences to capture animals that were going around the ends of the fence. To assess whether adding the pseudoecopassage design treatments affected animal choices we used chi-square analyses to compare the number of animals captured at the fence ends, to the number captured in the two pseudoecopassages on the outside of each pseudoecopassage replicate, and to the number captured in the two ecopassages on the inside of each pseudoecopassage replicate before and after design treatments were added to the replicates. To evaluate which pseudoecopassage design treatment was preferred we used two types of contingency table analyses: a chi-square test (Lesbarrères et al. 2004, Patrick et al. 2010) and a G-test (Woltz et al. 2008). Our null hypothesis was that all pseudoecopassages would be used equally. See Appendix 3: Pseudoecopassage Experiment for further detail.

Roadway Mortality

We monitored vertebrate roadway mortality along a 1.2 mile stretch of SR 78. Daily morning walking surveys to count carcasses went from the US Highway 33 exit to Burr Oak Blvd. Detected carcasses were identified and marked with a GPS unit then removed from the roadway to prevent double-counting later (Teixeira et al. 2013, Beckmann & Shine 2015). In addition, the roadway section the animal was found in was recorded; roadway sections were determined by habitat changes along SR 78 (Figure 5; labeled in Figure A5-1). Roadkill survey data was tabulated and summarized in graphical format for descriptive analysis. We also evaluated road section mortality data from six time frames: (1) before the pseudoecopassage experiment, (2) during the 2017 pseudoecopassage experiment, (3) during the 2018 pseudoecopassage experiment, (4) during the 2018 barrier-ecopassage system implementation, (5) after 2018 barrier-ecopassage system implementation occurred while repairs to the system were taking place, and (6) after the 2018 barrier-ecopassage system was fully completed. To assess whether the number of vertebrate carcasses detected in each roadway section for a given time frame varied we used chi-square tests, with the null hypotheses that all roadway sections would have equal amounts of carcasses in each time frame. To assess whether the six time frames had different roadway section mortality rates from each other we used chi-square tests for goodness-of-fit. Thus, our null hypotheses were that any given time frame would not have significantly different road section mortality frequencies as compared with the road section mortality frequencies of any other time frame. See Appendix 5: Roadway Mortality for additional detail.

Population Monitoring

To evaluate the impacts of roadway mortality rates and ecopassage use rates we needed amphibian population estimates (Andrews et al. 2015). Thus, we conducted dip net surveys, hoop net trapping, minnow trapping, pitfall trapping, spotlight surveys, and Spring Peeper call surveys to assess populations (Heyer et al. 1994, Olson et al. 1997, Enge 2001, Gunzburger 2007, Dodd 2010). All captured adult and large juveniles were measured, weighed, sexed, and marked using a batch marking technique (United States Geological Survey 2001, Ferner 2007, Dodd 2010). These captures were used to generate capture-recapture population estimates, while the Spring Peeper call surveys were used to generate area-density abundance estimates (Heyer et al. 1994, Pollock 2000, Gunzburger 2007, Mazerolle et al. 2007). We calculated four capture-recapture estimates that accounted for survey and trapping effort for eight species for each year (2015-2019) and averaged them to get a population estimate for each species for each year (Pollock 2000, Mazerolle et al. 2007). Spring Peeper area-density abundance estimates based on number of calling males, the female to male ratio, and wetland size were calculated for each wetland with available Spring Peeper breeding habitat for each year, 2015-2019 (Buckland 1987, Crouch & Patton 2002, Mazerolle et al. 2007). See Appendix 6: Population Monitoring for more detail.

Monitoring of 2013 Barrier-Ecopassage System (2015-2018)

We monitored the 2013 barrier-ecopassage system (Figure 3, Figure 4) for three and a half years (2015-2017 and February-May 2018). To monitor the effectiveness of the barrier fencing, one pitfall trap was placed at each end of the barrier fence (Mata et al. 2005, Baxter-Gilbert et al. 2015). We checked traps daily in the spring and fall of each year. We assessed the ecopassages using camera traps to obtain images of animals using the ecopassages (Pagnucco et al. 2011, Leeb et al. 2013). Spypoint Tiny-W3 cameras equipped with Jokie2 break-beam sensors were placed at each entrance of the ecopassages to take photographs of animals in the passages (Leeb et al. 2013). We assessed the effectiveness of the 2013 barrier-ecopassage system by tabulating and graphing the number of amphibians captured in the pitfall traps and the number detected by the ecopassage camera traps. See Appendix 7: Monitoring of 2013 Barrier-ecopassage System for further detail.

Monitoring of 2018 Barrier-Ecopassage System (2018-2019)

We monitored the 2018 barrier-ecopassage system (Figure 3, Figure 4) for one year (July 5, 2018 through July 5, 2019). To monitor barrier fence effectiveness two pitfall traps were placed at each end of the plastic barrier fence, one on each side of the fencing (Mata et al. 2005, Baxter-Gilbert et al. 2015). Pitfall traps were checked daily throughout the fall and spring. We used camera traps to assess the number of amphibians using the ecopassages (Pagnucco et al. 2011, Leeb et al. 2013, Hobbs & Brehme 2017). We placed Spypoint Tiny-W3 cameras on the sides of each ecopassage at the entrance angled so that the cameras viewed at least half (> 24 in.) of the entrance floor. We also installed 6-in. tall ramps that were buried into the soil 2 in. to get clear photos of amphibians entering the ecopassages (Hobbs & Brehme 2017). In the fall of 2018, we tested cameras with Jokie2 break-beam sensors in two ecopassages and cameras running on 30-second time lapse photography in three ecopassages. In the spring of 2019, we ran all cameras on 30-second time lapse photography. In the spring of 2019, we also placed two additional cameras facing straight down (each viewing about 13 in. of the passage entrance) in the west side entrances of the ecopassages to get dorsal views of amphibians for better identification. We assessed the effectiveness of the 2018 barrier-ecopassage system by tabulating and graphing the number of amphibians captured in the pitfall traps and the number detected by the ecopassage camera traps. See Appendix 10: Monitoring of 2018 Barrier-ecopassage System for greater detail.

Fence Behavioral Observations

We conducted behavioral observations of amphibians moving along the 2018 barrier fencing in place along State Route 78 near Nelsonville in Athens County, Ohio. Our behavioral observations were conducted on rainy nights and mornings (July 17, 2018-June 20, 2019) in order to observe animal behaviors along the fence. For each individual amphibian observed we recoded species, time, which side of the road the animal was on, direction of travel, length of fence traveled, whether the animal encountered an ecopassage, and the behaviors exhibited at an ecopassage. We also observed four behavioral elements: (1) if the curvature of the barrier fence directed amphibian movement, (2) if amphibians were able to climb up and/or over the 2018 barrier fencing, (3) the distance animals moved along the fence before turning away from the fence, and (4) movement patterns at the ends of the fencing. Curving the fence between passages, applying a lip to the top of the fence, having less than 150 ft. between ecopassages, and bending the fence ends have all been recommended as best management practices for amphibian fencing (Clevenger et al. 2001, Dodd et al. 2004, Aresco 2005b, Kruidering et al. 2005, Huijser et al. 2015, Huijser et al. 2016), but none have been empirically evaluated. See Appendix 12: Fence Behavioral Observations for additional detail.

Pseudofence Experiment

We used experimental pseudofences to assess the effectiveness of a 45-degree angle fence-end treatment for amphibians along SR 78. We used silt fencing with wood posts create our pseudofences. Each pseudofence was 13 ft. long with an attached 40 in. long fence-end treatment. The fence-end treatment was angled inwards at a 45-degree angle to test our hypothesis that an angled fence-end will turn amphibians around and prevent them from circumventing the barrier fence. One funnel trap was placed at each end of every pseudofence array. We designated traps located on the linear side of the pseudofence array as the ‘control’ traps, whereas ‘treatment’ traps were adjacent to the 40 in. fence-end treatment. In February 2019, we installed 13 pseudofence arrays adjacent to the wetland on the east side of the road along the road section where 62% of the mortality occurred in 2015-2016 (Figure 5). We used a paired t-test to analyze the difference in amphibian captures between control and treatment traps. Our null hypothesis was that there would be no difference in the number of amphibians captured between the control and treatment traps. See Appendix 13: Pseudofence Experiment for more detail.

Data Analysis

Data and analyses were carried out using Microsoft Excel, the statistical programming language R (R Core Team 2016), and ArcGIS (ESRI 2014). We also made comparisons between choice experiments and pseudoecopassage experiment (see Appendix 4: Comparison of Choice Experiments and Pseudoecopassage Experiment) and between the 2013 and 2018 barrier-ecopassage systems (see Appendix 11: Comparing Effectiveness of 2013 and 2018 Barrier-ecopassage Systems). All analyses were performed by the principal investigator and graduate student researchers.

Research Findings and Conclusions

We used 20 species of herpetofauna in our choice experiments (Table 1, Table 2, Figure A2-4). In our choice experiments, we found that herpetofauna preferred wider ecopassages (Table A2-1, Figure A2-5), ecopassages with full sky exposure (Table A2-2, Figure A2-6), and ecopassages with a full line-of-sight (Table A2-3, Figure A2-7). This was mirrored by the results of the pseudoecopassage experiment, where the majority of herpetofauna chose passages with a fully maintained line-of-sight and full sky exposure (Table A3-2, Figure A3-7). These two experiment types are consistent in that animals made the same choice despite experiment type (Table A4-1, Figure A4-2, Table A4-2, Figure A4-3).

Through our roadkill surveys, we detected a total of 21,302 vertebrate carcasses, of which 94.62% were amphibians (Table 5A-1, Figure 5A-4, Figure 5A-5). The average number of carcasses detected per survey varied by year and season (Table 5A-1). We tended to find the largest peaks in mortality in the spring, with smaller peaks in the fall (Figure A5-3). From 2015 to 2019 we recovered 14 amphibian species dead on the road (Table 1), and patterns of mortality varied annually by species group (Figure A5-5). We found that mole salamanders (Jefferson Salamander, Spotted Salamander, and Marbled Salamander) and Red-spotted Newts had a decrease in the number of carcasses detected each year, and Spring Peepers and Gray Treefrogs also show this trend (Figure A5-5). The declines in roadway mortality are likely due to decreases in population sizes over the study period (Figure A6-3, Figure A6-4).

We found that levels of roadway mortality significantly varied by roadway section in all six time frames evaluated (Table A5-2; all chi-square values >28.82, all P-values <0.001). All time frames also had significantly different road section mortality frequencies than all other time frames (all chi-square values >52.10, all P-values <0.001). Before the pseudoecopassage experiment was underway, 62% of the mortality was found in road section S4 (Figure 5, Table A5-2, Figure A5-6). While the pseudoecopassage experiment was in place mortality in S4 was still substantial, but once the 2018 barrier-ecopassage system was in place we saw mortality shift to road sections S3 and S5 (Table A5-2, Figure A5-6). Overall, the 2018 mitigation structure reduced mortality from 62.21% to 3.98% in the area it was located, though we do see evidence of a fence-end effect since mortality shifted to the adjacent two road sections.

We were able to calculate capture-recapture population estimates for eight amphibian species (Jefferson Salamander, Spotted Salamander, Marbled Salamander, Red-spotted Newt, American Toad, American Bullfrog, Green Frog, and Pickerel Frog; Figure A6-3) and area-density abundance estimates for Spring Peepers (Figure A6-4). All species, except the American Toad, show evidence of decline in population size since 2015 (Figure A6-3, Figure A6-4).

We captured 339 amphibians in the 2013 barrier-ecopassage system fence end pitfall traps and there was a decline in the number of amphibians captured each spring from 2015-2018 (Table A7-1, Figure A7-3). We detected a total of 35 individuals of seven amphibian species at the passage entrances (Table 1), with 12 individuals of three species detected at both ends of a passage (Table A7-1, Figure A7-3).

We captured 1,284 amphibians in the 2018 barrier-ecopassage system fence end pitfall traps (Figure A10-7 through A10-9). We detected a total of 4,564 amphibians at the ecopassage entrances, of which 2,521 were detected at both ends of an ecopassage, suggesting movement through the ecopassage (Table A10-1, Table A10-2). Twelve amphibian species were detected by the 2018 ecopassage camera traps (Table 1), with all species traveling through a passage, except the Red-backed Salamander (a non-migratory species) (Figure A10-7 through A10-9). We did not discover any amphibians that could climb over the downward-facing lip of the 2018 barrier fencing, but we did detect Spring Peepers and Gray Treefrogs that climbed the 2018 ecopassage walls and went up onto the road through the grated ecopassage tops with the ecopassage camera traps and during spotlight surveys.

We captured 56 individual amphibians from five species in the pseudofence experiment funnel traps. During the pseudofence experiment, significantly more amphibians were captured in control traps (n=43) compared to treatment traps (n=13), suggesting that a 45-degree angle bend in the fence was either avoided or turned amphibians around.

In conclusion, the 2018 mitigation structure was constructed with wider passages, greater sky exposure, and full line-of-sight (Table A11-1) due to the findings of our choice experiments and pseudoecopassage experiment. We detected 4,529 more amphibians in the 2018 ecopassages than in the 2013 ecopassages. We also found that amphibians detected in the 2018 ecopassages were 20.95% more likely to travel through an ecopassage than those detected in the 2013 ecopassages (Table A11-1). The addition of the 2018 mitigation structure reduced mortality along the road stretch it was introduced in by about 58% (Table A5-2, Figure A5-6). While amphibian populations did decline from 2015-2016 to 2019, the 2018 barrier-ecopassage mitigation structure may allow for population growth in the future.

Recommendations for Implementation of Research Findings

We recommend that the new structure be maintained, so that it decreases roadway mortality and increases connectivity among habitats. As the 2018 system greatly improved upon the 2013 system, any future amphibian barrier-ecopassage structures should have ecopassages that are as wide as possible, provide as much sky exposure as possible, and have a maintained line-of-sight (Woltz et al. 2008, Patrick et al. 2010, Hopkins & Kuchta 2017). We also recommend that the bottom substrate be natural soil (Lesbarrères et al. 2004, Woltz et al. 2008, Patrick et al. 2010, Clevenger and Huijser 2011, Gunson et al. 2016). Fencing between passages needs to be durable and long lasting, with a downward facing lip, and less than 150 ft. of fencing between passages (Clevenger and Huijser 2011, Gunson et al. 2016). In addition, the barrier fencing should be curved between passages to direct animals toward the ecopassages, and fence ends should be bent inward at a 45-degree angle to direct animals back toward the ecopassages (Kruidering et al. 2005, Clevenger & Huijser 2011, Huijser et al. 2015, Gunson et al. 2016).

Installation & Maintenance Recommendations

Maintenance of the 2018 barrier-ecopassage mitigation system is necessary throughout the life of the structure (Jacobson & Tonjes 2015). This includes the barrier fencing, as it is necessary to guide animals to the ecopassages (Cunnington et al. 2014, Baxter-Gilbert et al. 2015, Jacobson & Tonjes 2015, Markle et al. 2017). We recommend six maintenance visits to the 2018 barrier-ecopassage system annually to conduct inspections and make repairs (see timeline in Appendix 14: Recommended 2018 Barrier-ecopassage System Maintenance Plan). Recommended visits include conducting full inspections of the ecopassages and fencing, implementing repairs, investigating the surrounding landscape for erosion damage, mechanical removal of vegetation growing along the barrier fence and in front of ecopassages, and chemical removal of vegetation growing in the ecopassages. In addition, ecopassage cleanouts, drainpipe flushing, barrier fence replacement, and inspections of ecopassage concrete interiors will be needed. We also recommend that mesh be avoided in further erosion control measures as it entraps and kills wildlife (Mitchell et al. 2008, Kapfer & Paloski 2011; Figure A14-1). Finally, we recommend that winter road salting be avoided along the 2018 mitigation structure (Sanzo & Hecnar 2006, Karraker et al. 2008, Mitchell et al. 2008) and that maintenance personnel, equipment, and vehicles be inspected for invasive species before entering the 2018 mitigation site (Parendes & Jones 2000, Semlitsch 2003, Maerz et al. 2005, Kingsbury & Gibson 2012, Jacobson & Tonjes 2015); we recognize that these last two recommendations may be challenging to implement, yet they are important to consider. A full maintenance plan for the 2018 structure can be found in Appendix 14: Recommended 2018 Barrier-ecopassage System Maintenance Plan.

Ecopassage Recommendations

The 2018 barrier-ecopassage system provided greater connectivity than the 2013 system (Table A11-1) and greatly reduced mortality in the area where it was placed (Table A5-2, Figure A5-6). Like the 2018 structure, future amphibian barrier-ecopassage structures should have ecopassages that are as wide as possible, provide as much sky exposure as possible, and have a maintained line of sight (Woltz et al. 2008, Patrick et al. 2010, Hopkins & Kuchta 2017). We also recommend that the bottom substrate in ecopassages be natural soil (Lesbarrères et al. 2004, Woltz et al. 2008, Patrick et al. 2010, Clevenger and Huijser 2011, Gunson et al. 2016). Installing the best mitigation measures possible in future mitigation projects will reduce the negative impacts of roads on migratory amphibian populations. Many habitats are fragmented throughout Ohio, as well as the rest of the US, and would benefit from improved mitigation.

Fencing Recommendations

Barrier fencing between passages needs to be durable and long lasting, as fencing is necessary to guide animals to ecopassages (Cunnington et al. 2014, Baxter-Gilbert et al. 2015). A well-maintained fence is critical to the function of the ecopassage system. The bottom edge of wildlife exclusion barrier fencing must be buried at least 4-6 in. into the ground to prevent animals from going under the fence. It is also beneficial for barrier fencing to have a downward-projecting lip along the top edge to prevent animals from climbing over the fence, particularly Spring Peepers and Gray Treefrogs. Best practices manuals recommend less than 150 ft. of fencing between passages for amphibians (Clevenger and Huijser 2011, Gunson et al. 2016). However, we only detected one species, the Green Frog, that traveled this distance during our fence behavioral observations, so shorter fences between ecopassages may further improve the function of the ecopassage system; more work is needed on this element of ecopassage design (see Appendix 12: Fence Behavioral Observations).

We documented a fence-end effect despite bending the last 3-8 ft. of fence at each end away from the road at a 120- to 90-degree angle (Clevenger et al. 2001, Dodd et al. 2004, Cserkész et al. 2013, Huijser et al. 2016). We recommend bending the 2018 barrier fence end to a 45-degree angle (see Appendix 13: Pseudofence Experiment) and lengthening the amount of fence beyond the bend to limit the fence-end effect and thereby reduce roadway mortality (Kruidering et al. 2005, Huijser et al. 2015, Gunson et al. 2016).

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Appendix 1: Document Figures & Tables

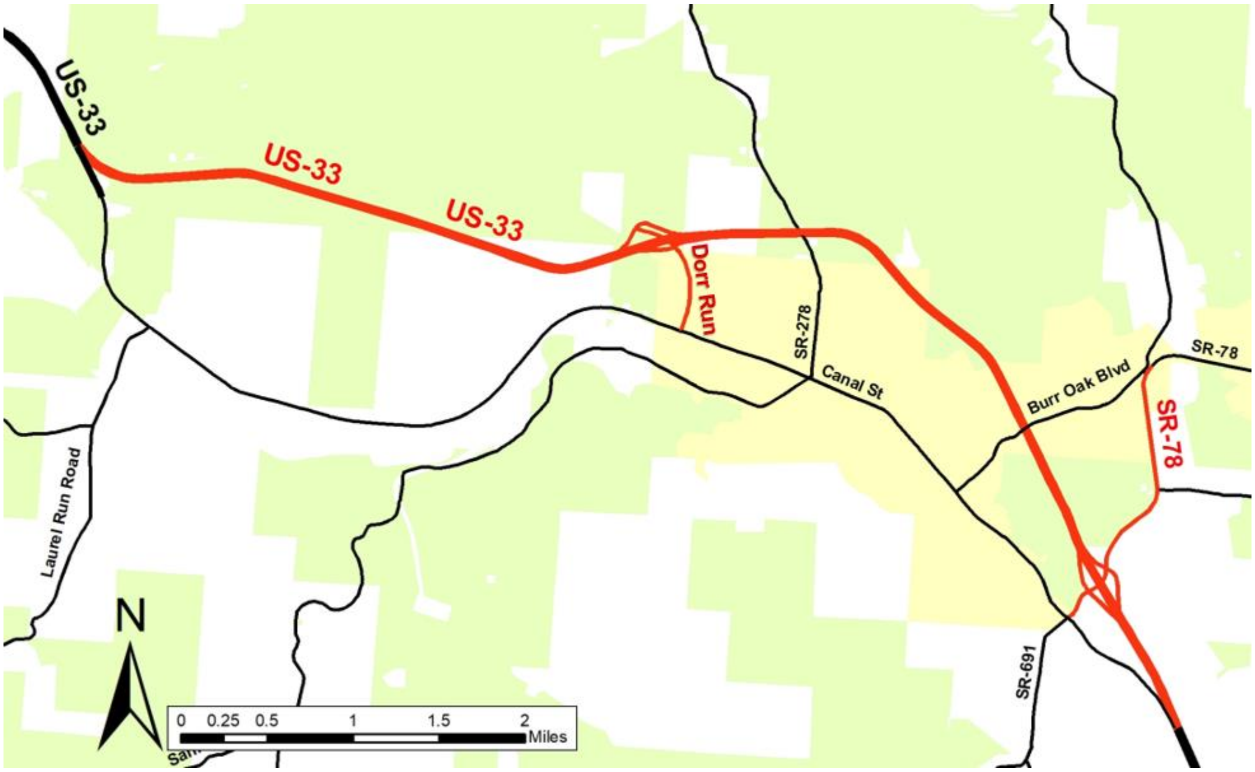

Figure 1. Map of the Nelsonville bypass (red) in Athens and Hocking Counties, Ohio. The bypass is made up of United States highway 33 (US-33) and two interchanges: the Dorr Run interchange and the State Route 78 (SR-78) interchange. Green areas represent the United States Forest Service – Wayne National Forest, and yellow areas represent the city of Nelsonville.

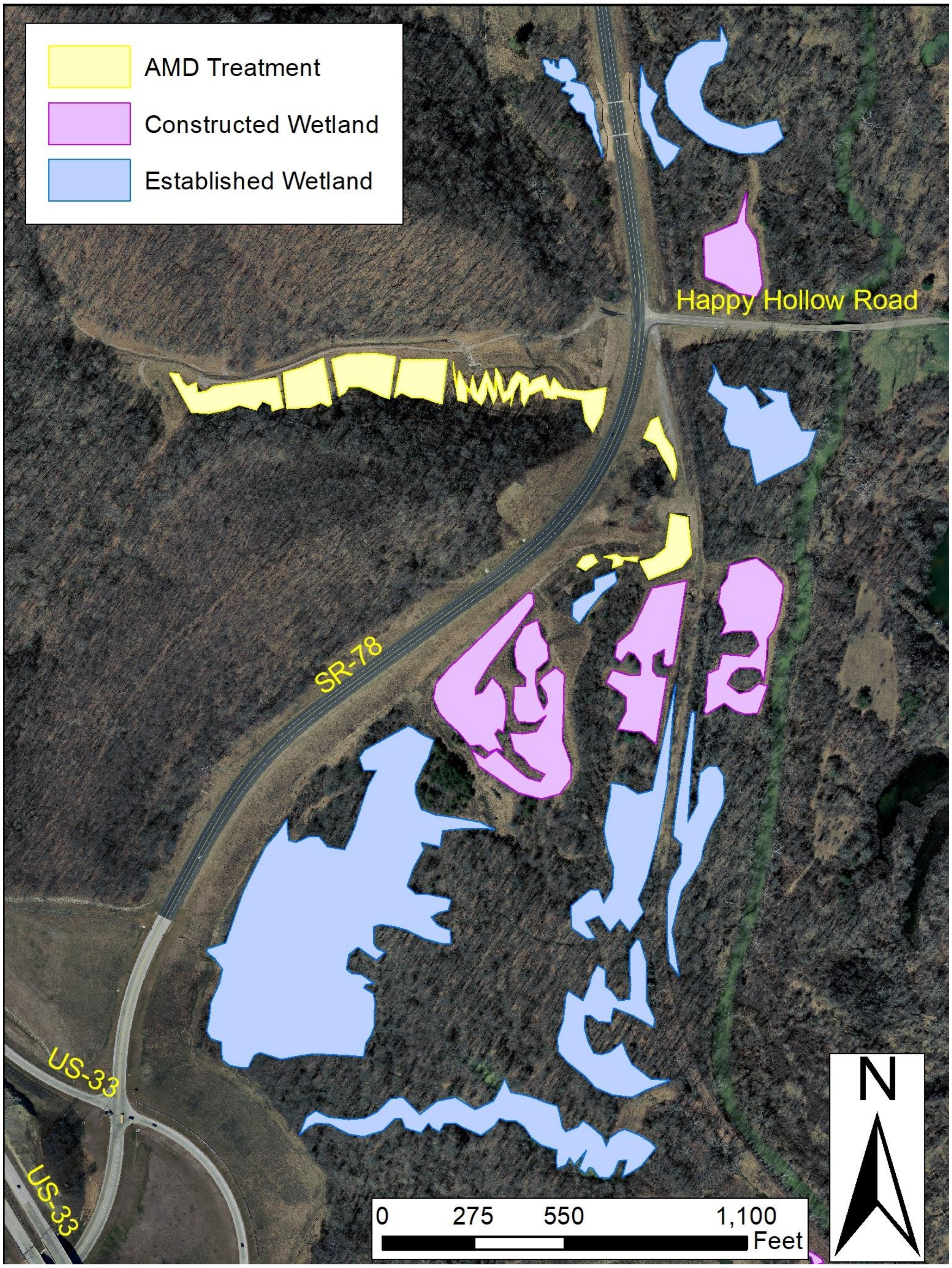

Figure 2. Map of wetland habitats along the State Route 78 interchange area of the Nelsonville bypass in Athens County, Ohio. Upland habitats are on the west side of State Route 78.

Figure 3. Images of the 2013 and 2018 barrier-ecopassage systems in place along State Route 78 near Nelsonville in Athens County, Ohio. Entrance view of a 2013 barrier-ecopassage system ecopassage (top left); 2013 ecopassage crossing the road (top center); 2013 barrier fence (top right). Entrance view of a 2018 barrier-ecopassage system ecopassage (bottom left); ecopassage crossing the road (bottom center); 2018 barrier fence (bottom right).

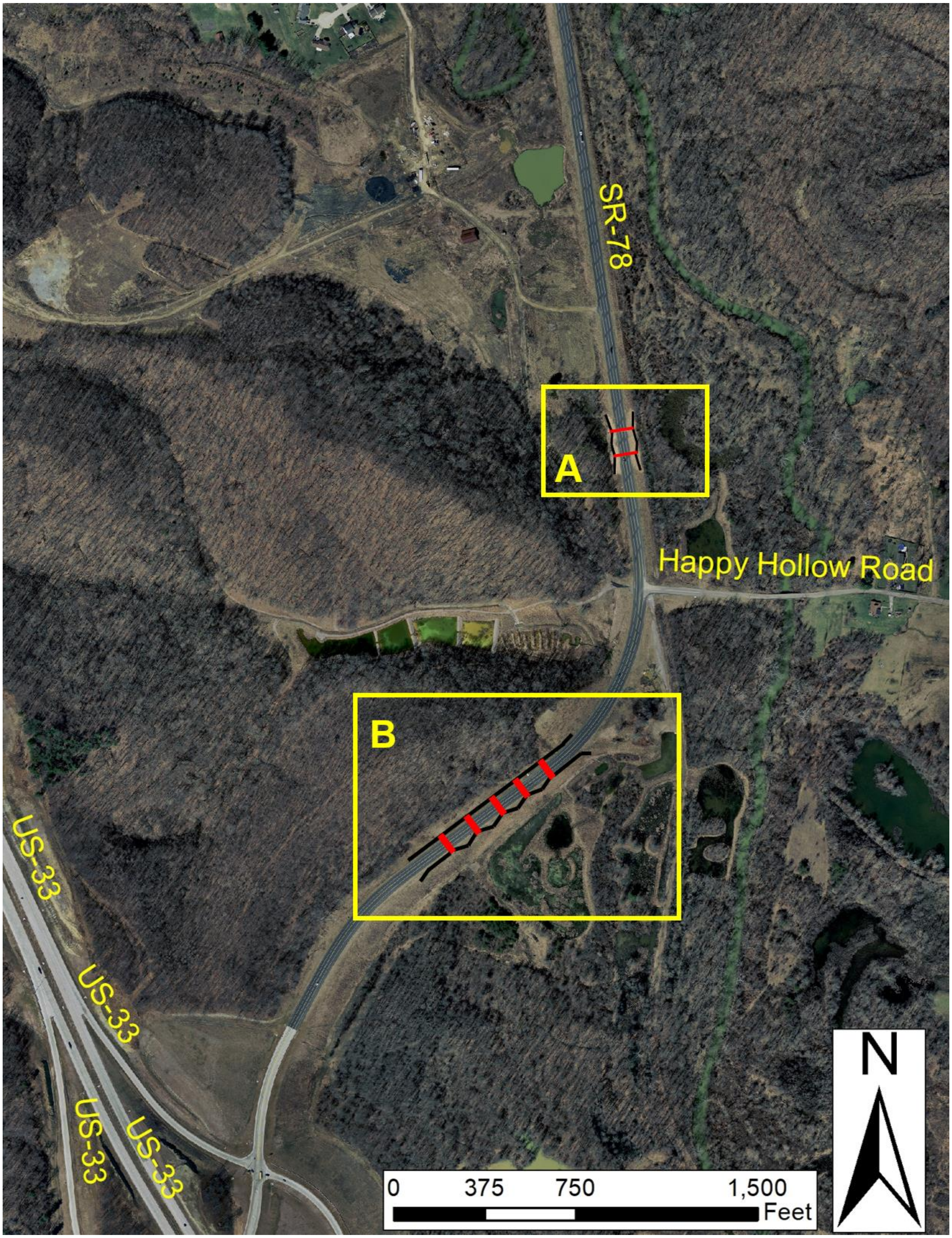

Figure 4. Map of 2013 (A) and 2018 (B) barrier-ecopassage systems in place along State Route 78 near Nelsonville in Athens County, Ohio. Ecopassages are red and barrier fencing is black.

Table 1. Amphibian species detected along State Route 78 near Nelsonville in Athens County, Ohio by each capture method 2015-2019. “Choice Exps” designates species used in the choice experiments and “Pseudo-eco Exp” designates species captured in the pseudoecopassage experiment.

Species	Scientific Name	Dip net surveys	Hoop net traps	Minnow traps	Pitfall traps	Spotlight surveys	Choice Exps	Pseudo-eco Exp	Roadkill Surveys	2013 Ecopassage camera traps	2018 Ecopassage camera traps
AMPHIBIANS											
Jefferson Salamander	*Ambystoma jeffersonianum*	X		X	X	X	X	X	X	X	X
Spotted Salamander	*Ambystoma maculatum*	X		X	X	X	X	X	X		X
Marbled Salamander	*Ambystoma opacum*			X	X	X	X	X	X	X	X
Red-spotted Newt	*Notophthalmus viridescens*	X		X	X	X	X	X	X	X	X
Long-tailed Salamander	*Eurycea longicauda*	X									
Eastern Red-backed Salamander	*Plethodon cinereus*				X	X		X	X		X
Northern Slimy Salamander	*Plethodon glutinosus*				X				X		
American Toad	*Anaxyrus americanus*	X		X	X	X	X	X	X	X	X
Unidentified Gray Treefrogs	*Hyla chrysoscelis/ versicolor*			X	X	X	X	X	X		X
Spring Peeper	*Pseudacris crucifer*	X		X	X	X	X	X	X	X	X
Western Chorus Frog	*Pseudacris triseriata*			X							
American Bullfrog	*Rana catesbeiana*	X	X	X	X	X	X	X	X	X	X
Green Frog	*Rana clamitans*	X	X	X	X	X	X	X	X	X	X
Pickerel Frog	*Rana palustris*	X		X	X	X	X	X	X		X
Northern Leopard Frog	*Rana pipiens*			X	X	X	X		X		
Wood Frog	*Rana sylvatica*			X	X	X	X	X	X		X

Figure 5. Percentages of mortality found in each roadway section along State Route 78 near Nelsonville in Athens County, Ohio, 2015-2016. 62% of roadway mortality was found in section S4, the fourth section south of the 2013 barrier-ecopassage mitigation structure.

Table 2. Reptile species detected along State Route 78 near Nelsonville in Athens County, Ohio by each capture method 2015-2019. “Choice Exps” designates species used in the choice experiments and “Pseudo-eco Exp” designates species captured in the pseudoecopassage experiment.

Species	Scientific Name	Dip net surveys	Hoop net traps	Minnow traps	Pitfall traps	Spotlight surveys	Choice Exps	Pseudo-eco Exp	Roadkill Surveys	2013 Ecopassage camera traps	2018 Ecopassage camera traps
REPTILES											
Copperhead	*Agkistrodon contortix*								X		
Worm Snake	*Carphophis amoenus*				X				X		
Black Racer	*Coluber constrictor*	X			X		X	X	X	X	X
Northern Ring-necked Snake	*Diadophis punctatus*				X		X	X	X		X
Eastern Hog-nosed Snake	*Heterodon platirhinos*	X							X	X	
Eastern Milksnake	*Lampropeltis triangulum*								X	X	X
Northern Watersnake	*Nerodia sipedon*	X		X	X	X	X	X	X	X	X
Rough Greensnake	*Opheodrys aestivus*								X		
Black Ratsnake	*Pantherophis spiloides*	X		X	X				X	X	
Red-bellied Snake	*Storeria occipitomaculata*				X				X		
Eastern Gartersnake	*Thamnophis sirtalis*	X		X	X	X	X	X	X	X	X
Eastern Smooth Earthsnake	*Virginia valeriae*				X						
Snapping Turtle	*Chelydra serpentina*	X	X	X	X		X	X	X	X	
Painted Turtle	*Chrysemys picta*	X	X	X	X	X	X	X	X	X	X
Eastern Musk Turtle	*Sternotherus odoratus*		X	X			X		X		
Eastern Box Turtle	*Terrapene carolina*	X			X	X	X		X	X	X

Appendix 2: Choice Experiments

METHODS -- We used choice experiments to assess amphibian and reptile preference of ecopassage entrance size, sky exposure, and line-of-sight (Woltz et al. 2008). Our choice experiments were behavioral tests in which an animal was placed in a central chamber under a bucket and given one minute to acclimate (Figure A2-1). Then we lifted the bucket from a hidden vantage point outside of the chamber and gave the animal 10 minutes to choose an exit passage. Behavioral observations were made throughout the time the animal was participating in the experiment.

We conducted the choice experiments in an open area southeast of the junction of Happy Hallow Rd. and State Route 78 (SR 78) near Nelsonville in Athens County, Ohio. We tested animals in the choice experiments March 31-November 7, 2017 and February 15-June 1, 2018. Tests were completed with animals captured along the roadway on rainy nights, in minnow traps, in hoop net traps, in pitfall traps at the fence ends of the 2013 barrier-ecopassage system along SR 78 (see Appendix 7: Monitoring of 2013 Barrier-ecopassage System), and in the pitfall traps of the pseudoecopassage experiment (see Appendix 3: Pseudoecopassage Experiment).

Our first experiment tested for preference of passage entrance size. We chose to test entrance size because other studies have suggested that larger entrance sizes may be preferred by most species (Woltz et al. 2008, Patrick et al. 2010). The setup of this experiment included a center chamber that was 4 ft. x 4 ft. x 4 ft. (Figure A2-2), with four exits that varied in size: 20 in. x 16 in. (the entrance size of the ecopassages installed in the 2013 barrier-ecopassage system along SR 78), 24 in. x 24 in., 40 in. x 24 in., and 40 in. x 44in. (Figure A2-2). All exit passages were 4 ft. in length.

Our second and third experiments tested for the influence of sky exposure and line-of-sight. We chose to assess these two variables because many amphibians navigate using cues from the sky, as well as landmarks that they can see in front of them (Adler 1982, Sinsch 1990, Phillips & Borland 1994, Phillips et al. 1995, Wilson 2001, Russell et al. 2005, Sinsch 2006). For both experiments, the setup included a center chamber that was 3 ft. x 3 ft. x 3 ft. All exits had 2 ft. x 2 ft. entrance sizes and were also 4 ft. in length (Figure A2-3). We tested for the influence of sky exposure on amphibian and reptile movement choices by comparing exit passages with no sky exposure (plywood board with no holes as top of passage) to exit passages with partial sky exposure (grate with 80% of the grate covered with black plastic as top of passage) and full sky exposure (grate as top of passage). We tested for preference of line-of-sight to the end of a passage by comparing eliminated line-of-sight (black plastic covering passage exit) with reduced line-of-sight (black plastic covering the bottom half of the passage exit and clear plastic covering the top half of the passage exit) and fully maintained line-of-sight (clear plastic covering the passage exit).

Between 2017 and 2018 a total of 1,280 animals from 20 species were used in the choice experiments (Table 1, Table 2, Figure A2-4). We tested 864 individual amphibians and 207 individual reptiles in the entrance size experiment. 349 amphibians and 86 reptiles were used in the sky exposure experiment, and 504 amphibians and 116 reptiles were used in the line-of-sight experiment. Most animals were run through two of the three choice tests and the orientation of the exit passages were changed regularly to account for directional bias.

To evaluate preference of passage parameters we used two types of contingency table analyses: a chi-square test (Patrick et al. 2010) and a G-test (Woltz et al. 2008). Our null hypotheses were that all exit passages for each choice experiment would be used equally, independent of the ecopassage parameter being manipulated in each choice experiment. We excluded all animals that chose to stay in the center chamber from our contingency table analyses. All statistical analyses were carried out in the statistical programming language R (R Core Team 2016).

RESULTS -- Amphibians and reptiles displayed preference for the 40 in. wide exit passages (Table A2-1). The 40 in. x 24 in. passage was used most frequently by both amphibians and reptiles (Figure A2-5). Six species of amphibian (Marbled Salamander, American Toad, American Bullfrog, Green Frog, Pickerel Frog, and Red-spotted Newt) and one species of reptile (Painted Turtle) showed statistical preference for the wider passages (Table A2-1). Two amphibian species (Jefferson Salamander and Spotted Salamander) and two reptile species (Snapping Turtle and Musk Turtle) chose the wider passages more often, but the preference was not statistically significant. Two reptile species (Black Racer and Ring-necked Snake) did not have any individuals choose the narrower passages (Table A2-1).

Amphibians and reptiles showed preference for the exit passage with full sky exposure (Table A2-2). Most amphibians and reptiles chose to use the passage with full sky exposure (Figure A2-6). Five species of amphibian (Marbled Salamander, American Toad, American Bullfrog, Green Frog, and Pickerel Frog) exhibited preference for the exit passage with full sky exposure (Table A2-2). Red-spotted Newts and Painted Turtles showed a preference for the full sky exposure passage, but the preference was not statistically significant. In addition, four amphibian species (Jefferson Salamander, Spotted Salamander, Northern Leopard Frog, and Wood Frog) and three reptile species (Black Racer, Northern Watersnake, and Eastern Gartersnake) did not have any individuals choose the exit passage with no sky exposure (Table A2-2).

Both amphibians and reptiles preferred the exit passage with a fully maintained line-of-sight to the end of the passage (Table A2-3, Figure A2-7). Seven species of amphibian (Spotted Salamander, American Toad, American Bullfrog, Green Frog, Pickerel Frog, Wood Frog, and Red-spotted Newt) and one species of reptile (Painted Turtle) displayed a statistical preference for the exit passage with full line-of-sight (Table

A2-3). Marbled Salamanders, Snapping Turtles, and Musk Turtles most often used the passage with a fully maintained line-of-sight, but the preference was not statistically significant. Three amphibian species (Gray Treefrog, Northern Leopard Frog, and Spring Peeper) and three reptile species (Black Racer, Ring-necked Snake, and Eastern Gartersnake) did not have any individuals choose the exit passage with no line-of-sight to the end of the passage (Table A2-3).

Figure A2-1. A Wood Frog being placed under the bucket in the central chamber of a choice experiment.

Figure A2-2. Entrance size choice experiment, with four exits differing in size.

Figure A2-3. Setup for sky exposure and line-of-sight choice experiments, with three exit passages that are all the same size.

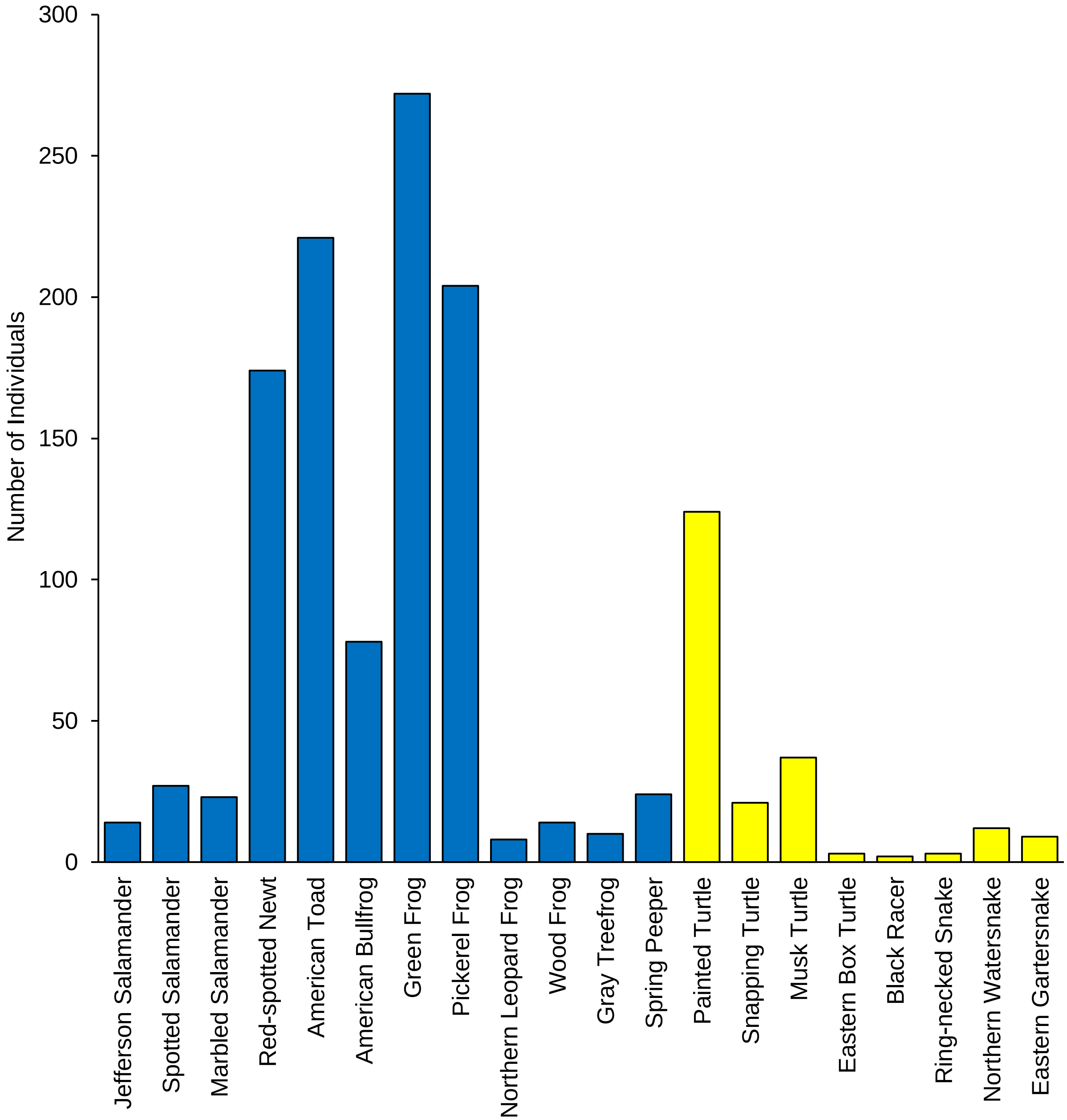

Figure A2-4. Number of individuals by species used in the choice experiments. Blue bars indicate amphibians and yellow bars indicate reptiles.

Table A2-1. The number of amphibians and reptiles of each species that chose each exit passage in the entrance size choice experiment. Individuals could choose passages with entrance sizes of 20 in. x 16 in., 24 in. x 24 in., 40 in. x 24 in., 40 in. x 44 in., or to stay in the center chamber. Chi-square and G-test analyses exclude animals that stayed in the center chamber.

	Stayed in Center	20in. x 16in.	24in. x 24in.	40in. x 24in.	40in. x 44in.	chi-square value	P-value	G-test value	P-value
AMPHIBIANS	160	85	101	320	198	199.58	**<0.001**	193.34	**<0.001**
Jefferson Salamander	2	0	2	8	2	6.00	0.049	5.55	0.063
Spotted Salamander	5	3	3	11	4	8.52	0.036	7.38	0.061
Marbled Salamander	2	5	1	10	5	7.76	**0.051**	8.59	**0.035**
American Toad	26	19	26	70	33	41.89	**<0.001**	38.03	**<0.001**
Gray Treefrog	8	1	0	1	0	NA	NA	NA	NA
American Bullfrog	7	3	11	32	18	28.38	**<0.001**	30.31	**<0.001**
Green Frog	32	22	25	63	66	38.40	**<0.001**	39.98	**<0.001**
Pickerel Frog	25	18	16	69	39	51.30	**<0.001**	49.09	**<0.001**
Northern Leopard Frog	2	0	1	4	1	3.00	0.223	2.77	0.250
Wood Frog	0	1	2	5	6	4.86	0.183	5.29	0.152
Red-spotted Newt	33	12	14	44	22	28.00	**<0.001**	25.62	**<0.001**
Spring Peeper	18	1	0	3	2	1.00	0.607	1.05	0.593
REPTILES	101	18	12	49	27	29.77	**<0.001**	28.31	**<0.001**
Painted Turtle	70	11	7	23	11	11.08	**0.011**	10.23	**0.017**
Snapping Turtle	7	2	1	7	3	6.38	0.094	5.96	0.114
Musk Turtle	16	3	2	9	6	6.00	0.112	6.04	0.110
Eastern Box Turtle	3	0	0	0	0	NA	NA	NA	NA
Black Racer	0	0	0	1	1	NA	NA	NA	NA
Ring-necked Snake	0	0	0	2	1	0.33	0.564	0.34	0.560
Northern Watersnake	3	2	0	4	3	0.67	0.717	0.68	0.712
Eastern Gartersnake	2	0	2	3	2	0.29	0.867	0.27	0.872

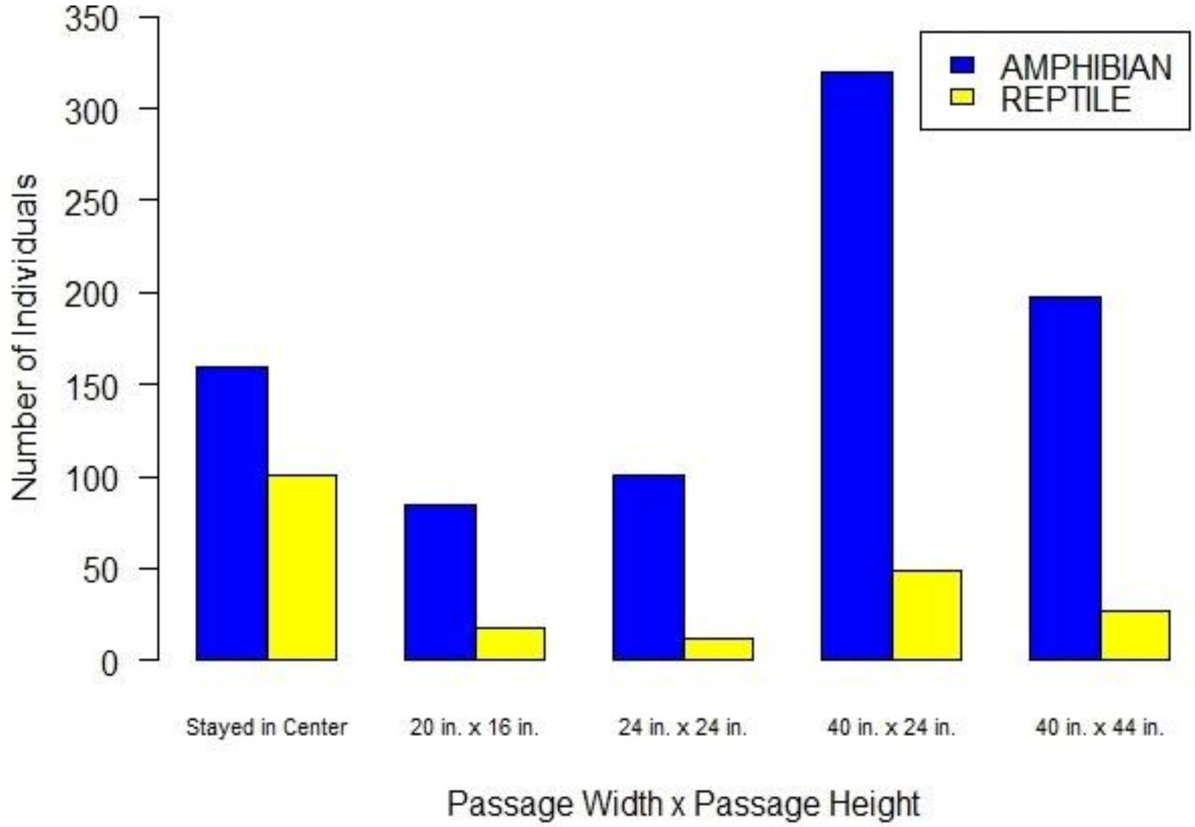

Figure A2-5. The number of individual amphibians and reptiles that chose each exit passage in the entrance size choice experiment. Individuals could choose passages with entrance sizes of 20 in. x 16 in., 24 in. x 24 in., 40 in. x 24 in., 40 in. x 44 in., or to stay in the center chamber.

Table A2-2. The number of amphibians and reptiles of each species that chose each exit passage in the sky exposure choice experiment. Individuals could choose passages with no sky exposure, partial sky exposure, full sky exposure, or to stay in the center chamber. Chi-square and G-test analyses exclude animals that stayed in the center chamber.

	Stayed in Center	No Sky Exposure	Partial Sky Exposure	Full Sky Exposure	chi-square value	P-value	G-test value	P-value
AMPHIBIANS	66	32	61	190	149.99	**<0.001**	143.69	**<0.001**
Jefferson Salamander	1	0	1	4	1.80	0.180	1.93	0.165
Spotted Salamander	1	0	0	4	NA	NA	NA	NA
Marbled Salamander	0	2	4	11	7.80	**0.019**	7.64	**0.022**
American Toad	19	7	18	56	48.96	**<0.001**	48.21	**<0.001**
Gray Treefrog	4	1	0	1	NA	NA	NA	NA
American Bullfrog	2	5	4	15	9.25	**0.010**	8.61	**0.013**
Green Frog	13	11	18	47	28.76	**<0.001**	27.44	**<0.001**
Pickerel Frog	14	4	9	39	41.35	**<0.001**	39.75	**<0.001**
Northern Leopard Frog	0	0	2	3	0.20	0.655	0.20	0.654
Wood Frog	0	0	2	1	0.33	0.564	0.34	0.560
Red-spotted Newt	4	2	2	7	4.55	0.103	4.20	0.122
Spring Peeper	8	0	1	2	1.00	0.607	1.05	0.593
REPTILES	42	8	8	28	18.18	**<0.001**	16.82	**<0.001**
Painted Turtle	29	4	4	11	5.16	0.076	4.79	0.091
Snapping Turtle	3	1	2	4	2.00	0.368	2.00	0.368
Musk Turtle	4	2	0	4	0.67	0.414	0.68	0.410
Eastern Box Turtle	2	0	0	0	NA	NA	NA	NA
Black Racer	0	0	0	1	NA	NA	NA	NA
Ring-necked Snake	0	1	0	0	NA	NA	NA	NA
Northern Watersnake	1	0	1	5	2.67	0.103	2.91	0.088
Eastern Gartersnake	3	0	1	3	1.00	0.317	1.05	0.306

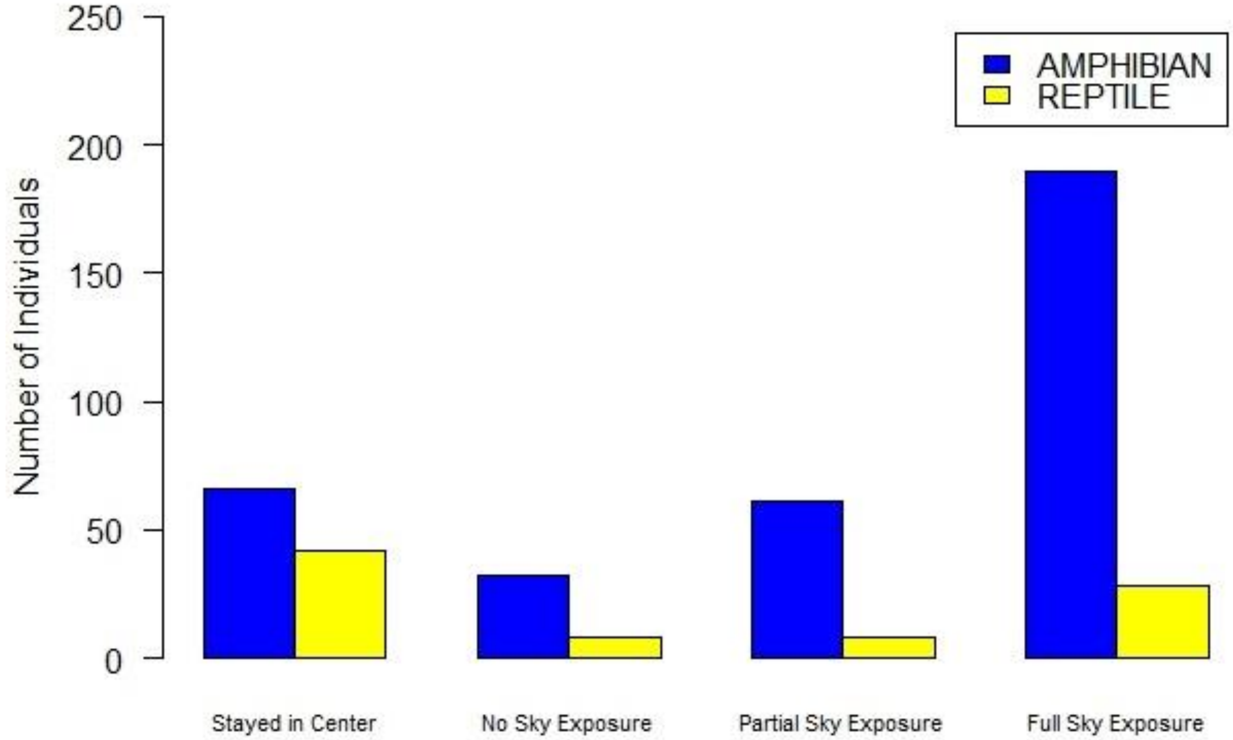

Figure A2-6. The number of individual amphibians and reptiles that chose each exit passage in the sky exposure choice experiment. Individuals could choose passages with no sky exposure, partial sky exposure, full sky exposure, or to stay in the center chamber.

Table A2-3. The number of amphibians and reptiles of each species that chose each exit passage in the line-of-sight choice experiment. Individuals could choose passages with no line-of-sight, partial line-of-sight, full line-of-sight, or to stay in the center chamber. Chi-square and G-test analyses exclude animals that stayed in the center chamber.

	Stayed in Center	No Line-of-sight	Partial Line-of-sight	Full Line-of-sight	chi-square value	P-value	G-test value	P-value
AMPHIBIANS	119	39	69	277	261.84	**<0.001**	247.70	**<0.001**
Jefferson Salamander	2	1	1	4	3.00	0.223	2.77	0.250
Spotted Salamander	6	1	3	11	11.20	**0.004**	11.06	**0.004**
Marbled Salamander	0	1	0	5	2.67	0.103	2.91	0.088
American Toad	16	5	8	28	22.88	**<0.001**	21.54	**<0.001**
Gray Treefrog	3	0	0	1	NA	NA	NA	NA
American Bullfrog	10	3	9	26	22.47	**<0.001**	22.60	**<0.001**
Green Frog	24	8	25	77	70.49	**<0.001**	70.75	**<0.001**
Pickerel Frog	16	8	9	57	63.60	**<0.001**	59.32	**<0.001**
Northern Leopard Frog	1	0	0	2	NA	NA	NA	NA
Wood Frog	2	0	1	8	5.44	**0.020**	6.20	**0.013**
Red-spotted Newt	29	12	12	57	50.00	**<0.001**	46.26	**<0.001**
Spring Peeper	10	0	1	1	NA	NA	NA	NA
REPTILES	58	14	7	37	25.48	**<0.001**	24.77	**<0.001**
Painted Turtle	44	10	4	17	8.19	**0.017**	8.68	**0.013**
Snapping Turtle	4	1	0	6	3.57	0.059	3.96	0.047
Musk Turtle	8	2	2	7	4.54	0.103	4.20	0.122
Eastern Box Turtle	1	0	0	0	NA	NA	NA	NA
Black Racer	0	0	0	1	NA	NA	NA	NA
Ring-necked Snake	0	0	1	1	NA	NA	NA	NA
Northern Watersnake	1	1	0	3	1.00	0.317	1.05	0.306
Eastern Gartersnake	0	0	0	2	NA	NA	NA	NA

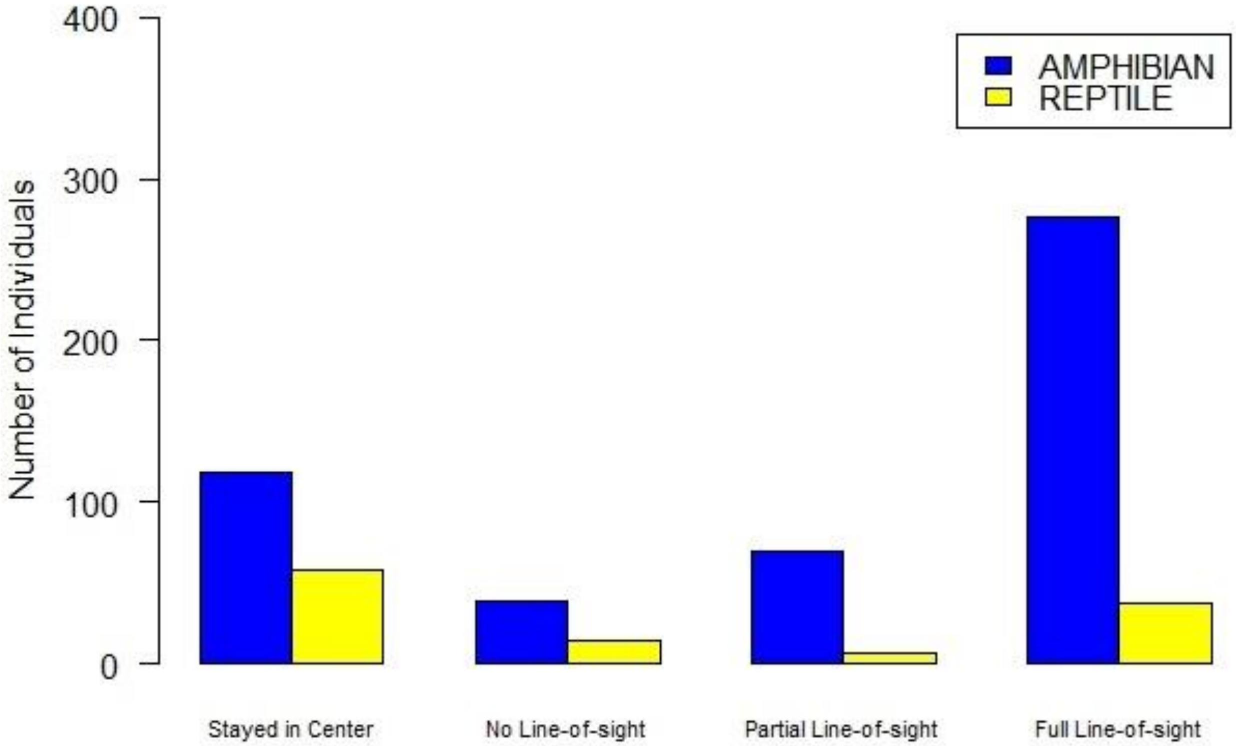

Figure A2-7. The number of individual amphibians and reptiles that chose each exit passage in the line-of-sight choice experiment. Individuals could choose passages with no line-of-sight, partial line-of-sight, full line-of-sight, or to stay in the center chamber.

Appendix 3: Pseudoecopassage Experiment

METHODS -- We created a pseudoecopassage experiment to test for ecopassage preference along State Route 78 (SR 78) near Nelsonville in Athens County, Ohio. Silt fence was installed on both sides of the road to prevent amphibians and reptiles from accessing the road. Along this silt fence we made openings and installed 'pseudoecopassage' replicates; each replicate tested four alternative pseudoecopassage designs. Thus, animals approaching the fence would follow the fence in one direction until they encountered a pseudoecopassage replicate or reached the end of the fence. There may also have been animals that directly encountered the pseudoecopassage replicates without approaching the fence. A pseudoecopassage is a 4 ft. long tunnel placed perpendicular to the silt fence and ending in a pitfall trap. Animals found in the trap were transported across the road by hand. Thus, our pseudoecopassages simulated the connectivity provided by ecopassages and allowed us to test different designs.

Our pseudoecopassage experiment assessed two features that may impact the choices of migratory amphibians and reptiles: sky exposure and line-of-sight. We chose to test these two variables because many amphibians navigate using cues from the sky, as well as visual landmarks (Adler 1982, Sinsch 1990, Phillips & Borland 1994, Phillips et al. 1995, Wilson 2001, Russell et al. 2005, Sinsch 2006). If access to navigational cues from the sky are impeded and there was not a direct line-of-sight through a passage, we predicted amphibians would avoid the passage. We tested four pseudoecopassage designs: (1) full sky exposure and full line-of-sight, (2) partial sky exposure and full line-of-sight, (3) full sky exposure and no line-of-sight, and (4) partial sky exposure and no line-of-sight. Full sky exposure passages had a grated top, while partial sky exposure passages had a grated top with 80% of the grate covered with black plastic (Figure A3-1). Full line-of-sight passages had clear plastic covering the passage exit, and no line-of-sight passages had black plastic covering the passage exit (Figure A3-1). Our pseudoecopassages all had 24 in. x 24 in. entrances to control for entrance size. The pseudoecopassages also had a natural soil substrate as previous research suggests that ecopassages with natural substrates are preferred (Lesbarrères et al. 2004, Woltz et al. 2008, Patrick et al. 2010). The substrate extended to the pitfall trap where animals were captured at the end of the pseudoecopassage.

Replicates of each of the four pseudoecopassage designs were placed 100 ft. apart along the silt fence barrier (Figure A3-2, Figure A3-3). In 2017, 8 replicates of four pseudoecopassage design treatments were in place (covering 500 ft. along SR 78) and in 2018 14 replicates were in place (covering 800 ft. along SR 78). The order of the four pseudoecopassage treatments was random among the experimental replicates (Figure A3-1, Figure A3-2). Pitfall traps were also installed at both ends of both barrier fences to capture animals circumventing the fence ends.

The pseudoecopassage experiment was conducted in an area along the SR 78 site that included 62% of recorded mortality in 2015 and 2016 (Figure 5). Construction and installation of the initial eight pseudoecopassage replicates was completed on March 24, 2017, and the six replicates added in 2018 were completed on February 12th. The sky exposure and line-of-sight design treatments were added on April 20th in 2017 and on February 20th in 2018. Pitfall traps were checked daily March 24-November 11, 2017 and February 12-June 1, 2018. Animals found in each pseudoecopassage pitfall trap were considered users of the pseudoecopassage that lead to the trap. A total of 188 animals (amphibians and small mammals) were captured in the pseudoecopassage and fence end pitfall traps before the design treatments were added, and 1,347 animals (amphibians, reptiles, and small mammals) were captured after the treatments were added (Figure A3-4). Many of the amphibians and reptiles captured in the pseudoecopassage pitfall traps were used in the choice experiments as well (see Appendix 2: Choice Experiments and Appendix 4: Comparison of Choice Experiments and Pseudoecopassage Experiment).

To assess whether adding the pseudoecopassage design treatments affected animal choices we used chi-square analyses to compare the number of animals captured at the fence ends, to the number captured in the two pseudoecopassages on the outside of each pseudoecopassage replicate, and to the number captured in the two ecopassages on the inside of each pseudoecopassage replicate before and after design treatments were added to the replicates. If the frequencies of animals trapped at the fence ends, in the outside pseudoecopassages, and in the inside pseudoecopassages, were the same before and after design treatments were added that would indicate that the treatments did not affect animal choices.

To evaluate which pseudoecopassage type was preferred we used two types of contingency table analyses: a chi-square test (Lesbarrères et al. 2004, Patrick et al. 2010) and a G-test (Woltz et al. 2008). Our null hypothesis was that all pseudoecopassages would be used equally. The animals captured in the pitfall traps at the silt fence ends were not used in analyses of pseudoecopassage preference. All statistical analyses were carried out in the statistical programming language R (R Core Team 2016).

RESULTS -- Before the pseudoecopassage design treatments were implemented amphibians and mammals preferred the outer pseudoecopassages, and after the treatments were implemented amphibians, mammals, and reptiles still preferred the outer pseudoecopassages (Table AS3-1). However, after the treatments were added the frequency with which outer passages and inner passages were used shifted and amphibians were more likely to use inner passages (shift from 12.7% before to 32.4% after). In addition, after the treatments were added the frequency with which amphibians and mammals were found in the fence end pitfall traps increased (from 6.4% to 25.4%

for amphibians, and 13.3% to 22.7% for mammals) (Table A3-1, Figure A3-5, Figure A3-6). These results indicate that adding the design treatments impacted choices made by amphibians and mammals since they were more likely to bypass an outer pseudoecopassage and use an inner pseudoecopassage with a preferred design treatment, or to bypass all the pseudoecopassage design treatments and be caught in a fence end pitfall trap.

Both amphibians and reptiles preferred the pseudoecopassage design treatment with a fully maintained line-of-sight and full sky exposure regardless of the location of the treatment within the pseudoecopassage replicates (Table A3-2, Figure A3-7). The small mammals caught displayed preference for the pseudoecopassage with no line-of sight to the end of the passage and full sky exposure (Table A3-2, Figure A3-7). Six species of amphibian (Marbled Salamander, American Toad, Green Frog, Pickerel Frog, Red-spotted Newt, and Spring Peeper) showed preference for the pseudoecopassages with a full line-of-sight and full sky exposure (Table A3-2). American Bullfrogs and Wood Frogs avoided the pseudoecopassages with no line-of-sight and partial sky exposure, but this was not statistically significant (Table A3-2). All of the Gray Treefrogs, Painted Turtles, Ring-necked Snakes, and Northern Watersnakes chose to use pseudoecopassage with full line-of-sight and full sky exposure (Table A3-2).

Figure A3-1. Pseudoecopassage replicates along State Route 78 near Nelsonville in Athens County, Ohio. The order of the design treatments was randomized for each pseudoecopassage replicate. There were eight pseudoecopassage replicates in 2017 and 14 pseudoecopassage replicates in 2018.

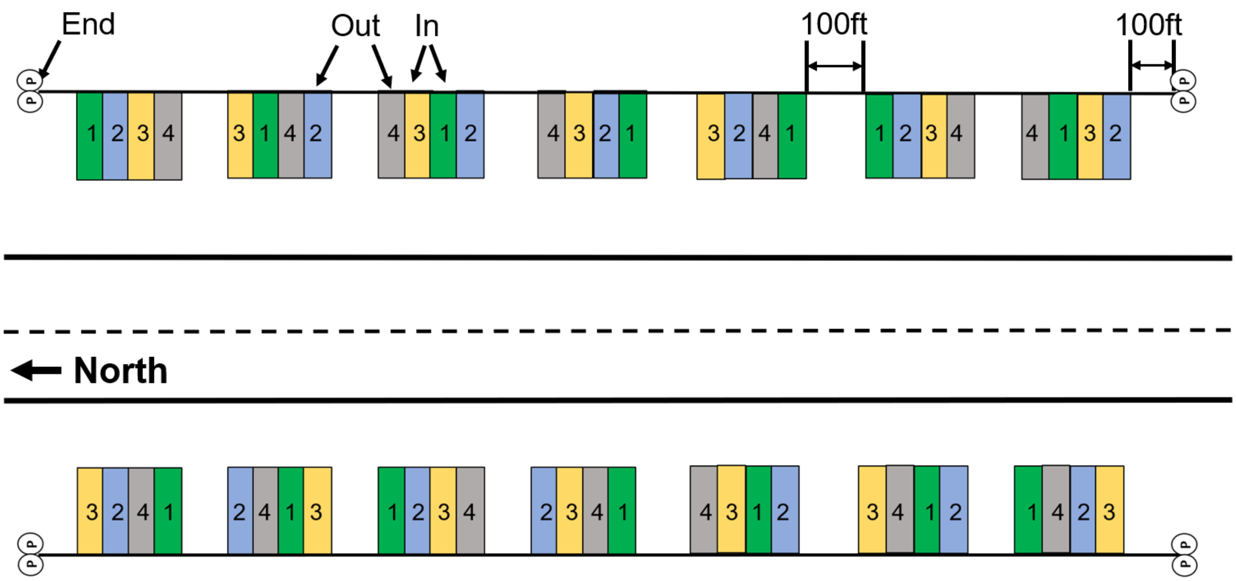

Figure A3-2. The randomized pattern of all 14 pseudoecopassage replicates along the silt fence barriers on either side of SR 78 near Nelsonville, Athens County, Ohio. The four northmost replicates were present in 2017 and 2018. The three southmost replicates were present only in 2018. Pseudoecopassage replicates were placed 100ft. apart along the fence line. Boxes with numbers were pseudoecopassages, and each had a pitfall trap at the end nearest to the road. The treatments are as follows: 1 = full sky exposure and full line-of-sight; 2 = partial sky exposure and full line-of-sight; 3 = full sky exposure and no line-of-sight; 4 = partial sky exposure and no line-of-sight. Circles with P's indicate pitfall traps at the end of each fence (End). The two pseudoecopassages in the middle of each replicate were the inside passages (In) and the two pseudoecopassages in each replicate that connected directly to the silt fence were the outside passages (Out).

Figure A3-3. Pseudoecopassage experiment in place along State Route 78 near Nelsonville in Athens County, Ohio.

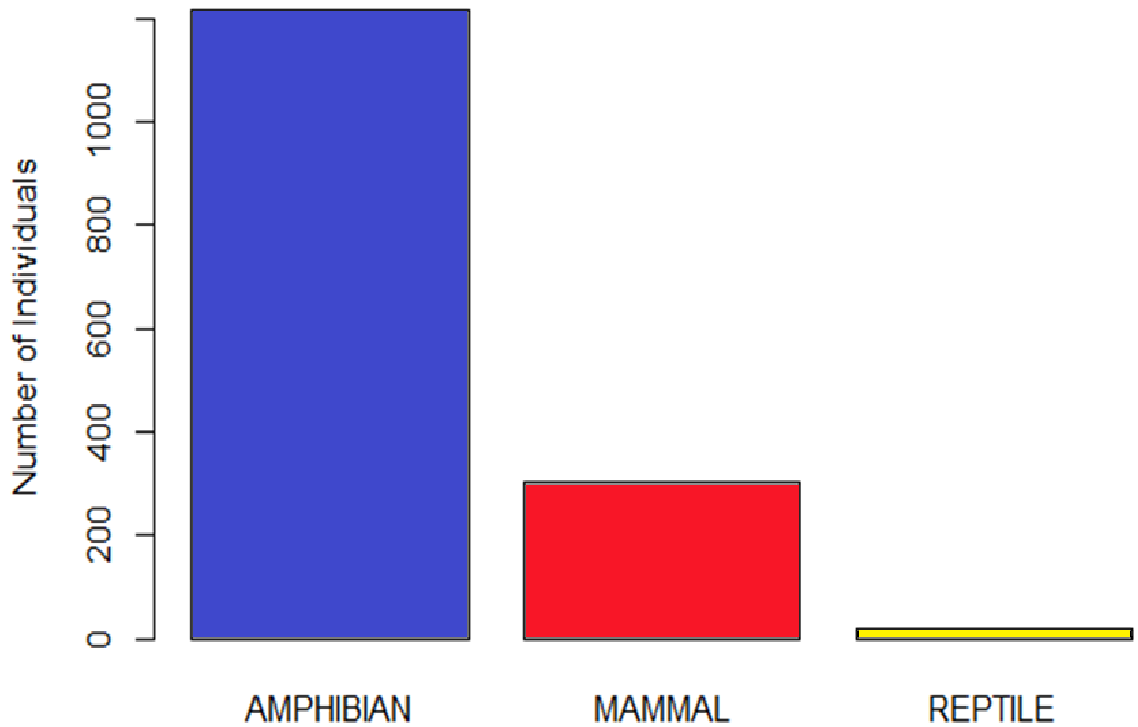

Figure A3-4. Number of individual amphibians, mammals, and reptiles captured in the pseudoecopassage experiment, March 24-November 11, 2017 and February 12-June 1, 2018.

Table A3-1. Number of amphibians, mammals, and reptiles captured in the pitfall traps at the fence ends (END), in the inside pseudoecopassages (IN), and in the outside pseudoecopassages (OUT) before and after design treatments were added to the pseudoecopassages. The first chi-square test evaluated whether each pitfall trap type was entered randomly, and the second chi-square test evaluated whether frequencies of usage of each pitfall trap type remained the same after the treatments were added.

	END (%)	IN (%)	OUT (%)	Pitfall chi-square value	Pitfall P-value	Freq. chi-square value	Freq. P-value
AMPHIBIANS BEFORE	11 (6.4)	22 (12.7)	140 (80.9)	177.38	**<0.001**	1096.1	**<0.001**
AMPHIBIANS AFTER	264 (25.4)	338 (32.4)	440 (42.2)	44.97	**<0.001**		
MAMMALS BEFORE	2 (13.3)	3 (20.0)	10 (66.7)	7.60	**0.022**	24.39	**<0.001**
MAMMALS AFTER	65 (22.7)	62 (21.6)	160 (55.7)	64.94	**<0.001**		
REPTILES	1 (5.6)	7 (38.8)	10 (55.6)	7.00	**0.030**		

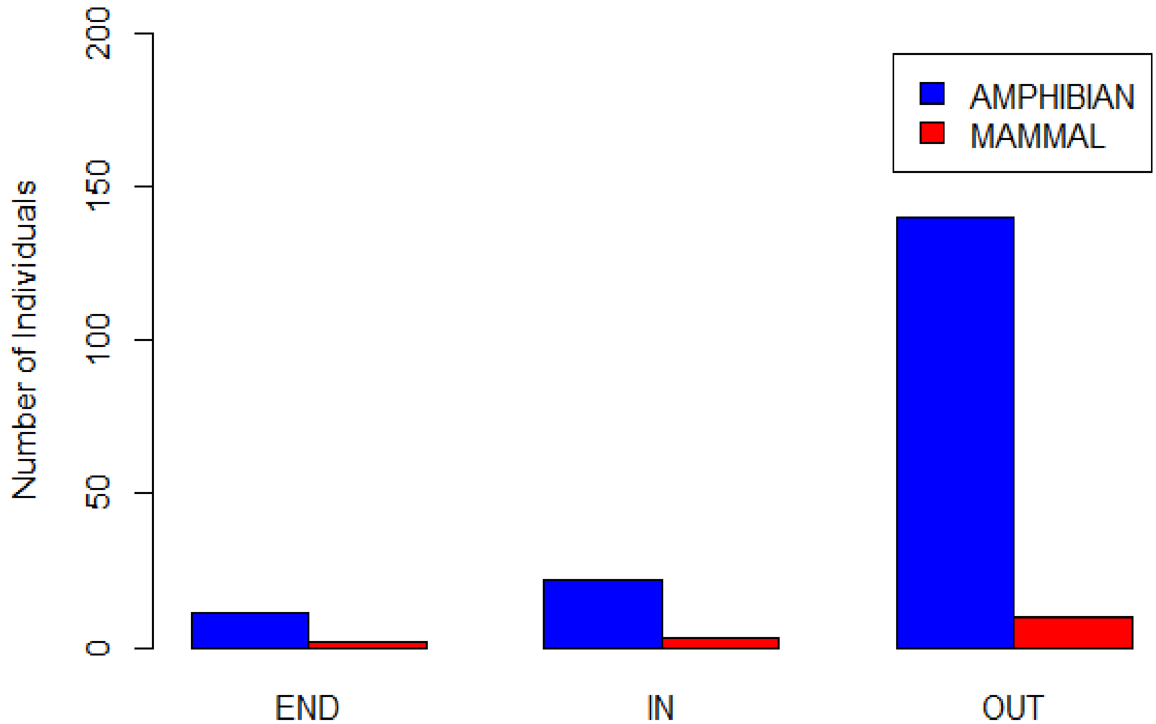

Figure A3-5. The number of individual amphibians and mammals captured at the fence ends (END), and in the pseudoecopassages on the inside (IN) and outside (OUT) of the pseudoecopassage replicates before the design treatments were implemented on April 20th in 2017 and on February 20th in 2018. No reptiles were captured before the pseudoecopassage design treatments were implemented.

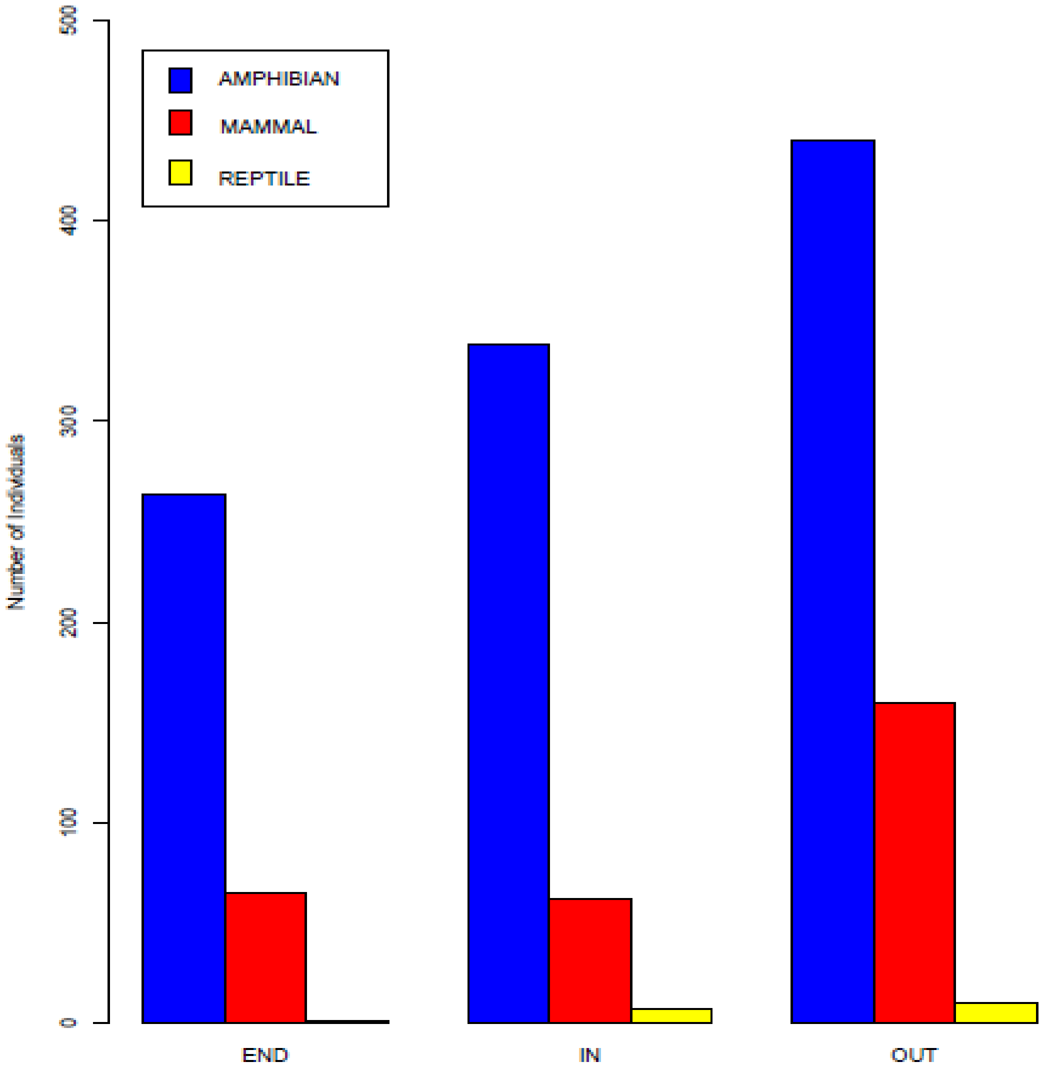

Figure A3-6. The number of individual amphibians, mammals, and reptiles captured at the fence ends (END), and in the pseudoecopassages on the inside (IN) and outside (OUT) of the pseudoecopassage replicates after the design treatments were implemented on April 20th in 2017 and on February 20th in 2018.

Table A3-2. The number of amphibians, reptiles, mammals, and individuals of each species that chose each pseudoecopassage in the pseudoecopassage experiment 2017-2018. Individuals could choose pseudoecopassages with full line-of-sight & full sky exposure, full line-of-sight & partial sky exposure, no line-of-sight & full sky exposure, or no line-of-sight & partial sky exposure.

	Full Line-of-sight & Full Sky Exposure	Full Line-of-sight & Partial Sky Exposure	No Line-of-sight & Full Sky Exposure	No Line-of-sight & Partial Sky Exposure	chi-square value	P-value	G-test value	P-value
AMPHIBIANS	397	256	78	47	411.91	**<0.001**	431.14	**<0.001**
Jefferson Salamander	3	1	1	1	2	0.572	1.73	0.631
Spotted Salamander	2	2	1	0	2.2	0.532	3.31	0.346
Marbled Salamander	13	8	1	0	20.55	**<0.001**	24.95	**<0.001**
American Toad	135	91	17	8	177.03	**<0.001**	197.14	**<0.001**
Gray Treefrog	1	0	0	0	NA	NA	NA	NA
American Bullfrog	4	5	1	0	6.8	0.079	8.86	0.031
Green Frog	80	40	7	13	94.8	**<0.001**	94.67	**<0.001**
Pickerel Frog	77	37	17	10	77.07	**<0.001**	73.92	**<0.001**
Wood Frog	1	4	1	0	6	0.112	6.22	0.101
Red-backed Salamander	0	1	0	0	NA	NA	NA	NA
Red-spotted newt	47	26	14	10	34.18	**<0.001**	32.73	**<0.001**
Spring Peeper	35	40	18	5	31.55	**<0.001**	37.19	**<0.001**
REPTILES	12	3	2	0	19.94	**<0.001**	19.81	**<0.001**
Painted Turtle	5	0	0	0	NA	NA	NA	NA
Snapping Turtle	1	1	0	0	2	0.572	2.77	0.428
Black Racer	1	0	0	0	NA	NA	NA	NA
Ring-necked Snake	1	0	0	0	NA	NA	NA	NA
Northern Watersnake	1	2	0	0	3.67	0.3	4.5	0.212
Eastern Gartersnake	3	2	0	0	5.4	0.145	7.13	0.068
MAMMALS	56	58	70	38	9.42	**0.024**	9.82	**0.02**

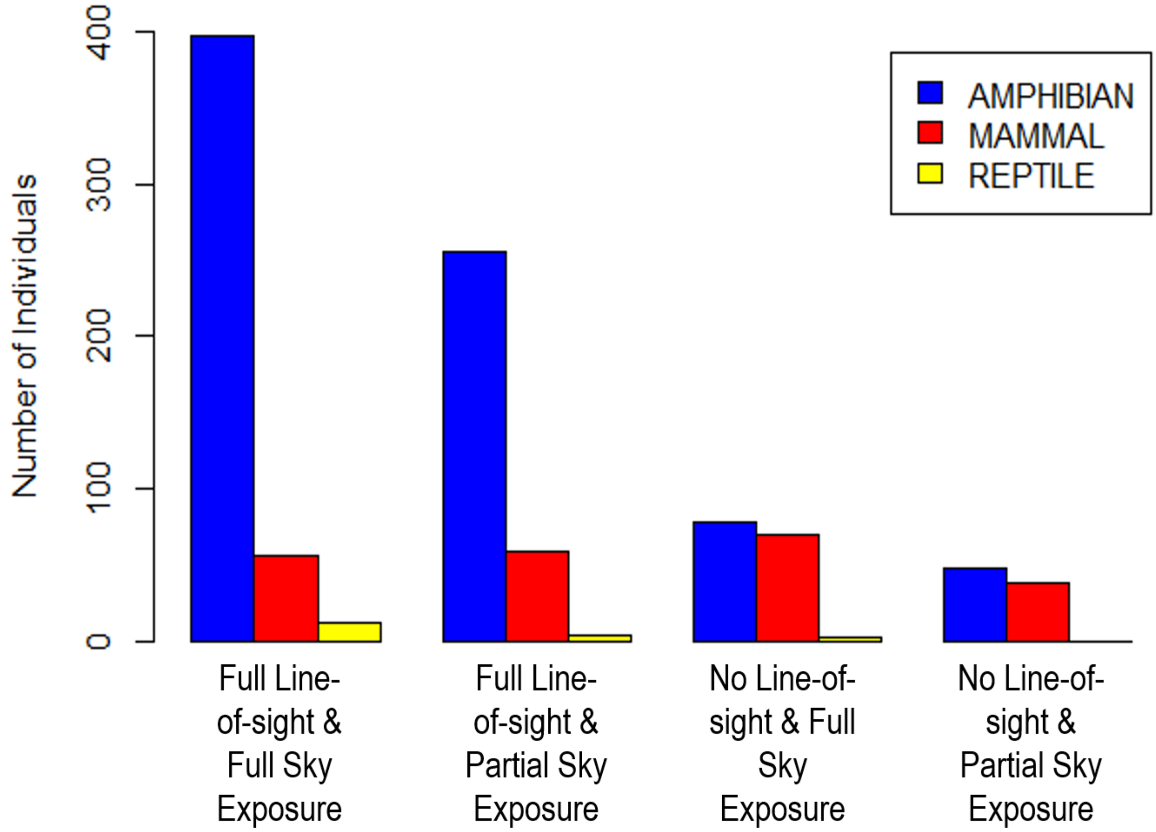

Figure A3-7. The number of individual amphibians, mammals, and reptiles that chose each exit passage in the pseudoecopassage experiment in place along State Route 78 near Nelsonville in Athens County, Ohio, 2017-2018. Individuals could choose pseudoecopassages with full line-of-sight & full sky exposure, full line-of-sight & partial sky exposure, no line-of-sight & full sky exposure, or no line-of-sight & partial sky exposure.

Appendix 4: Comparison of Choice Experiments & Pseudoecopassage Experiment

METHODS -- Previous studies have suggested that larger entrance sizes are generally preferred by amphibians and reptiles (Woltz et al. 2008, Patrick et al. 2010). In addition, greater sky exposure (Woltz et al. 2008, Gunson et al. 2016) and the ability to see all the way across an ecopassage (Clevenger & Huijser 2011) have been recommended to improve ecopassage use by amphibians and reptiles, but neither have been empirically tested.

To increase our knowledge of passage preferences by amphibians and reptiles we used manipulative choice experiments to assess amphibian and reptile preference of ecopassage entrance size, sky exposure, and line-of-sight and an observational pseudoecopassage experiment to assess sky exposure and line-of-sight (see Appendix 2: Choice Experiments and Appendix 3: Pseudoecopassage Experiment). By using both a manipulative and a passive observational experiment we could analyze whether these two types of experiments produced similar results. If both experimental types produce similar results, our inferences regarding behavioral choices are strengthened.

Thus, we tested amphibians and reptiles captured in the pseudoecopassage experiment in the choice experiments as well. We then used a chi-square test and a G-test to assess whether they made the same choice in both experiments more often than would be expected randomly. An example of making the same choice would be an animal that chose full sky exposure in the choice experiment and was trapped in a pseudoecopassage with full sky exposure. An individual making a different choice would be an animal that chose full sky exposure or no sky exposure in the choice experiment but was trapped in a pseudoecopassage with partial sky exposure. We used both a chi-square test and a G-test because the number of individuals of each species captured in the pseudoecopassage experiment varied. We also excluded all animals that chose to stay in the center chamber during the choice experiments from our analyses. All statistical analyses were carried out in the statistical programming language R (R Core Team 2016).

We captured a total of 369 amphibians and 17 reptiles across 16 species in the pseudoecopassages that were used in the sky exposure and line-of-sight choice experiments (Figure A4-1). 150 amphibians and 13 reptiles were tested in the sky exposure choice experiment and 219 amphibians and 4 reptiles were tested in the line-of-sight choice experiment.

RESULTS -- Most amphibians and reptiles made the same choices in the sky exposure choice experiment as they did in the pseudoecopassage experiment, indicating that the two experimental types produced concordant results (Table A4-1, Figure A4-2). Four species of amphibian (Marbled Salamander, American Toad, Green Frog, and Pickerel

Frog) consistently made the same choice concerning sky exposure (Table A4-1). Five species of amphibian (Jefferson Salamander, Spotted Salamander, Wood Frog, Red-spotted Newt, and Wood Frog) and four species of reptile (Painted Turtle, Snapping Turtle, Northern Watersnake, and Eastern Gartersnake) did not have any individuals make a different choice in the sky exposure experiment than they had made in the pseudoecopassage experiment (Table A4-1). During the sky exposure choice experiment, 18.0% of the amphibians tested and 30.8% of the reptiles tested stayed in the center rather than using any of the exit passages.

Amphibians regularly made the same choices in the line-of-sight choice experiment as they did in the pseudoecopassage experiment indicating that the two experimental types produced congruent results (Table A4-2, Figure A4-3). Reptiles did not consistently make the same choice concerning line-of-sight; however, this may be due to small sample size (n=4; Table A4-1). Green Frogs and Pickerel Frogs consistently made the same choice concerning line-of-sight (Table A4-1). Marbled Salamanders often made the same choice, but the trend was not statistically significant. Spotted Salamanders, Black Racers, and Eastern Gartersnakes did not have any individuals make a different choice in the line-of-sight choice experiment than they had made in the pseudoecopassage experiment (Table A4-1). 24.2% of the amphibians tested and 25% of the reptiles tested stayed in the center during the line-of-sight choice experiment.

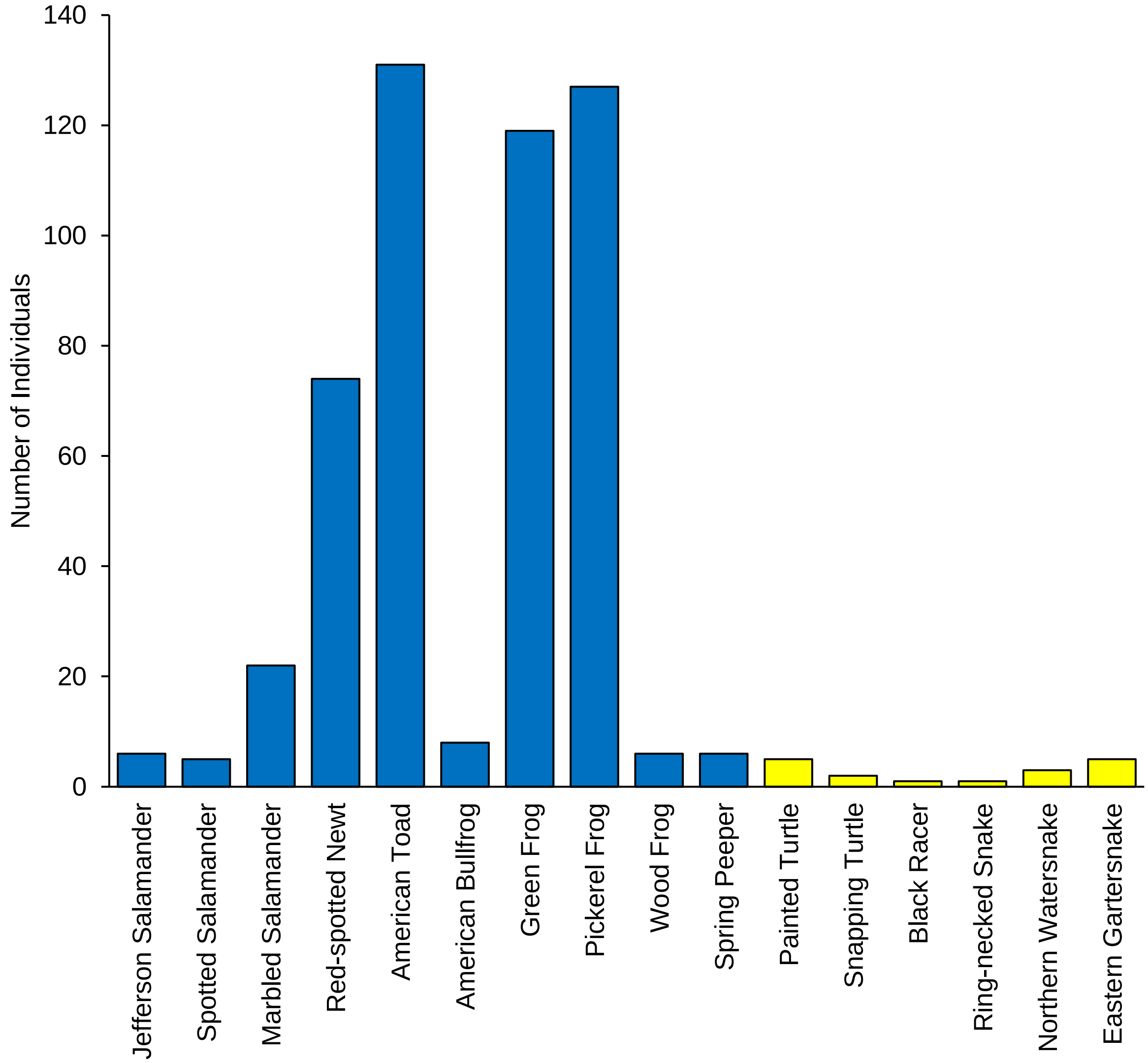

Figure A4-1. The number of individuals of each species of amphibian and reptile that were caught in pseudoecopassage pitfall traps and then tested in the sky exposure or line-of-sight choice experiments. Blue bars indicate amphibians and yellow bars indicate reptiles.

Table A4-1. The number of amphibians and reptiles of each species that made the same choice or a different choice in the sky exposure choice experiment and the pseudoecopassage experiment. Chi-square and G-test analyses exclude animals that stayed in the center chamber.

	Same Choice	Different Choice	Stayed in Center	chi-square value	P-value	G-test value	P-value
AMPHIBIANS	106	17	27	149.99	**<0.001**	143.69	**<0.001**
Jefferson Salamander	2	0	0	NA	NA	NA	NA
Spotted Salamander	1	0	0	NA	NA	NA	NA
Marbled Salamander	12	4	0	4.00	**0.046**	4.19	**0.041**
American Toad	41	8	10	22.22	**<0.001**	24.31	**<0.001**
Green Frog	21	3	4	13.50	**<0.001**	15.19	**<0.001**
Pickerel Frog	24	2	9	18.62	**<0.001**	21.94	**<0.001**
Wood Frog	1	0	0	NA	NA	NA	NA
Red-spotted Newt	3	0	2	NA	NA	NA	NA
Spring Peeper	1	0	2	NA	NA	NA	NA
REPTILES	8	1	4	5.44	**0.020**	6.18	**0.013**
Painted Turtle	3	0	2	NA	NA	NA	NA
Snapping Turtle	1	0	1	NA	NA	NA	NA
Ring-necked Snake	0	1	0	NA	NA	NA	NA
Northern Watersnake	1	0	0	NA	NA	NA	NA
Eastern Gartersnake	3	0	1	NA	NA	NA	NA

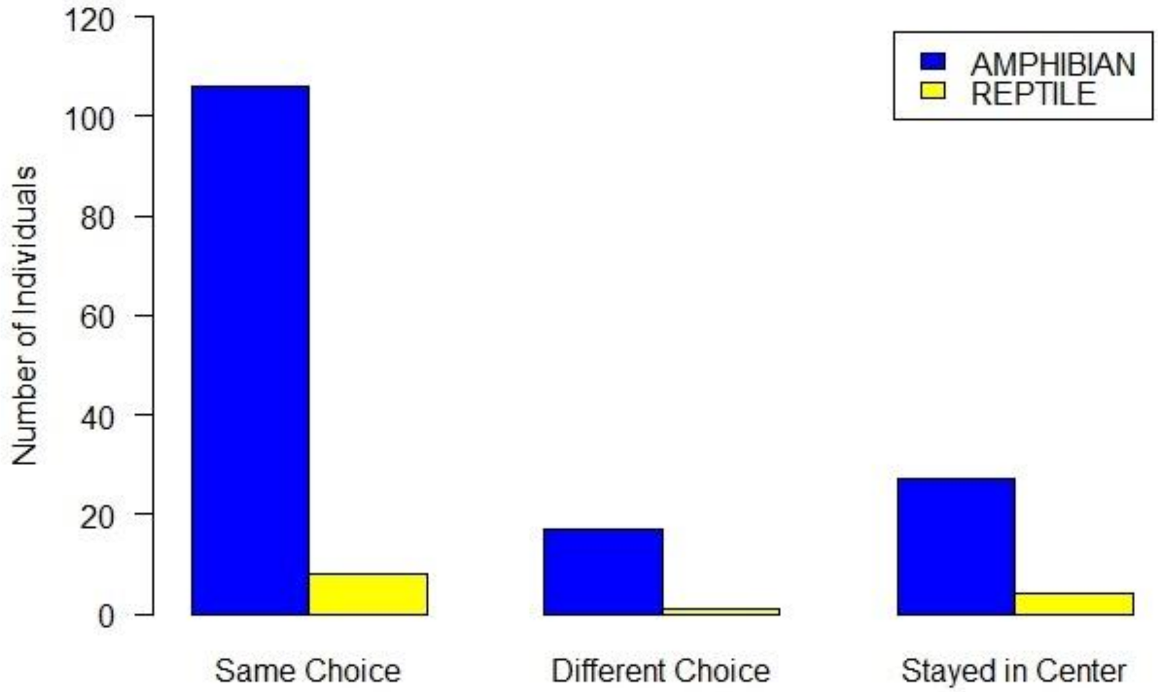

Figure A4-2. The number of individual amphibians and reptiles that made the same choice or a different choice in the sky exposure choice experiment and the pseudoecopassage experiment, and the number of animals that chose to stay in the center chamber during the choice experiment.

Table A4-2. The number of amphibians and reptiles of each species that made the same choice or a different choice in the line-of-sight choice experiment and the pseudoecopassage experiment. Chi-square and G-test analyses exclude animals that stayed in the center chamber.

	Same Choice	Different Choice	Stayed in Center	chi-square value	P-value	G-test value	P-value
AMPHIBIANS	111	55	53	18.89	**<0.001**	19.27	**<0.001**
Jefferson Salamander	1	2	1	0.33	0.564	0.34	0.560
Spotted Salamander	2	0	1	NA	NA	NA	NA
Marbled Salamander	5	1	0	2.67	0.103	2.91	0.088
American Toad	10	8	12	0.22	0.637	0.22	0.637
American Bullfrog	1	4	3	1.80	0.180	1.93	0.165
Green Frog	31	13	11	7.36	**0.007**	7.58	**0.006**
Pickerel Frog	40	11	9	16.49	**<0.001**	17.52	**<0.001**
Wood Frog	3	1	1	1.00	0.317	1.05	0.306
Red-spotted Newt	18	14	13	0.50	0.480	0.50	0.479
Spring Peeper	0	1	2	NA	NA	NA	NA
REPTILES	2	1	1	0.33	0.564	0.34	0.560
Black Racer	1	0	0	NA	NA	NA	NA
Northern Watersnake	0	1	1	NA	NA	NA	NA
Eastern Gartersnake	1	0	0	NA	NA	NA	NA

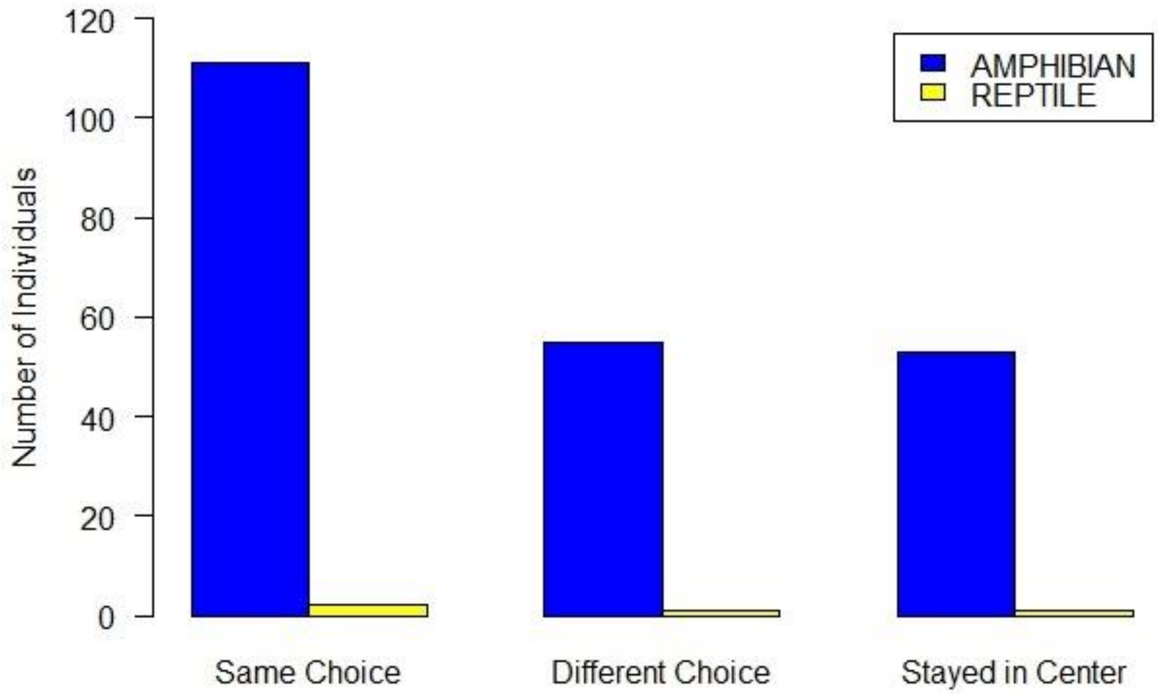

Figure A4-3. The number of individual amphibians and reptiles that made the same choice or a different choice in the line-of-sight choice experiment and the pseudoecopassage experiment, and the number of animals that chose to stay in the center chamber during the choice experiment.

Appendix 5: Roadway Mortality

METHODS – We monitored vertebrate roadway mortality along State Route 78 (SR 78) near Nelsonville in Athens County, Ohio. This highway was newly constructed in 2013. SR 78 is a two-lane highway with 8-ft. wide shoulders and a speed limit of 50 mph. We conducted daily walking surveys from the US Highway 33 exit to Burr Oak Blvd., a 1.2-mile stretch (Figure A5-1) to count the number of vertebrate carcasses. We chose to do walking surveys because small animal carcasses are easily missed during driving surveys (Enge & Wood 2002, Slater 2002, Dodd et al. 2004, Crawford et al. 2014). It was also not feasible to conduct slow (<20 mph) driving surveys (as studies in other locations have done) due to the high levels of faster traffic on SR 78 (Fahrig et al. 1995, Ramp et al. 2006, Orlowski 2007, Sillero 2008), and because even at 20 mph many small carcasses would be missed. We conducted our surveys in the morning between 6:00 am and 9:00 am to avoid missing carcasses removed by traffic or scavengers while still having good visibility (Salter 2002, Antworth et al. 2005, Santos et al. 2011). Detected carcasses were identified and marked with a GPS unit (Figure A5-2) then removed from the roadway to avoid double-counting the same carcass (Teixeira et al. 2013, Beckmann and Shine 2015). In addition, the roadway section the animal was found in was recorded; the roadway sections were determined by habitat changes along the SR 78 road stretch surveyed (Figure A5-1).

We monitored roadkill for 137-265 days each year from 2015 through 2019 (Table A5-1). Roadway mortality surveys occurred between March 12-November 23, 2015, February 4-November 14, 2016, January 12-November 15, 2017, January 26-November 12, 2018, and February 4-July 5, 2019 with additional surveys occurring as needed due to weather earlier during each year (Figure A5-3).

Roadkill survey data was tabulated and summarized in graphical format for descriptive analysis. We also evaluated road section mortality data from six time frames: (1) before the pseudoecopassage experiment (2015-early spring 2017), (2) during the 2017 pseudoecopassage experiment, (3) during the 2018 pseudoecopassage experiment, (4) during the 2018 barrier-ecopassage system implementation, (5) after 2018 barrier-ecopassage system implementation occurred while repairs to the fence were taking place, and (6) after the 2018 barrier-ecopassage system was fully completed (2018-2019) (see Appendix 3: Pseudoecopassage Experiment and Appendix 9: Implementation of 2018 Barrier-ecopassage System). To assess whether the number of vertebrate carcasses detected in each roadway section for a given time frame varied we used chi-square tests, with the null hypotheses that all roadway sections would have equal amounts of carcasses in each time frame. To assess whether the six time frames had different roadway section mortality rates from each other we used chi-square tests for goodness-of-fit. Thus, our null hypotheses were that any given time frame would not have significantly different road section mortality frequencies as compared with the road section mortality frequencies of any other time frame. For all analyses of roadway section mortality, we summed the number of carcasses from the west and east side of the road (ex. WS4+ES4=S4; road sections labeled in Figure A5-1).

RESULTS -- Through our vertebrate roadkill surveys we detected a total of 21,302 carcasses, of which 20,155 were amphibian carcasses (94.62% of all carcasses) (Table 5A-1, Figure 5A-4, Figure 5A-5). Reptiles accounted for 3.42% (n=728) of the carcasses, birds were 1.15% (n=246) of all carcasses, and mammals made up 0.81% (n=173) of the detected carcasses (Figure A5-5). The average number of carcasses detected per survey varied by year and season (Table 5A-1). In general, we found the largest peaks of vertebrate mortality in the spring, with smaller peaks in the fall (Figure A5-3). From 2015 to 2019 we detected 14 amphibian species dead on the road (Table 1), with patterns of mortality that varied annually by species group (Figure A5-5). In particular, mole salamanders (Jefferson Salamander, Spotted Salamander, and Marbled Salamander) and Red-spotted Newts showed a decrease in the number of carcasses detected each year, and Spring Peepers and Gray Treefrogs also showed this trend (Figure A5-5). These declines in roadkilled animals are likely due to population declines in these species (see Appendix 6: Population Monitoring).

We found that levels of roadway mortality significantly varied by roadway section in all six time frames evaluated (Table A5-2; all chi-square values >28.82, all P-values <0.001). Before the pseudoecopassage experiment was underway most of the mortality was found in road section S4 (62.21%; Table A5-2, Figure A5-6). While the 2017 pseudoecopassage experiment was occurring (the pseudoecopassage experiment was occupying 500 ft. of S4) road section S4 had the greatest number of carcasses, but more carcasses were found in S3 and S5 than had been previously (Figure A5-6). During the 2018 pseudoecopassage experiment (while the pseudoecopassage experiment was occupying all of S4 and 100 ft. of S5) section S5 had the greatest number of carcasses. While implementation of the 2018 barrier-ecopassage system was occurring (June 4-29, 2018), SR 78 was closed to traffic from the US Highway 33 exit to Happy Hallow Road (road sections S2 through S6), and mortality levels were highest in N3 and S1. While repairs were being made to the 2018 barrier-ecopassage system fencing (June 29-October 14, 2018) mortality was high in S2, S3, and S5, and once the 2018 barrier-ecopassage system was completed mortality was highest in S3 (Table A5-2, Figure A5-6).

All time frames had significantly different road section mortality frequencies than all other time frames (all chi-square values >52.10, all P-values <0.001). Thus, the pseudoecopassage experiment and 2018 barrier-ecopassage system changed roadkill patterns along SR 78 from the roadkill pattern found before the pseudoecopassage experiment was in place (Figure A5-6).

Figure A5-1.Roadway sections surveyed for vertebrate carcasses along a 1.2 mile stretch of State Route 78 near Nelsonville in Athens County, Ohio. The US Highway 33 exit is at the southwestern edge of the figure and the Burr Oak Blvd intersection is at the north edge of the figure. For analyses we combined the number of carcasses found on each side of the road for each section (ex. WS5 + ES5 = S5).

Figure A5-2. Amphibian carcasses (Spring Peeper-far left, Spotted Salamander-middle left, Red-spotted Newt-middle right, and Pickerel Frog-far right) found along State Route 78 near Nelsonville in Athens County, Ohio.

Table A5-1. Number of vertebrate carcasses detected, number of surveys conducted, average number of vertebrate carcasses detected, and number of surveys with no vertebrate carcasses detected per year along a 1.2 mile stretch of State Route 78, near Nelsonville in Athens County, Ohio, 2015-2019. We did not collect summer 2015 roadway mortality data and fall 2019 road mortality data is not yet available.

	2015	2016	2017	2018	2019
TOTAL NUMBER OF CARCASSES	**7590**	**6188**	**3236**	**2282**	**2006**
NUMBER OF SURVEYS	194	225	265	225	137
AVERAGE NUMBER OF CARCASSES PER SURVEY	39.12	27.50	12.21	10.14	14.64
TOTAL NUMBER OF SPRING CARCASSES	6434	5402	1611	1098	1959
NUMBER OF SPRING SURVEYS	102	124	123	108	123
AVERAGE NUMBER OF CARCASSES PER SPRING SURVEY	63.08	43.56	13.10	10.17	15.93
TOTAL NUMBER OF SUMMER CARCASSES	N/A	262	627	301	47
NUMBER OF SUMMER SURVEYS	N/A	26	58	45	14
AVERAGE NUMBER OF CARCASSES PER SUMMER SURVEY	N/A	10.08	10.81	6.69	3.36
TOTAL NUMBER OF FALL CARCASSES	1156	524	998	883	N/A
NUMBER OF FALL SURVEYS	92	75	84	72	N/A
AVERAGE NUMBER OF CARCASSES PER FALL SURVEY	12.57	6.99	11.88	12.26	N/A
NUMBER OF SURVEYS WITH NO CARCASSES DETECTED	5	6	36	58	33

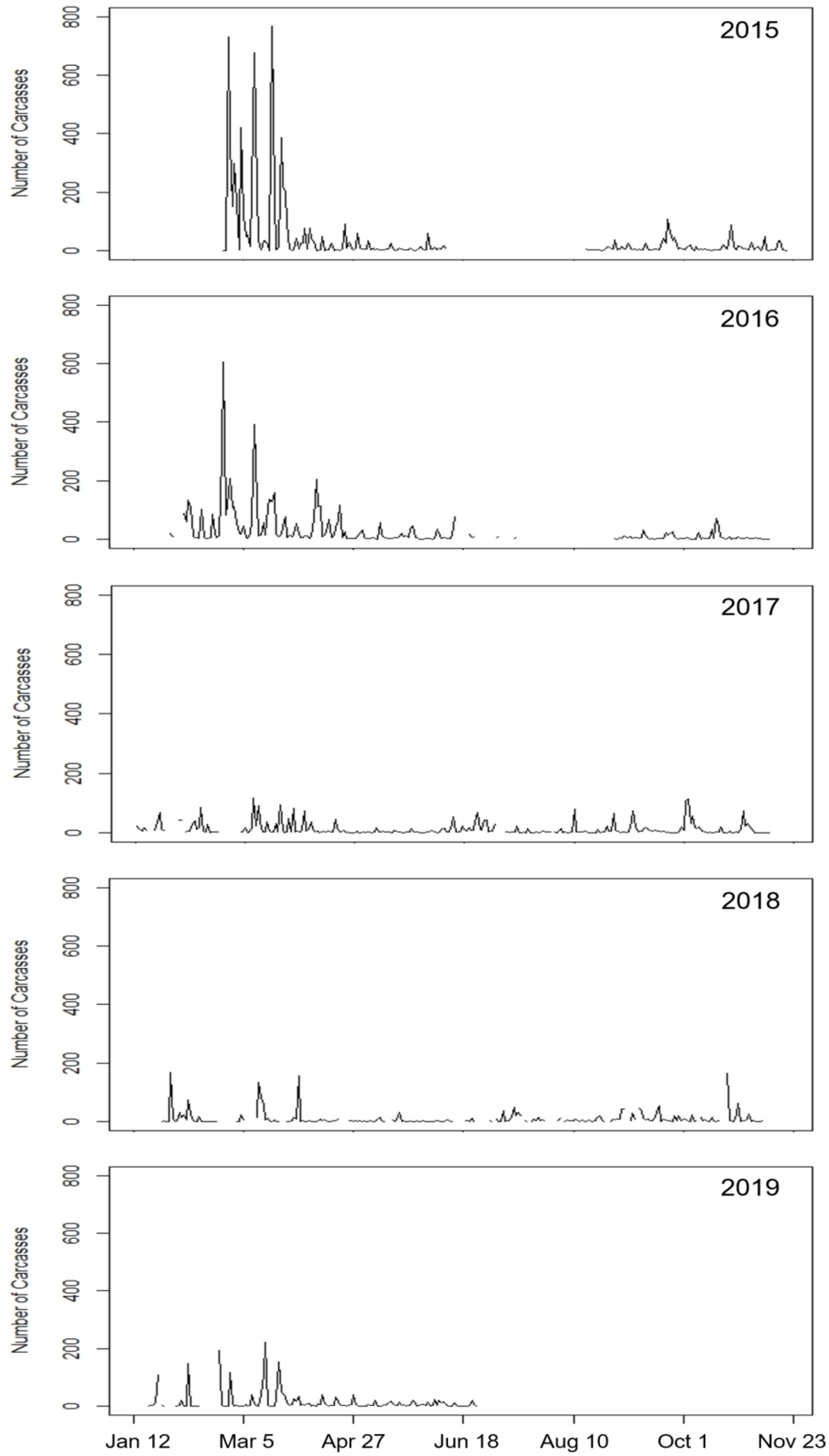

Figure A5-3. Number of vertebrate carcasses detected each day along a 1.2 mile stretch of State Route 78 near Nelsonville in Athens County, Ohio, 2015-2019.

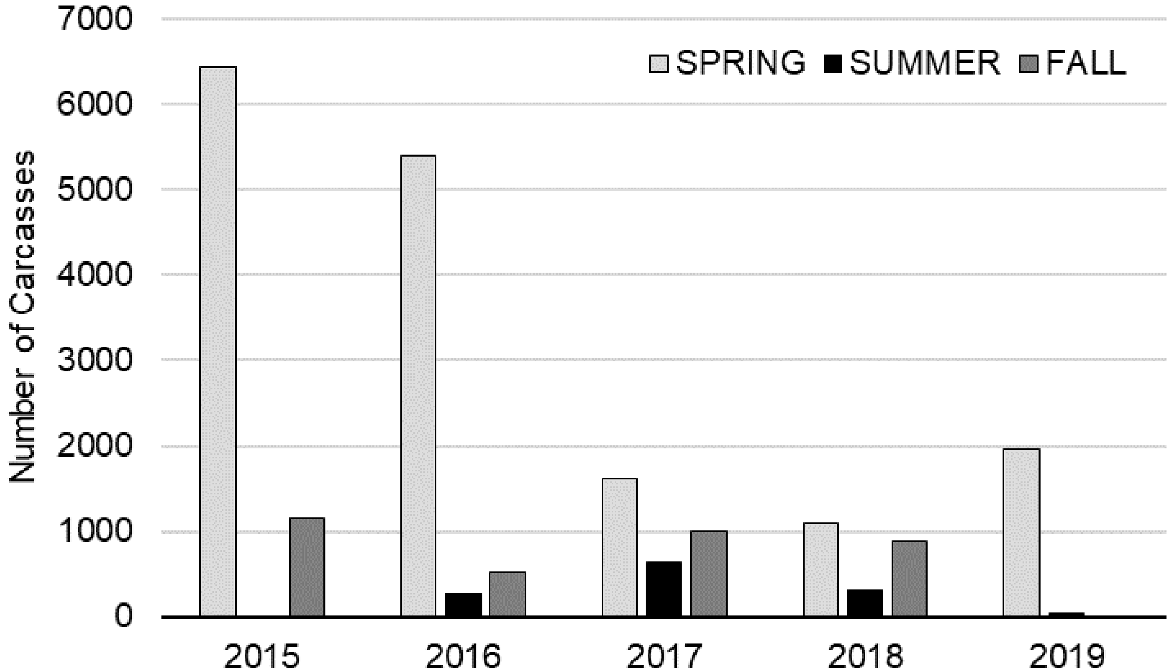

Figure A5-4. Number of vertebrate carcasses detected each season along a 1.2 mile stretch of State Route 78, near Nelsonville in Athens County, Ohio, 2015-2019. We did not collect summer 2015 roadway mortality data and fall 2019 road mortality data is not yet available.

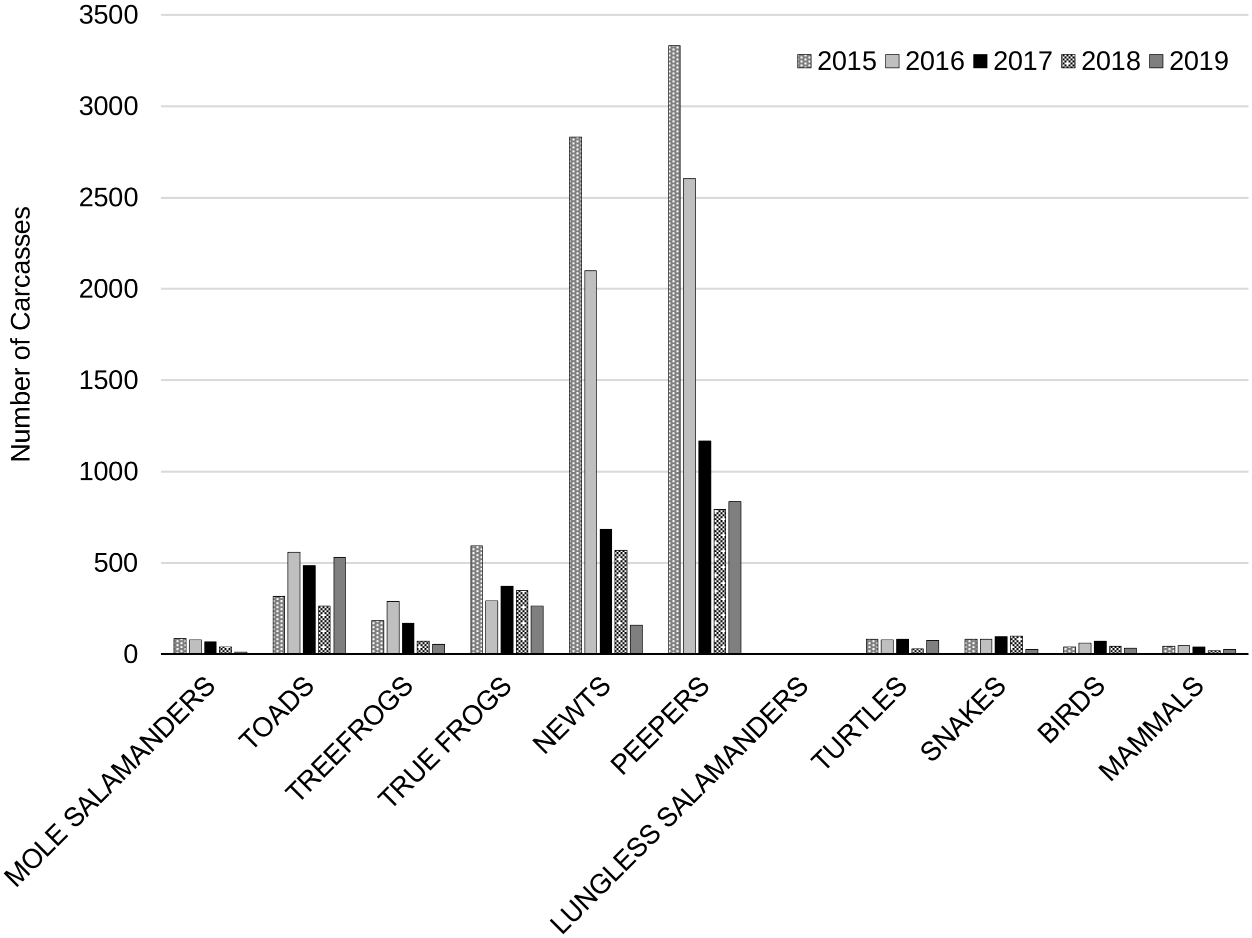

Figure A5-5. Number of carcasses detected among wildlife groups along a 1.2 mile stretch of State Route 78, near Nelsonville in Athens County, Ohio, 2015-2019.

Table A5-2. Number of vertebrate carcasses detected in each roadway section (west and east lanes combined) along a 1.2 mile stretch of State Route 78, near Nelsonville in Athens County, Ohio, 2015-2019. Roadway sections are pictured in Figure A5-1. The 2018 barrier-ecopassage system covered all of S4 and half of S5.

	N4	N3	N2	N1	FENCE	S1	S2	S3	S4	S5	S6
2015-2017 Pre-Pseudoecopassage Experiment	34	122	238	88	118	567	858	1276	8925	1694	427
2017 Pseudoecopassage Experiment	35	56	61	24	9	169	242	506	882	450	159
2018 Pseudoecopassage Experiment	14	47	32	22	6	89	118	181	214	261	68
2018 Barrier-Ecopassage Implementation	1	7	3	1	0	7	5	1	0	1	1
2018 Barrier-ecopassage System in Need of Fence Repairs	20	25	26	15	20	55	122	122	38	113	105
2018-2019 Completed Barrier-ecopassage System	38	93	108	51	85	284	327	663	93	345	251

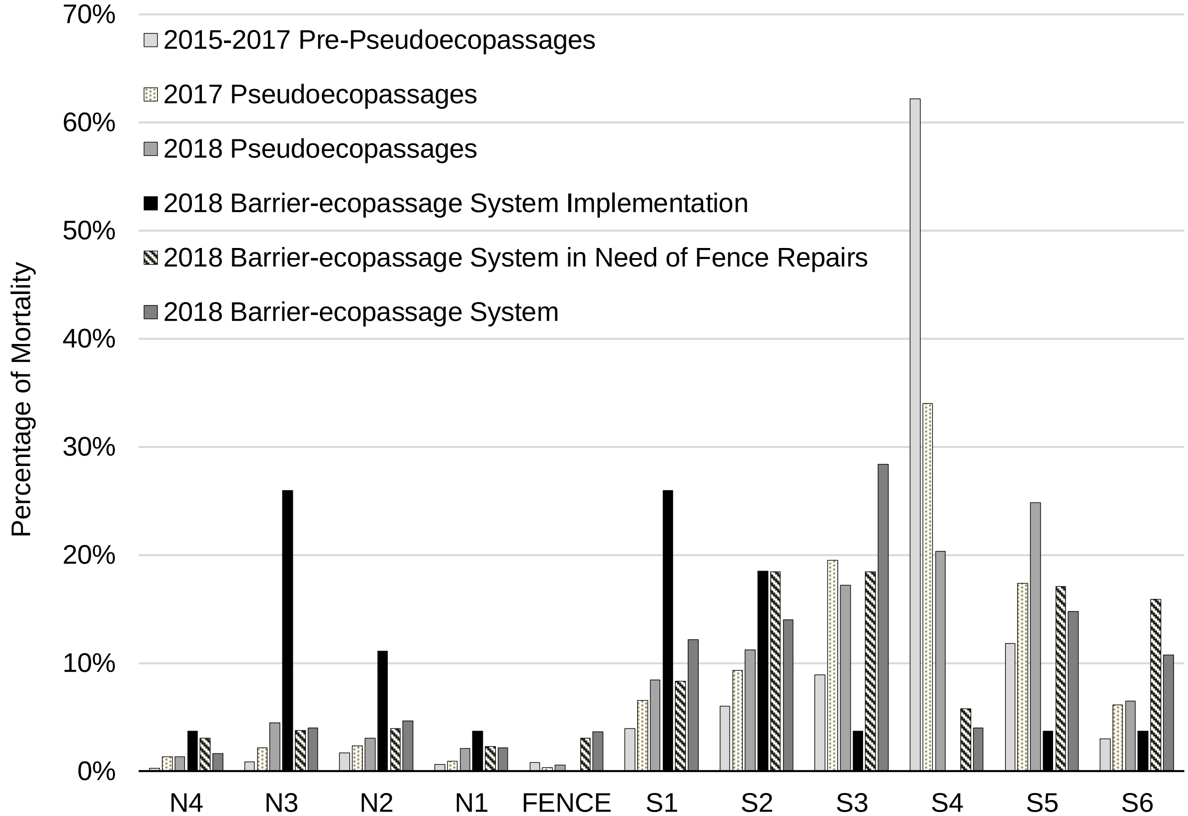

Figure A5-6. Percentage of vertebrate road mortality occurring in each roadway section (west and east lanes combined) monitored along a 1.2 mile stretch of State Route 78, near Nelsonville in Athens County, Ohio, 2015-2019. Roadway sections are pictured in Figure A5-1. The 2018 barrier-ecopassage system covered all of S4 and half of S5.

Appendix 6: Population Monitoring

METHODS -- To evaluate the impacts of roadway mortality rates and ecopassage use rates, population estimates were required (Andrews et al. 2015). To assess amphibian populations, we used a variety of sampling techniques, including:

(1) DIP NET SURVEYING – Dip net surveys were conducted in spring of 2015 (March-June) in 12 wetlands (Figure A6-1, numbered wetlands). Three surveys were conducted in each wetland unless it dried up before they could all be performed. We also conducted a single dip net survey in wetlands 1, 2, 3, 5, 7, and 8 in 2016 (Table A6-1). Surveys were completed using a standard aluminum-frame dip net that measured 11.8 in. x 15.4 in. with 1/8 in. mesh and a fiberglass handle. A dip net sweep was classified as pulling the basket of the dip net across the substrate on the bottom of the wetland for a 3 ft. length (Gunzburger 2007). All suitable amphibian habitats within a wetland were surveyed.

(2) HOOP NET TRAPPING – Circular hoop net traps made of string netting with diameters of either 24 in. or 36 in. were placed in deeper wetlands (wetlands 3, 2, 9, 10, and 51; wetlands pictured in Figure A6-1) to capture larger frogs and turtles. Hoop net traps were always placed with at least 10% of the trap above the surface of the water (Dodd 2010, McDiarmid et al. 2012; Figure A6-2). Hoop net traps were used regularly from the spring through summer of 2015 and 2017, and intermittently at other times.

(3) MINNOW TRAPPING – Rectangular Promar® collapsible minnow traps were placed in wetlands where water levels were sufficient to submerge trap openings. The double-opening minnow traps measured 10 in. x 10 in. x 17 in. and were constructed of maroon 1/8 in. polyethylene mesh. Each trap had a zippered opening to assist in safely removing captured individuals. To keep the top 1-2 in. of the traps above water floats were used (Olson et al. 1997, Dodd 2010). Minnow traps were deployed in 11 wetlands (all except wetland 10, wetlands labeled in Figure A6-1). Three rounds of minnow trapping in each wetland were conducted in the spring and one round of minnow trapping occurred in the fall. During each round minnow traps were placed in each wetland for 3-5 nights and checked each morning (Figure A6-2).

(4) PITFALL TRAPPING – Our pitfall traps were five-gallon buckets buried in the ground and level with the ground's surface. Pitfall traps contained some soil and a sponge at the bottom to prevent desiccation of amphibians. The bottoms of buckets were perforated to allow excess rainwater to drain and to prevent drowning of captured animals (Heyer et al. 1994, Enge 2001, Dodd 2010). Four pitfall traps were placed at the fence ends of the 2013 barrier-ecopassage system 2015-2018 (see Appendix 7: Monitoring of 2013 Barrier-

ecopassage System). Twelve traps were placed along a 500-ft. barrier in 2016 and 2017 before the pseudoecopassage experiment was in place (see Appendix 3: Pseudoecopassage Experiment). The 2017 pseudoecopassage experiment setup involved 40 pitfall traps, and the 2018 pseudoecopassage experiment had 64 pitfall traps. Eight pitfall traps were installed at the fence ends of the 2018 barrier-ecopassage system on September 21, 2018.

(5) SPOTLIGHT SURVEYS – Surveys were conducted on rainy nights when the most amphibians are migrating along State Route 78 (SR 78) near Nelsonville in Athens County Ohio to capture migrating amphibians (Sexton et al. 1990, Petranka 1998, Pfingsten et al. 2013). Head lamps were used to detect amphibians and individuals were captured by hand (Heyer et al. 1994, Dodd 2010, Figure A6-2).

(6) SPRING PEEPER CALL SURVEYS – Spring Peepers were identified to species based on their calls. The number of calling males detected within a 10-ft. radius by sound and/or sight were recorded during five-minute samples. Surveys were conducted at least one hour after sunset (Heyer et al. 1994, Gunzburger 2007, Dodd 2010). We conducted our call surveys throughout each spring (March-May). Surveys were completed at four wetlands in the spring of 2015, at nine wetlands in 2016 and 2017, and at 10 wetlands in 2018 and 2019.

All adult animals captured were measured, weighed, sexed, and marked using a batch marking technique (United States Geological Survey 2001, Ferner 2007, Dodd 2010; Figure A6-2). In 2015 and 2016 the second right toe (from the body) was clipped, in 2017 the third right toe was clipped, in 2018 the second left toe was clipped, and in 2019 the third right toe was clipped.

We conducted 35 dip net surveys, 674 hoop nets trap nights (one trap open for one night), 16,605 minnow trap nights, 21,792 trap nights, and 222 spotlight surveys from 2015 to 2019 (Table A6-1). Throughout 2015-2019 we completed 1,536 Spring Peeper call surveys (Table A6-1). Adult animals captured were used to generate capture-recapture population estimates, while the Spring Peeper call surveys were used to generate area-density abundance estimates for Spring Peepers (Heyer et al. 1994, Pollock 2000, Gunzburger 2007, Mazerolle et al. 2007, Dodd et al. 2010).

We generated capture-recapture estimates for eight amphibian species (Jefferson Salamander, Spotted Salamander, Marbled Salamander, Red-spotted Newt, American Toad, American Bullfrog, Green Frog, and Pickerel Frog) that had high enough capture levels throughout the study period (Table A6-2). We calculated four capture-recapture estimates for each species for each year (2015-2019): (1) a Lincoln-Petersen estimate using captures and recaptures for a given year, (2) a Lincoln-Petersen estimate with removal using captures, recaptures, and the number of roadkilled animals, (3) a Chapman estimate using captures and recaptures for a given year, and (4) a Chapman estimate with removal using captures, recaptures, and the number of roadkilled animals (Pollock 2000, Mazerolle et al. 2007). All four of our estimates were scaled by capture effort each year. Capture effort was calculated by taking the average capture rate for each method in that year and multiplying it by the number of surveys or traps nights (Mazerolle et al. 2007). In late-May through June 2019 we captured 525 juvenile Green Frogs and three juvenile Pickerel Frogs that we marked but excluded from population analyses as there was not an opportunity to recapture these individuals. Captured toadlets and metamorphic peepers and treefrogs were not included as captures because they were not marked.

Spring Peeper area-density abundance estimates were calculated for each wetland (N_w). We used the equation $N_w=(n_m+n_f)*(A_w/$ 314 sq. ft.) to calculate the number of adult Spring Peepers per wetland (Buckland 1987, Crouch & Patton 2002, Mazerolle et al. 2007). The variable n_m is the average calling male density per 10-ft. radius circle in a wetland. The variable n_f is the expected number of females per 10-ft. radius circle in a wetland. We calculated the expected number of females by multiplying the average calling male density in a wetland by the female to male ratio of captured spring peepers each year. The variable A_w is the area of the wetland containing suitable Spring Peeper breeding habitat. Spring Peeper suitable breeding habitat was identified as water less than 12 in. deep with vegetation present for egg deposition; suitable habitat was identified in the field and mapped into ArcMap to calculate area size. A_w is divided by 314 sq. ft. as this is the area of a 10-ft. radius circle.

The programs Microsoft Excel and the statistical programming language R (R Core Team 2016) were used to process data and generate population estimates from collected data.

RESULTS -- Using all our sampling methods we detected 16 amphibian species (Table 1, Table A6-2). We were able to calculate capture-recapture population estimates for eight amphibian species (Jefferson Salamander, Spotted Salamander, Marbled Salamander, Red-spotted Newt, American Toad, American Bullfrog, Green Frog, and Pickerel Frog; Figure A6-3). We caught the most Red-spotted Newts (Table A6-2), although newts population estimates drop from over 10,000 individuals in 2015 and 2016 to below 2,000 individuals in 2017-2019 (Figure A6-3). The three mole salamander species (Jefferson Salamander, Spotted Salamander, and Marbled Salamander) all have relatively low population sizes throughout the study period, and their population estimates decrease over time (Figure A6-3). The American Bullfrog and Green Frog both have decreasing population trends as well (Figure A6-3). Conversely, the American Toad population was stable except for the 2018 population estimate, which was relatively low (Figure A6-3).

We estimated an abundance of 21,407 Spring Peepers in 2015 from four wetlands (Figure A6-4). In 2016 we estimated 19,990 Spring Peepers from nine wetlands, and 14,444 Spring Peepers in 2017. We estimated there were 14,546 and 14,903 Spring Peepers in 2018 and 2019 respectively. Wetland 2 (see Figure A6-1) had the highest number of peepers in all years, followed by wetland 7 (Figure A6-4).

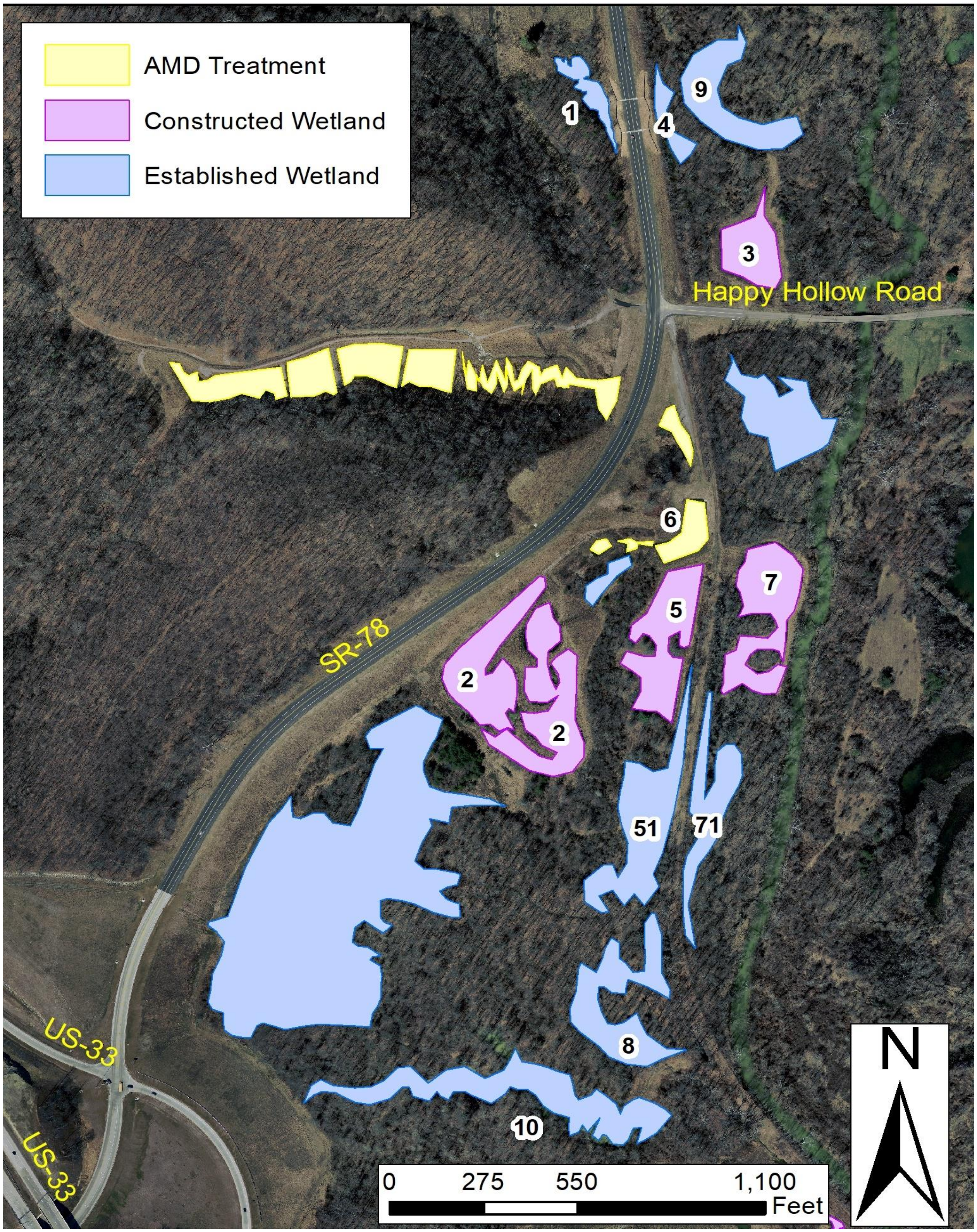

Figure A6-1. Map of wetlands along State Route 78 near Nelsonville in Athens County, Ohio. Yellow wetlands are part of the Coe Hollow Acid Mine Drainage (AMD) Treatment Project, purple wetlands were constructed by the Ohio Department of Transportation in 2009, and blue wetlands were established naturally occurring wetlands before 2009. Wetland identification numbers, for wetlands that maintain productive standing water for amphibians, are labeled in this figure. Amphibians were only captured in one AMD Treatment wetland, wetland 6.

Table A6-1. Number of dip net surveys, hoop net trap nights, minnow trap nights, pitfall trap nights, and spotlight surveys conducted each year 2015-2019.

	2015	2016	2017	2018	2019
DIP NET SURVEYS	29	6	0	0	0
HOOP NET TRAP NIGHTS	240	0	376	34	24
MINNOW TRAP NIGHTS	1881	1910	2643	3435	2736
PITFALL TRAP NIGHTS	772	3120	10480	6332	1088
SPOTLIGHT SURVEYS	35	61	45	42	39
CALL SURVEYS	72	350	366	368	380

Figure A6-2. Images of a 36 in. hoop net trap (top left), C. B. Hopkins checking a minnow trap (top center), an American Toad found during a spotlight survey (top right), K. E. Harman measuring a Green Frog (bottom left), two Jefferson Salamanders in a pitfall trap (bottom center), and a Spotted Salamander released after measurements were taken (bottom right).

Table A6-2. Number of animals captured (and recaptured) of each amphibian species along State Route 78 near Nelsonville in Athens County, Ohio, 2015-2019.

	2015	2016	2017	2018	2019
Jefferson Salamander	14(1)	33(5)	10(3)	16(2)	6(3)
Spotted Salamander	21(3)	27(6)	13(2)	24(5)	16(7)
Marbled Salamander	12(1)	22(3)	24(5)	8(2)	5(2)
Red-spotted Newt	1019(68)	1005(73)	153(19)	220(27)	288(61)
Long-tailed Salamander	1(0)	0(0)	0(0)	0(0)	0(0)
Eastern Red-backed Salamander	0(0)	6(0)	2(0)	4(0)	1(0)
Slimy Salamander	0(0)	1(0)	0(0)	0(0)	0(0)
American Toad	74(7)	205(27)	183(22)	155(29)	276(55)
American Bullfrog	53(3)	61(5)	89(10)	36(8)	41(7)
Green Frog	148(8)	145(17)	303(29)	84(11)	626(17)
Pickerel Frog	33(4)	86(12)	260(21)	31(9)	51(14)
Northern Leopard Frog	8(0)	9(1)	4(0)	3(0)	0(0)
Wood Frog	5(0)	6(0)	9(1)	6(1)	3(1)
Gray Treefrog	3(0)	44(0)	11(0)	3(0)	2(0)
Spring Peeper	257(8)	280(13)	168(7)	243(14)	308(20)
Western Chorus Frog	0(0)	1(0)	0(0)	1(0)	0(0)

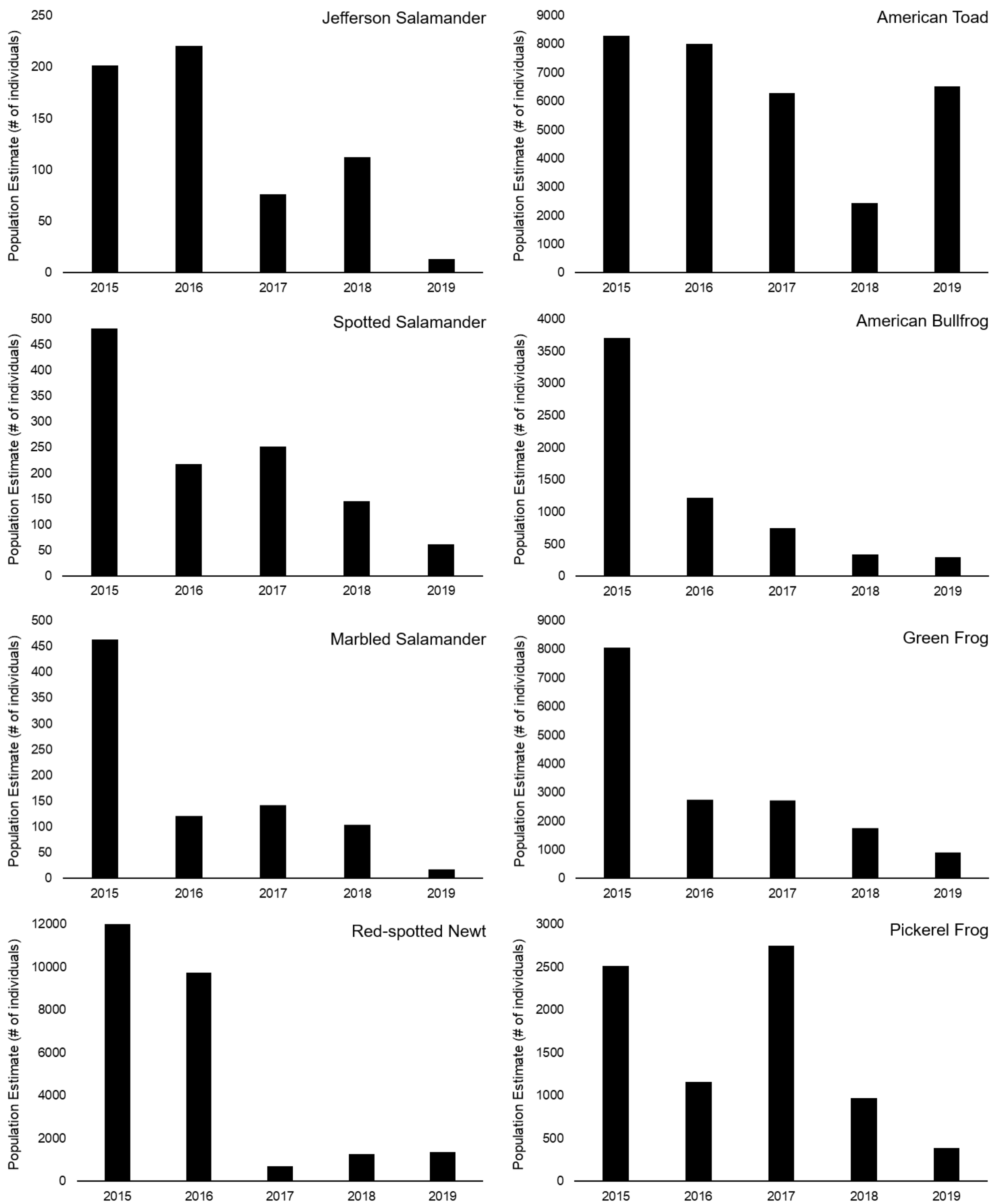

Figure A6-3. Average of four population size estimates for eight amphibian species along State Route 78 near Nelsonville in Athens County, Ohio, 2015-2019.

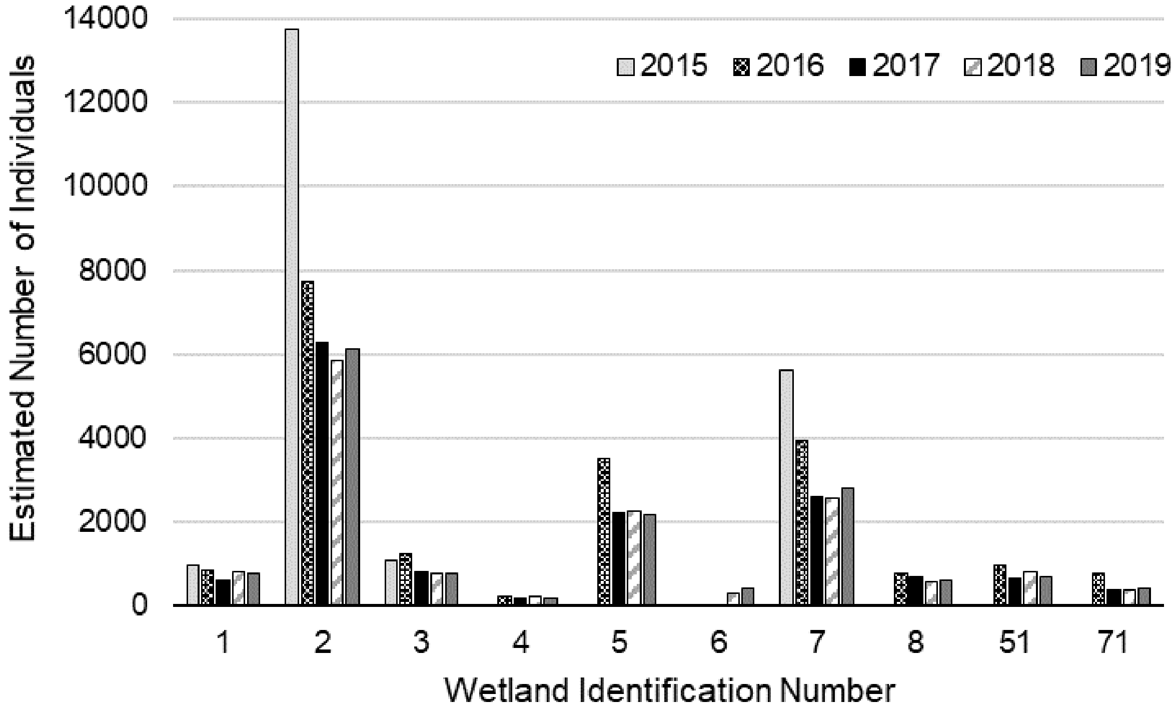

Figure A6-4. Estimated number of individual Spring Peepers breeding in each wetland along State Route 78 near Nelsonville in Athens County, Ohio, 2015-2019. Wetland numbers are provided in Figure A6-1. Wetlands 1, 2, 3, and 7 were evaluated in 2015 and wetland 6 was evaluated in 2018 and 2019.

Appendix 7: Monitoring of 2013 Barrier-Ecopassage System (2015-2018)

METHODS -- We monitored the barrier-ecopassage system installed by the Ohio Department of Transportation (ODOT) along State Route 78 (SR 78) near Nelsonville in Athens County, Ohio in 2013 for three and a half years (2015-2017 and February-May 2018). The 2013 barrier-ecopassage system consisted of two 60 ft. long ecopassages connected by 500 ft. of barrier fencing (Figure 3, Figure 4, Figure A7-1). The ecopassages were made of treated concrete with an entrance size of 20 in. wide x 16 in. tall; there were small perforations (1 in. x 1.5 in.) running the length of the ecopassages to allow for some sky exposure, and the curved plastic fencing was 2 ft. tall and was buried 4 in. into the ground (Figure 3). The barrier fencing and ecopassages were made specifically for amphibians by ACO Polymer Products, Inc. While monitoring the 2013 barrier-ecopassage system we made repairs to the fence and documented structural defects that we could not mend.

To monitor the effectiveness of the barrier fencing, one pitfall trap was placed at each end of the plastic barrier fence (Mata et al. 2005, Baxter-Gilbert et al. 2015, see Appendix 6: Population Monitoring for more about pitfall traps). We checked traps daily in the spring and fall of each year. Each animal captured was measured, weighed, sexed, and marked (United States Geological Survey 2001, Ferner 2007, Dodd 2010).

We assessed the ecopassages using camera traps to obtain images of animals using the ecopassages (Pagnucco et al. 2011, Leeb et al. 2013). Spypoint Tiny-W3 cameras equipped with Jokie2 break-beam sensors were placed at each entrance of the ecopassages to take photographs of amphibians in the passages (Leeb et al. 2013, Figure A7-2). We used handmade wooden blocks to funnel animals within the view of the camera, which we measured at about 10 in. in width. Animals that entered the ecopassage would cross an infrared light beam at the entrance produced by a sensor which triggered the camera to take a photo (Figure A7-2). Break-beam sensor cameras were examined and the batteries were replaced every five to eight days. Cameras with break-beam sensors were in place for 523 nights from April 16, 2015 to May 23, 2018 (Table A7-1).

We assessed the effectiveness of the 2013 barrier-ecopassage system by tabulating and graphing the number of amphibians captured in the pitfall traps and the number detected by the ecopassage camera traps. Tables and graphs were created in Microsoft Excel.

RESULTS -- We captured 339 amphibians in the 2013 barrier-ecopassage system fence end pitfall traps (Table A7-1, Figure A7-3). There was a decline in the number of amphibians captured each spring throughout the study (Table A7-1, Figure A7-3) likely due to a combination of population declines (see Appendix 6: Population Monitoring) and the inability of the barrier fence to guide animals as irreparable fence issues accumulated over time.

Frogs and salamanders were detected in the ecopassages (Figure A7-4). We detected a total of 35 amphibians at the passage entrances, of which 12 were detected at both ends of a passage (Table A7-1, Figure A7-3). Thus, 34.29% of amphibians entering an ecopassage crossed through. Seven amphibian species (Jefferson Salamander, Marbled Salamander, Red-spotted Newt, American Toad, American Bullfrog, Green Frog, and Spring Peeper) were detected by the ecopassage camera traps (Table 1). Marbled Salamanders (n=3), Green Frogs (n=7), an American Bullfrog, and an American Toad were seen traversing the whole length of an ecopassage.

We found 9.69 times more animals at the barrier fence ends than we detected in the ecopassages (Table A7-1, Figure A7-3). Thus, there were amphibians at this location along SR 78 and the fencing appeared to be guiding individuals, but the ecopassages were not facilitating the movement of many amphibians.

During our monitoring of the 2013 barrier-ecopassage system we began making repairs to the plastic barrier fence in 2016 as needed. By 2017 there were several areas that we were no longer able to repair due to severe fence cracking and erosion (Figure A7-5). In 2017 we also discovered that the concrete blocks making up the ecopassages were separating from each other (Figure A7-5); by 2018 there were several inches between some of the blocks. Therefore, in 2018 during implementation of the 2018 barrier-ecopassage system by ODOT crews the 2013 ecopassages were filled in with concrete to prevent any hazards to driver safety and road integrity, functionally removing the 2013 barrier-ecopassage system (Figure A7-6, see Appendix 9: Implementation of 2018 Barrier-ecopassage System).

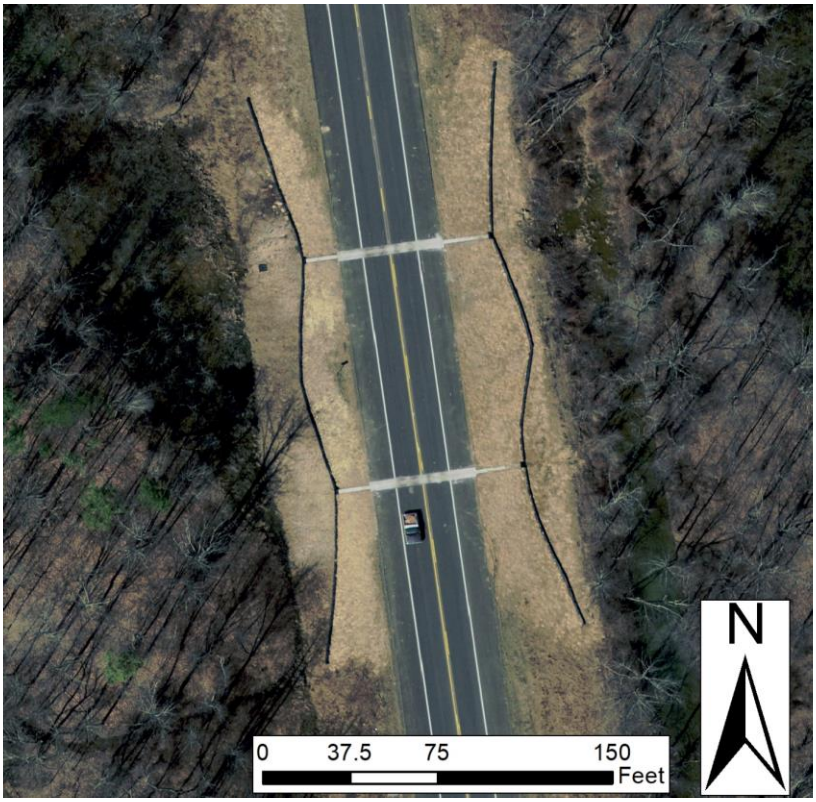

Figure A7-1. Aerial view of 2013 barrier-ecopassage system in place along State Route 78 near Nelsonville in Athens County, Ohio.

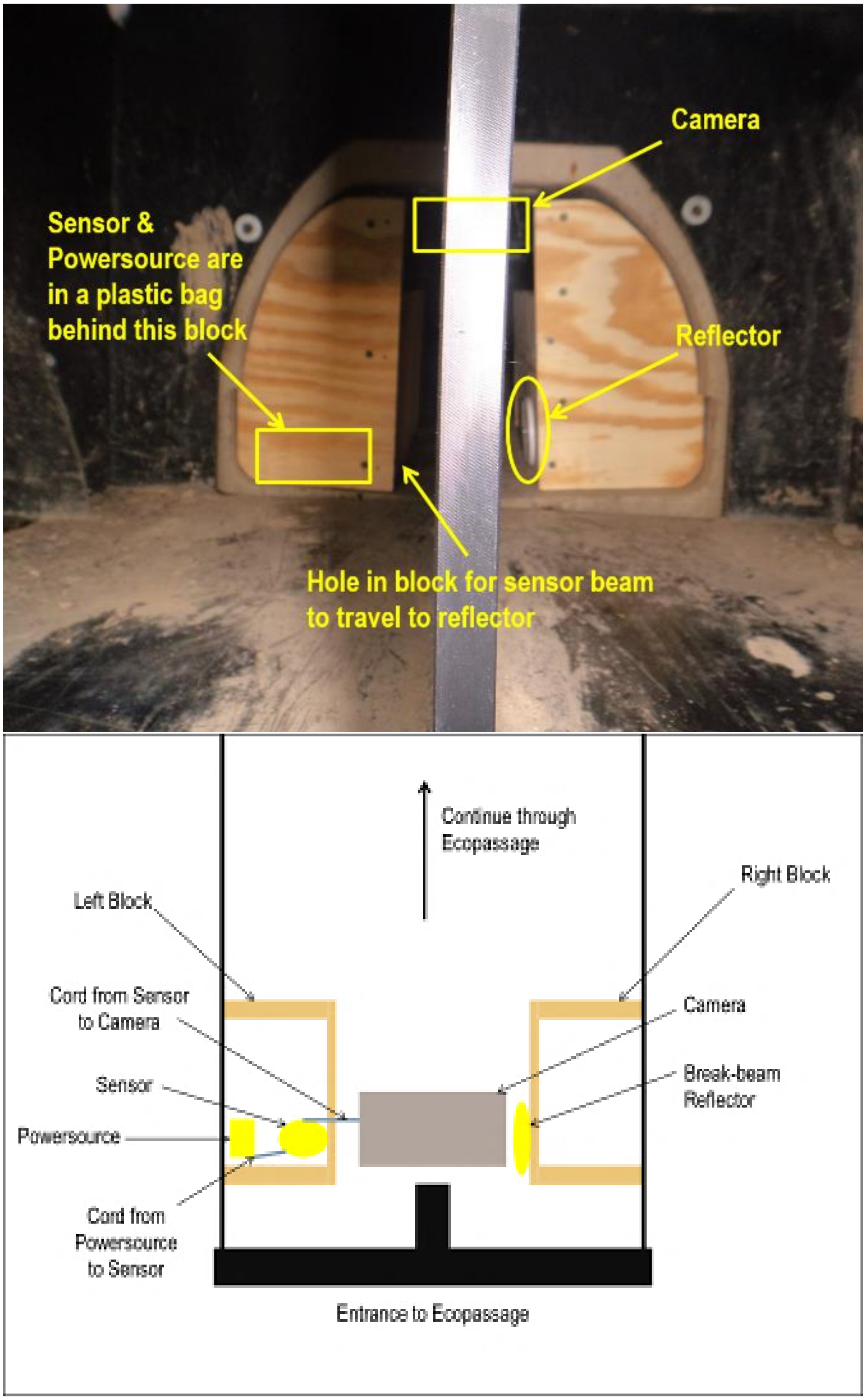

Figure A7-2. Image of the break-beam sensor camera system in place in the 2013 ecopassages along State Route 78 near Nelsonville in Athens County, Ohio (top) and schematic of the break-beam sensor camera system from a top-down view (bottom). Note that the central divider at the forefront of the photo does not extend into the tunnel.

Table A7-1. Number of nights the 2013 barrier-ecopassage system camera traps were in place, number of amphibians detected in ecopassages (at one or more ecopassage entrances), number of amphibians traveling through an ecopassage (detected at both the west side and east side of an ecopassage), and the number of amphibians captured in the pitfall traps at the ends of the barrier fencing along State Route 78 near Nelsonville in Athens County, Ohio, 2015-2018.

	Spring 2015	Fall 2015	Spring 2016	Fall 2016	Spring 2017	Fall 2017	Spring 2018
Number of ecopassage camera trap nights	61	76	102	68	97	64	55
Number of amphibians detected in ecopassages	10	3	7	5	4	3	3
Number of amphibians traveling through an ecopassage	2	2	2	2	1	1	2
Number of amphibians captured in fence end pitfall traps	101	32	84	39	34	28	21

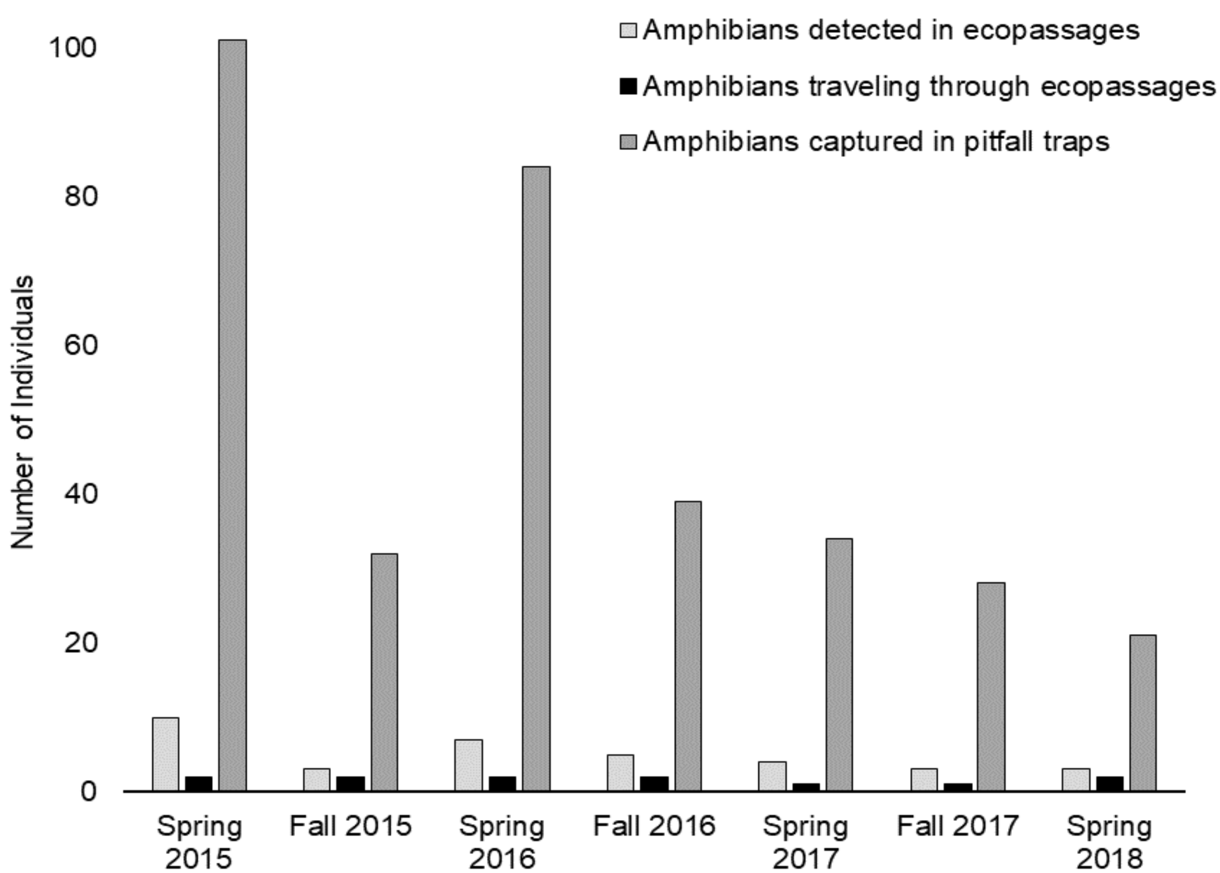

Figure A7-3. Number of individual amphibians detected in 2013 ecopassages (at one or more ecopassage entrances), number of amphibians traveling through a 2013 ecopassage (detected at both the west side and the east side of an ecopassage), and the number of amphibians captured in the pitfall traps at the ends of the 2013 barrier fencing along State Route 78 near Nelsonville in Athens County, Ohio, 2015-2018.

Figure A7-4. Amphibians detected by the 2013 barrier-ecopassage system camera traps. Green Frog (left), Marbled Salamander (center), and American Toad (right).

Figure A7-5. Maintenance concerns of the 2013 barrier-ecopassage system in place along State Route 78 near Nelsonville in Athens County, Ohio. Gaps and cracks in the plastic barrier fencing (left) and separation of concrete blocks that make up the ecopassages (right).

Figure A7-6. The 2013 ecopassages in place along State Route 78 near Nelsonville in Athens County, Ohio after they were filled in with concrete on June 27, 2019.

Appendix 8: Recommendations for 2018 Barrier-Ecopassage System

We recommended that a new mitigation structure be constructed along State Route 78 (SR 78) near Nelsonville in Athens County, Ohio in the area of highest amphibian mortality, a stretch of roadway southwest of the 2013 mitigation structure (Figure 5). Based on our experimental and observational studies, we recommended that the new mitigation structure have ecopassages that were as wide as possible, provided as much sky exposure as possible, and included a maintained line-of-sight (see Appendix 2: Choice Experiments and Appendix 3: Pseudoecopassage Experiment). We also recommended that the bottom substrate be natural soil, as prior studies have shown this is preferred over alternatives such as concrete and plastic (Lesbarrères et al. 2004, Woltz et al. 2008, Patrick et al. 2010, Clevenger and Huijser 2011, Gunson et al. 2016). Fencing between passages needs to be durable and long lasting, and best practices manuals recommend no more than 150 ft. of fencing between passages for most small animals (Clevenger and Huijser 2011, Gunson et al. 2016). In addition, best practices suggest that it is beneficial for barrier fencing to restrict the abilities of climbing amphibians by including a downward-projecting lip along the top edge. Table A8-1 contains a matrix of recommended mitigation structures ranked by the inferred amount of connectivity provided.

Steps to implement a new mitigation structure include: (1) consideration of recommended mitigation features based on benefits (reduced mortality and improved connectivity) and costs (time, money, materials, risks/obstacles, etc.); (2) designing the structure; (3) installing the structure; (4) monitoring the new structure to assess effectiveness; and (5) maintaining the structure into the future.

Implementing mitigation structures entails some obstacles that must be overcome. Any new structure must be safe for humans and vehicle traffic. One strategy to ensure human and vehicle safety is using barrier and ecopassage materials that are prefabricated and come with safety approval. Another concern is that the construction of new mitigation could slow down traffic while construction and installation are taking place, which effects community members who use the road. An additional obstacle to installing the best mitigation structure possible is the existence of infrastructure already in place at the site, such as water control structures, present roadway features, gas lines, and other objects. Preexisting infrastructure should be considered during the design process to reduce risks. Erosion also needs to be accounted for when constructing a new mitigation structure. Another important consideration is the short-term and long-term maintenance of the mitigation structure (Jacobson & Tonjes 2015).

Mitigation installations require monitoring to assess their effectiveness, after which time they still require short- and long-term maintenance. The 2013 barrier-ecopassage system was an example of mitigation deterioration, as the hard-plastic barrier fence experienced significant cracking and soil eroded from underneath;

moreover, the blocks that made up the ecopassages began to separate (Figure A7-5). A final risk is our incomplete understanding of wildlife behavior and movement patterns that may result in an imperfectly functioning mitigation structure. In our study, we used a site with 2.5 years of existing data on which to base mitigation decisions. Data on mortality and movement patterns prior to mitigation installation is rare and precious. In addition, we completed a full literature review, and used up-to-date science and best practices to inform mitigation design.

By installing the best mitigation possible, the goal is to reduce the negative impacts of SR 78 on amphibian populations by reducing mortality and maintaining population and habitat connectivity. Our research on ecopassages is applicable to migratory amphibian management along other highways. Many habitats are fragmented throughout Ohio, the rest of the United States, and the world, and would benefit from improved mitigation systems.

We recommended that a new structure be implemented in mid- to late-fall of 2017. This timing allowed most amphibians and reptiles to migrate prior to installation. After the new mitigation system was installed, we monitored the effectiveness of the structure through mortality surveys, camera and pitfall trapping, and amphibian population studies. Our evaluation of the success of the new barrier-ecopassage structure is relevant to future regulatory and non-regulatory mitigation along two-lane highways in Ohio and beyond.

Table A8-1. Matrix of recommended mitigation structures ranked by estimated amount of connectivity provided. The amount of connectivity provided is based on the findings of our research, findings by other studies, case studies of successful projects, best practices manuals, and other literature.

Rank	Mitigation Structure	Line-of-sight	Sky Exposure	Passage Width	Sub-strate
1	700 ft. long, 24 in. tall, grated bridge	fully maintained	75-90%	700 ft.	natural
2	6 to 4 ecopassages, each 60 in. wide x 24 in. tall. Fencing between passages, and at each end	fully maintained	75-90%	This width was used in successful projects in Canada and Vermont (Gunson et al. 2016)	natural
3	7 to 5 ecopassages, each 48 in. wide x 24 in. tall. Fencing between passages and at each end	fully maintained	75-90%	This width preferred by amphibians in choice experiment	natural
4	7 to 5 ecopassages, each 48 in. wide x 24 in. tall. Fencing between passages and at each end	fully maintained	75-90%	This width preferred by amphibians in choice experiment	concrete
5	7 to 5 ecopassages, each 36 in. wide x 24 in. tall passages. Fencing between passages and at each end	fully maintained	75-90%	Not as wide as amphibians preferred	natural
6	7 to 5 ecopassages each 36 in. wide x 24 in. tall. Fencing between passages and at each end.	fully maintained	75-90%	Not as wide as amphibians preferred	concrete
7	7 to 5 ecopassages each 48 in. wide x 24 in. tall. Fencing between passages and at each end.	fully maintained	20-40%	This width was preferred by amphibians in choice experiment	concrete
8	7 to 5 ecopassages each 36 in. wide x 24 in. tall. Fencing between passages and at each end	fully maintained	20-40%	Not as wide as amphibians preferred	concrete
9	7 to 5 ecopassages each 48 in. wide x 24 in. tall. Fencing between passages and at each end.	reduced	75-90%	This width was preferred by amphibians in choice experiment	concrete
10	7 to 5 ecopassages each 24 in. wide x 24 in. tall. Fencing between passages and at each end.	reduced	20-40%	Not as wide as amphibians preferred	concrete

Appendix 9: Implementation of 2018 Barrier-Ecopassage System

We submitted our interim report with recommendations for the new 2018 amphibian barrier-ecopassage system to be placed along State Route 78 (SR 78) near Nelsonville in Athens County, Ohio at the end of June 2017 (see Appendix 8: Recommendations for 2018 Barrier-ecopassage System, Table A9-1).

A list of materials needed for implementation and design plans were created (Table A9-2, Figure A9-1, Figures A9-3 through A9-8). The chosen design was to have five ecopassages with 900 ft. of barrier fencing on each side of the road (Figure 3, Figure 4, Figure A9-1, Figure A9-2). The ecopassages were 48 in. wide by 36 in. tall concrete utility trenches with grated tops (Trenwa 2013) that were about 54 ft. long (Figure 3, Figures A9-3 through A9-8). Four to eight inches of soil was added to the bottom of each passage to provide a natural substrate and to reduce effect of crowning the ecopassages on the line-of-sight through the ecopassages. Ecopassages were spaced about 150 ft. apart (Figure 4, Figure A9-1). AMX40 flexible plastic fencing from Animex® Wildlife Exclusion Fencing & Mitigation Solutions was chosen for the barrier fencing (Animex 2016). The AMX40 fencing is 32 in. tall when installed and has a lip at the top to prevent climbing animals from going up over the fence. Ideally, the fencing is curved from the center between ecopassages towards the ecopassages on the east side of the road, to funnel animals toward ecopassages (Figure 4).

Materials required for implementation of the 2018 barrier-ecopassage structure were purchased in August and September of 2017, and implementation was set to take place in October 2017 (Table A9-1). However, implementation was postponed until June 2018. The barrier-ecopassage structure was installed by Ohio Department of Transportation (ODOT) crews from May 29-July 5, 2018 (Table A9-1). The implementation plan for ODOT crews included: sawing the roadway, excavating road cuts for ecopassage installation, installing the ecopassages, adding soil to the ecopassages, installing ecopassage grated tops, creating headwalls for the ecopassage entrances, installing barrier fencing, installing guard rail for motorist safety, installing drainage pipes to prevent erosion, and adding other erosion control methods (rock bank, hay, biodegradable mesh, etc.). Figures A9-9 through A9-11 depict some of the implementation process carried out by ODOT crews. While ODOT crews installed the 2018 barrier-ecopassage system, they also filled in the degraded 2013 ecopassages, effectively removing the 2013 barrier-ecopassage system (see Appendix 7: Monitoring of 2013 Barrier-ecopassage System, Figure A7-6).

Upon completion of work by ODOT crews there were still some concerns regarding the implementation of the 2018 barrier-ecopassage system. The plastic AMX40 fencing had not been buried in the ground along the whole length, the fencing also had no drainage holes drilled in the bottom 4 in. of the fence, the top lip of the fencing had not been folded properly, the fencing was incorrectly attached to the fence posts, and the fencing did not properly interface with the headwalls to prevent animals from trespassing (Figure A9-12). There were also areas where erosion control measures were needed (Figure A9-12).

In the fall of 2018, we spent several days making repairs to the barrier fence with the help of personnel from ODOT, the Ohio Department of Natural Resources, the United States Fish and Wildlife Service, and the United States Army Corps of Engineers (Table A9-1). We implemented 120- to 90-angle bends (with 3-8 ft. of fencing going past the bend) at the fence ends to deter animals from going around the fence (Huijser et al. 2015, Huijser et al. 2016, Gunson et al. 2016). We also attached the fencing to the ecopassage headwalls and seeded bare soils to reduce erosion (Figure A9-13). In addition, one day was spent building a rock bank to reduce erosion along the barrier fence (Figure A9-14). On October 14, 2018 we deemed the 2018 barrier-ecopassage mitigation structure fully implemented. We continued evaluating the barrier fencing throughout the rest of 2018 every 2-3 weeks (Table A9-1).

In the spring of 2019, we began inspecting the barrier fence weekly for areas where dirt had eroded along the fence and near the headwalls (Table A9-1). Upon locating areas where the fence had become permeable due to erosion (Figure A9-15) we replaced the missing dirt. After the first heavy spring rains many holes appeared along the fence and near the headwalls, creating trespass opportunities that needed to be filled in with dirt (Figure A9-15). In addition, there were areas where the hillside was unstable and was sliding into the ditch (Figure A9-15). During the first heavy spring rains one of the ecopassages also began flooding (Figure A9-16). We constructed an earthen dam to prevent future flooding and eventually added sandbags to the dam to further reduce the chances of flooding (Figure A9-16). We added drainage holes to the bottom 4 in. of the fence in some areas and placed widow screen at the bottom of the fence in others to allow water to flow through while reducing the erosion of sediments. During our spring fence inspections, we also observed if the fence had separated from the fence posts or if the fencing had separated from the headwalls and made repairs as needed.

After the heavy spring rains in 2019 passed, we continued inspecting the barrier fence and making repairs every 2-3 weeks. With less frequent and lighter bouts of rain, the barrier fence held up well and required few repairs. There was recurrent erosion at the headwalls of ecopassage 5 that we blocked with rocks and a sandbag (ecopassages labeled in Figure A9-2). We also seeded all the bare soils to reduce erosion. Our last observation of the fence for the spring was on June 27, 2019 (Table A9-1).

We conducted our first fall fence evaluation on August 21, 2019 (Table A9-1). Over the summer vegetation had completely covered some of the ecopassage entrances and exceeded the height of the barrier fencing in some locations, and we manually removed this vegetation (Figure A9-17 and Figure A9-18). We also found some areas where the fencing needed to be reattached to the headwalls. We also found that the ecopassage 5 headwall continued to experience further erosion (Figure A9-19). However, we did not find any cracks in the fence, areas where the fence was not attached to the posts, or other holes under the fence.

Table A9-1. Timeline of activities involved in the implementation, maintenance, and repair of the 2018 barrier-ecopassage system from June 30, 2017 till August 24, 2019.

DATE	ACTIVITY
June 30, 2017	Submission of interim report with 2018 barrier-ecopassage system recommendations
July 19, 2017	Planning meeting at State Route 78 field site to discuss 2018 barrier-ecopassage system
July 21, 2017	Submission of final interim report with 2018 barrier-ecopassage system recommendations
July 24, 2017	List of materials needed for implementation of 2018 barrier ecopassage system created (see Table A9-2)
July 24, 2017	Design plans for 2018 barrier-ecopassage system created (see Figure A9-1, Figures A9-3 through A9-8)
August 10, 2017	Full list of price quotes for materials needed for implementation of 2018 barrier ecopassage system compiled
August 28, 2017	Materials for implementation of 2018 barrier ecopassage system purchased through Ohio Department of Transportation District 10
August 28, 2017	Organization of crews for implementation of 2018 barrier ecopassage system handled by Ohio Department of Transportation District 10
August 28, 2017	Ecopassage locations were surveyed, staked, and painted
September 22, 2017	Road closure signs posted for implementation of 2018 barrier-ecopassage system beginning October 2017
September 27, 2017	Implementation of 2018 barrier ecopassage system postponed from October 2017 till June 2018
April 23, 2018	Planning meeting to discuss implementation of 2018 barrier-ecopassage system
May 23, 2018	Road closure signs posted for implementation of 2018 barrier-ecopassage system beginning June 2018
May 29, 2018	Implementation of 2018 barrier ecopassage system begins
May 29, 2018	Passage locations were re-surveyed, staked, and painted
May 30-31, 2018	Sawed roadway
June 4, 2018	State Route 78 closed from US Highway 33 west bound exit ramp to Happy Hallow Road for implementation of 2018 barrier-ecopassage system
June 4-6, 2018	Excavated road cuts
June 6-12, 2018	Installed ecopassages
June 8-27, 2018	Installed drainage pipes and other erosion solutions
June 12-28, 2018	Filled gaps between ecopassage concrete blocks
June 13-15, 2018	Added soil to the ecopassages
June 13-19, 2018	Installed ecopassage grated tops
June 18-22, 2018	Created headwalls for the ecopassage entrances
June 18-July 3, 2018	Added control measures to reduce erosion (rock bank, hay, biodegradable mesh, etc.)
June 25-26, 2018	Installed guard rail
June 27, 2018	Filled in 2013 barrier-ecopassage system to prevent safety hazards
June 27-July 5, 2018	Installed barrier fencing
June 28, 2018	State Route 78 reopened from US Highway 33 west bound exit ramp to Happy Hallow Road

July 5, 2018	Implementation of 2018 barrier ecopassage system ends
July 23, 2018	Expressed concerns about fencing, headwalls, and erosion
July 24, 2018	Planning meeting at State Route 78 field site to discuss 2018 mitigation structure continued implementation and repairs
August 2, 2018	Purchased materials for continued implementation and repairs of 2018 barrier-ecopassage system
August 22, 2018	Worked on burying the 2018 barrier fence, properly attaching the fence to the fence posts, and folding the top lip of the fence
August 23, 2018	Worked on burying the 2018 barrier fence, properly attaching the fence to the fence posts, and folding the top lip of the fence
September 7, 2018	Received dirt from ODOT District 10; Trenched the ground to improve fencing installation, worked on burying the 2018 barrier fence, and added 120- to 90-degree angle fence end treatments
September 20, 2018	Worked on attaching fencing to headwalls
September 26, 2018	Planning meeting at State Route 78 field site to discuss 2018 mitigation structure continued implementation, repairs, and erosion concerns
September 27, 2018	Worked on burying the 2018 barrier fence
September 28, 2018	Seeded exposed soils along 2018 barrier-ecosystem mitigation structure to reduce erosion
October 12, 2018	Received rock from ODOT District 10; Worked on moving rock to rebuild banks along ditch, burying the fence, and properly attaching all barrier fencing to fence posts
October 14, 2018	Worked on burying the 2018 barrier fence and properly attaching the fence to the fence posts
October 14, 2018	2018 barrier-ecopassage system was fully implemented
October 14-December 28, 2018	Barrier fence inspection conducted every 2-3 weeks
February 2, 2019	First fence inspection of 2019, also filled in several areas where dirt had eroded along the fence and near the headwalls
February 2-April 27, 2019	Weekly barrier fence inspections were conducted, areas of erosion were filled in with dirt, and fence integrity was maintained with repairs
February 6-8, 2019	Discovered an ecopassage flooding and built an earthen dam and dug out ditch to prevent future flooding
February 15, 2019	Dug out ditch to prevent ecopassage flooding
February 26-27, 2019	Added sandbags to bolster the earthen dam
February 27, 2019	Patched crack in the barrier fence
March 28, 2019	Dug out ditch to prevent ecopassage flooding
April 28-June 27, 2019	Barrier fence inspections were conducted every 2-3 weeks, areas of erosion were filled in with dirt, and fence integrity was maintained with repairs
June 4, 2019	Added rocks and a sandbag to reduce erosion near one of the ecopassage headwalls
June 5, 2019	Obtained seed from ODOT
June 21, 2019	Seeded exposed soils along 2018 barrier-ecosystem mitigation structure to reduce erosion
August 21, 2019	First fall 2019 fence inspection
August 21-23, 2019	Weed whacked vegetation growing along barrier fence and in front of ecopassages
August 24, 2019	Reattached fencing to headwalls where necessary

Table A9-2. List of materials needed for 2018 barrier-ecopassage system to be implemented along State Route 78 near Nelsonville in Athens County, Ohio. Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

Item 606 Guardrail Type MGS: 900’

Item 606 Guardrail Type MGS With Long Posts: 900’

Item 606 Anchor Assembly, MGS Type E: 4 Each

Item 626 Barrier Reflector (A2): 20 Each

Item 659 Class 2 Seed Mix: 130 lbs

Item 670 Ditch Erosion Protection: 580 SY

Straw Bales: 20 each

Item 602 Concrete Masonry: 25 CY

Item 613 Low Strength Mortar Backfill (Type 2): 164 CY

Trenwa 48” x 36” Road Crossing XL Trench with Bar Grating: 268’

Item 611 4” Conduit, Type F: 400’ with 20 end caps, 17 ninety-degree ells and 13 tees (connects to existing 4” 707.31 underdrain)

Item 611 Precast Reinforced Concrete outlet: 5 Each

Animex AMX40 Fencing with stakes and hardware: 1800’

Item 611 Structural Backfill: 101 CY

Natural Sand: 30 CY

Item 442 Asphalt Concrete Surface Course, 12.5MM, Type A (448): 206 CY

All specification references and item numbers are found in the 2016 ODOT Construction and Materials Specifications Book

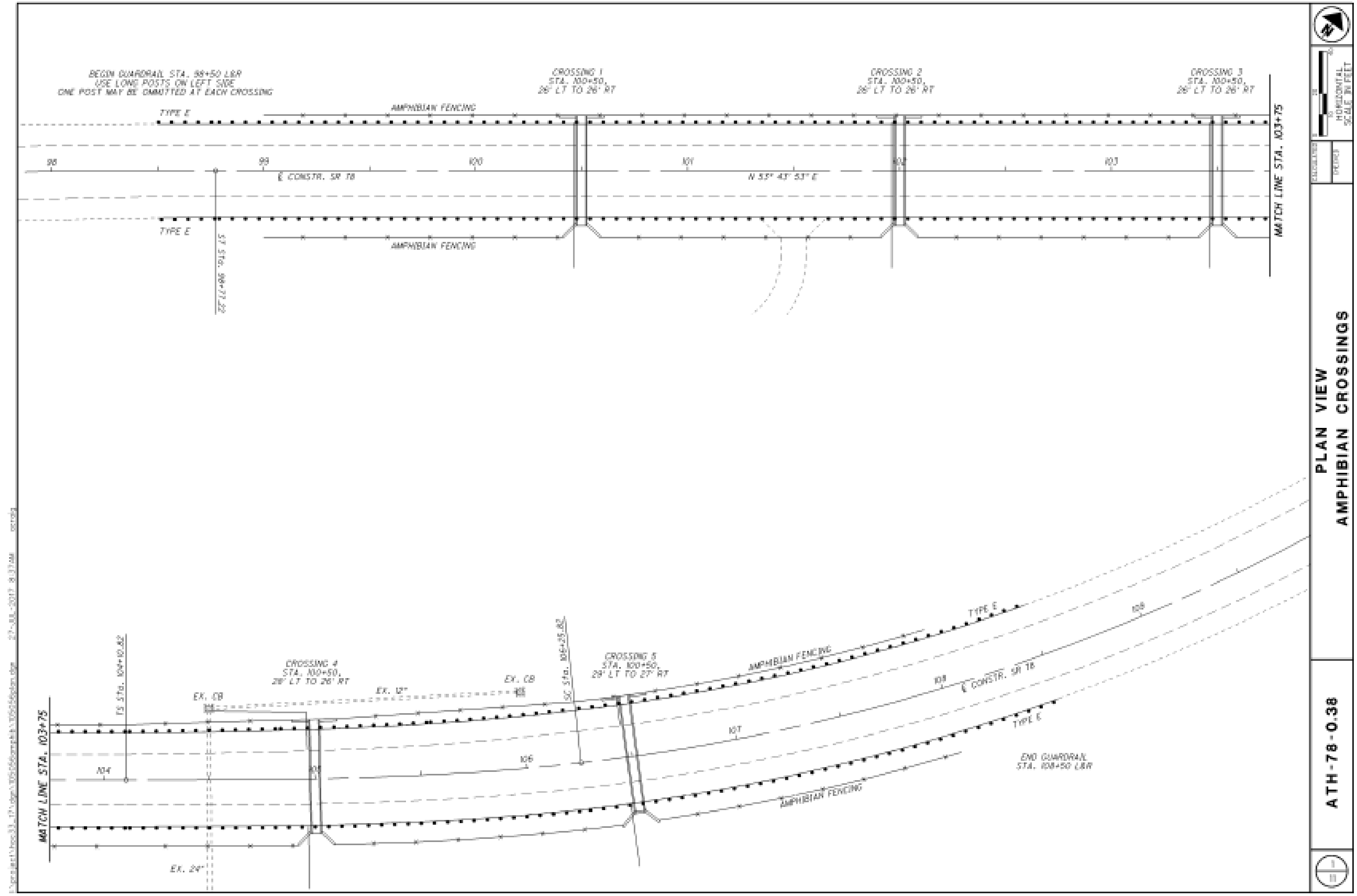

Figure A9-1. Design plan view for 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio. Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

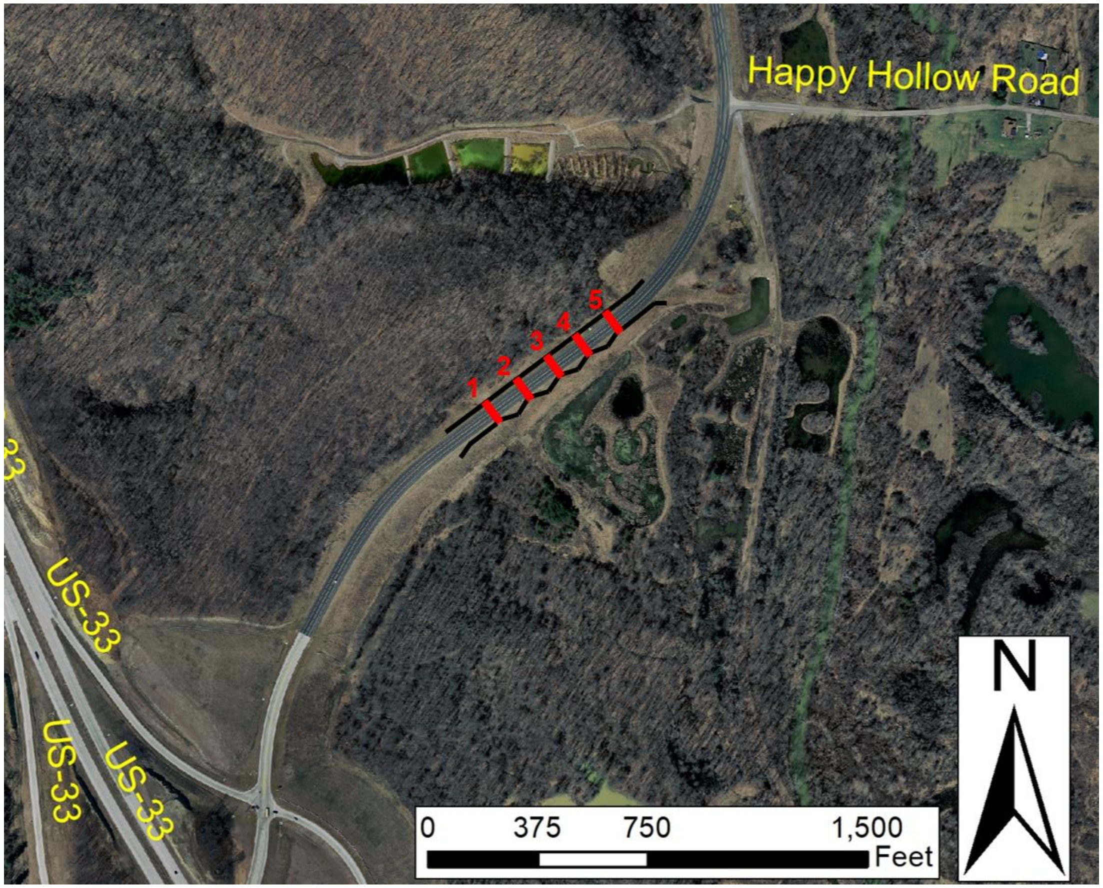

Figure A9-2. 2018 barrier-ecopassage system in place along State Route 78 near Nelsonville in Athens County, Ohio. Ecopassages are designated 1-5 with ecopassage 1 being the south-most and ecopassage 5 being the north-most.

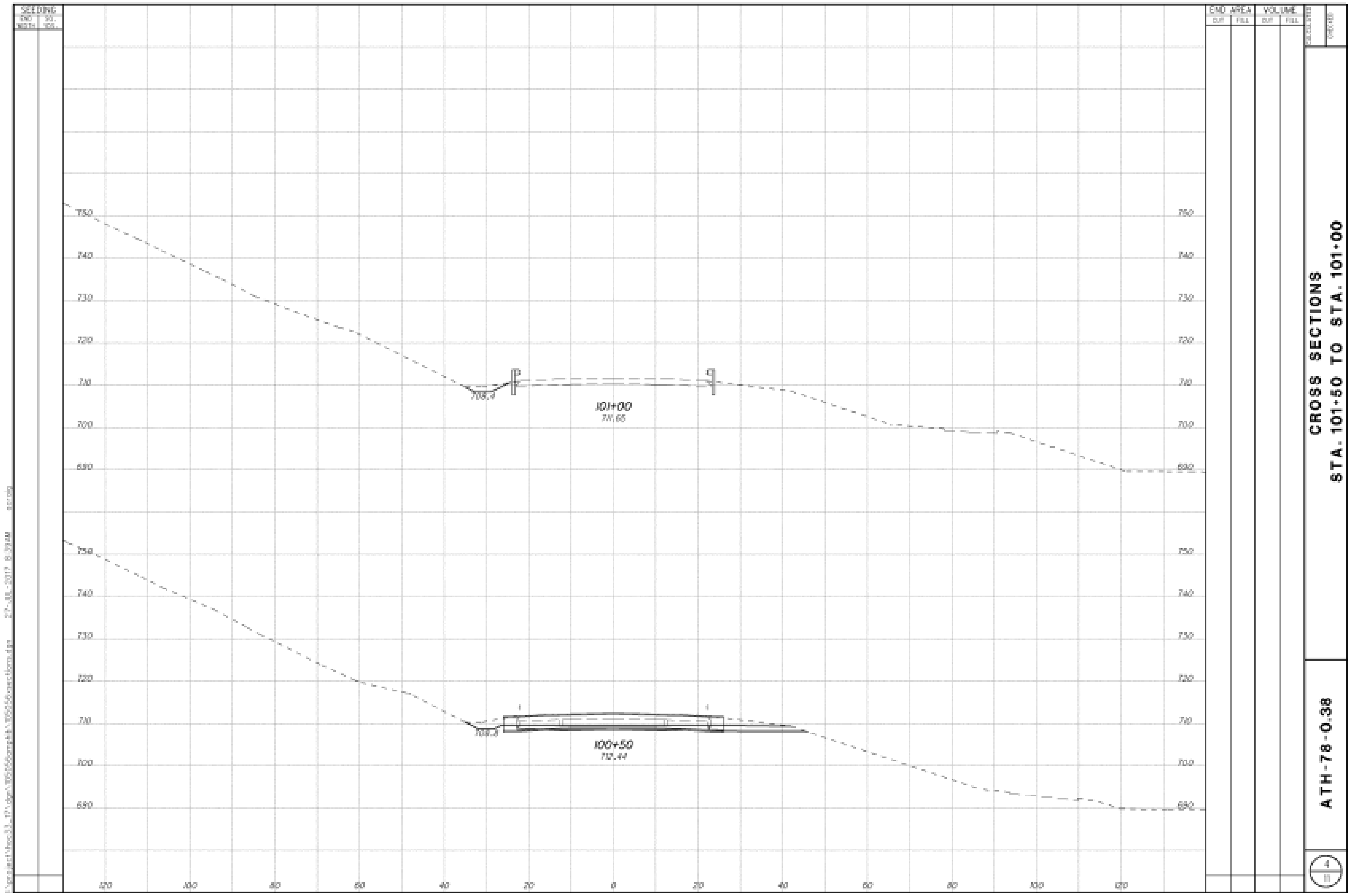

Figure A9-3. Design plan cross section for ecopassage 1 (south-most) of the 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio (ecopassages are labeled in Figure A9-2). Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

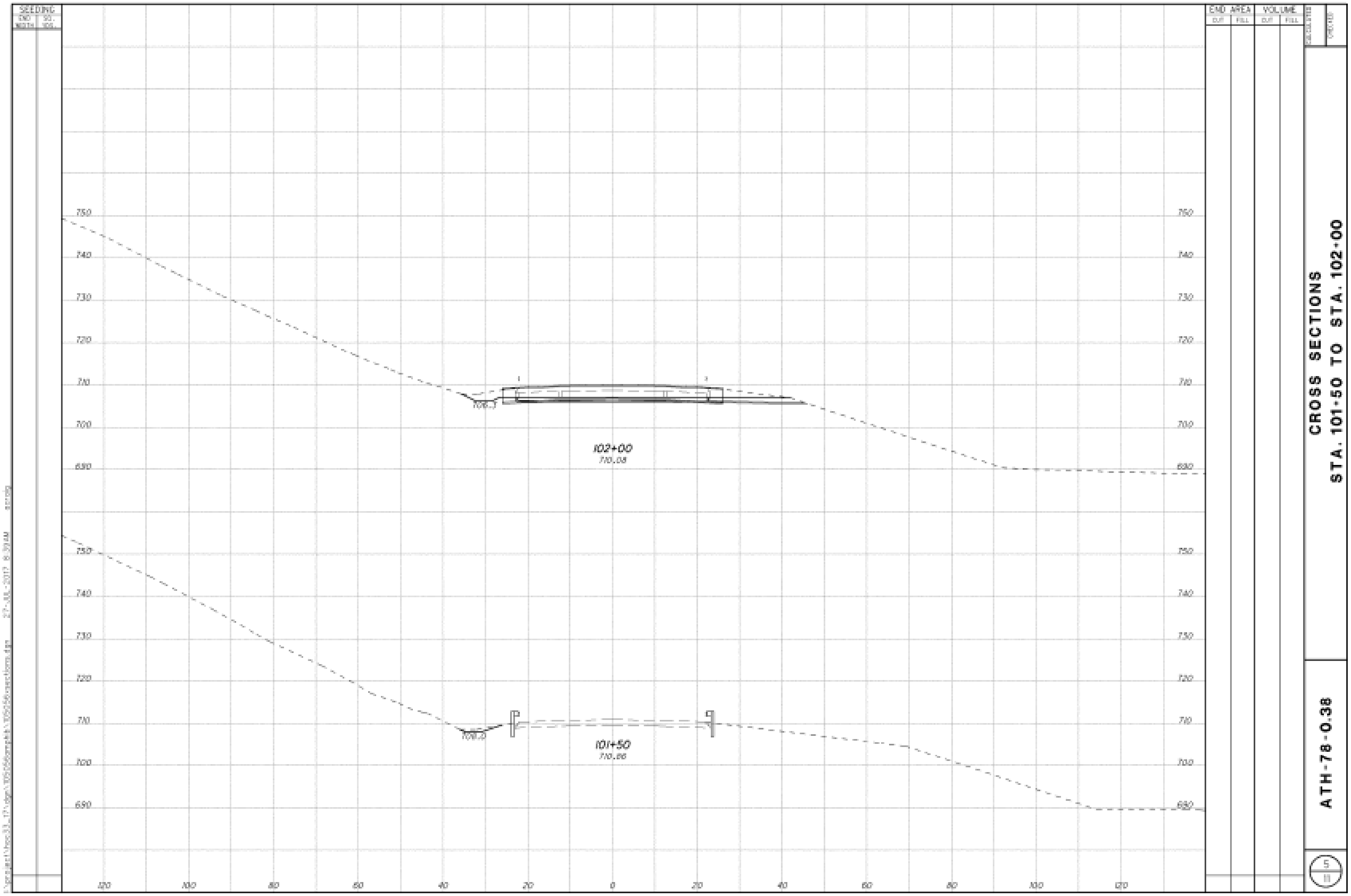

Figure A9-4. Design plan cross section for ecopassage 2 of the 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio (ecopassages are labeled in Figure A9-2). Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

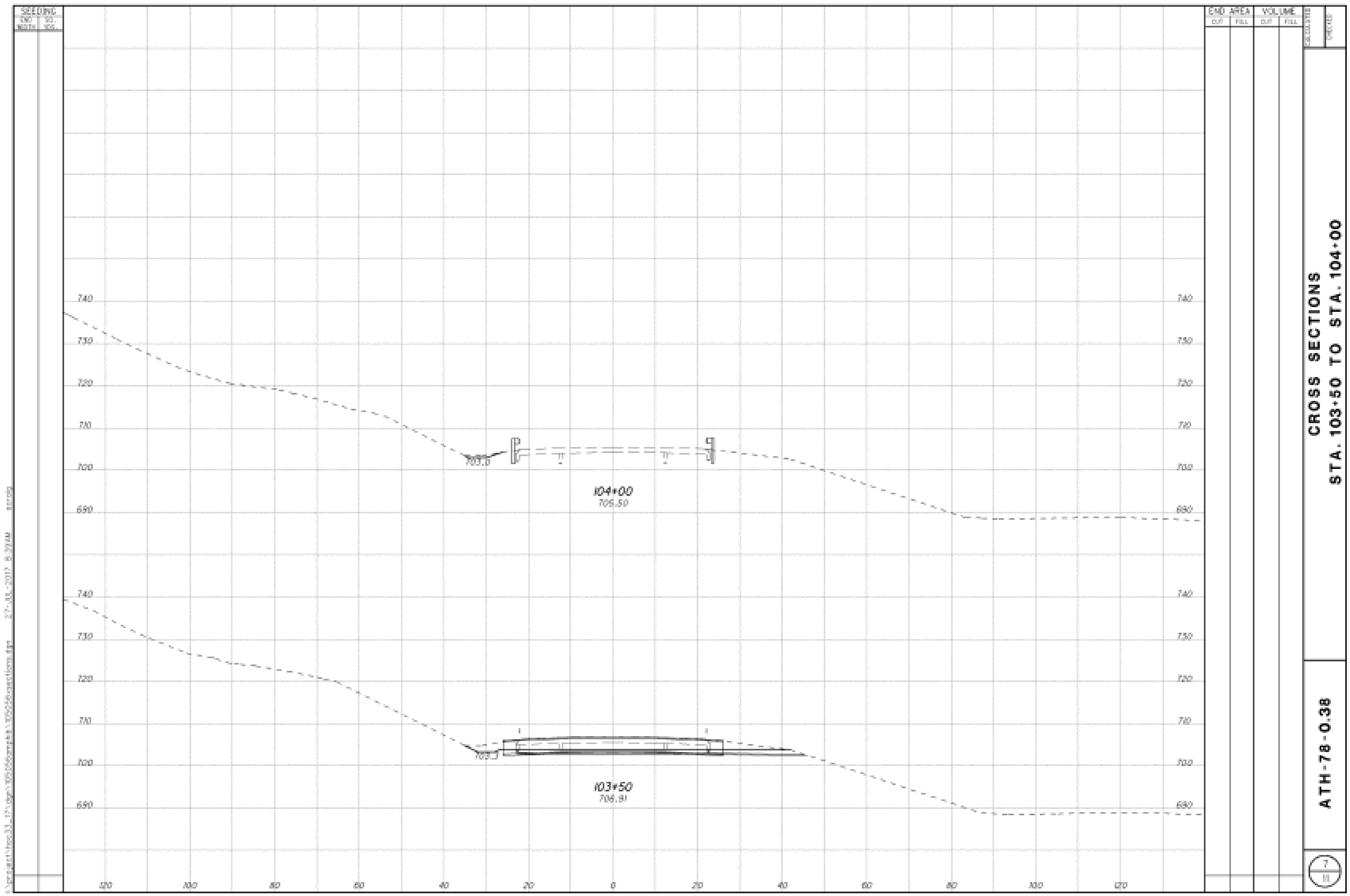

Figure A9-5. Design plan cross section for ecopassage 3 of the 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio (ecopassages are labeled in Figure A9-2). Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

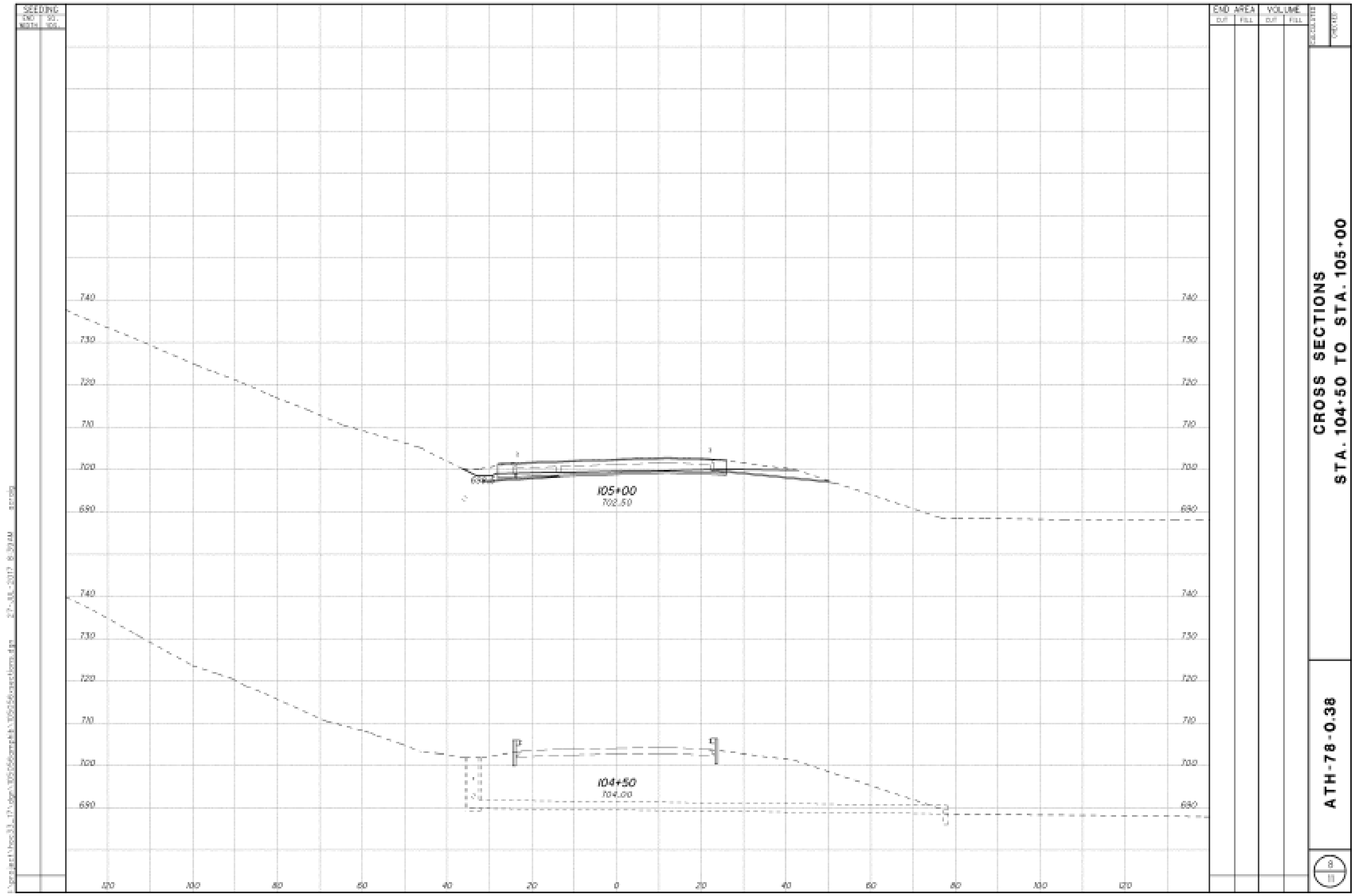

Figure A9-6. Design plan cross section for ecopassage 4 of the 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio (ecopassages are labeled in Figure A9-2). Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

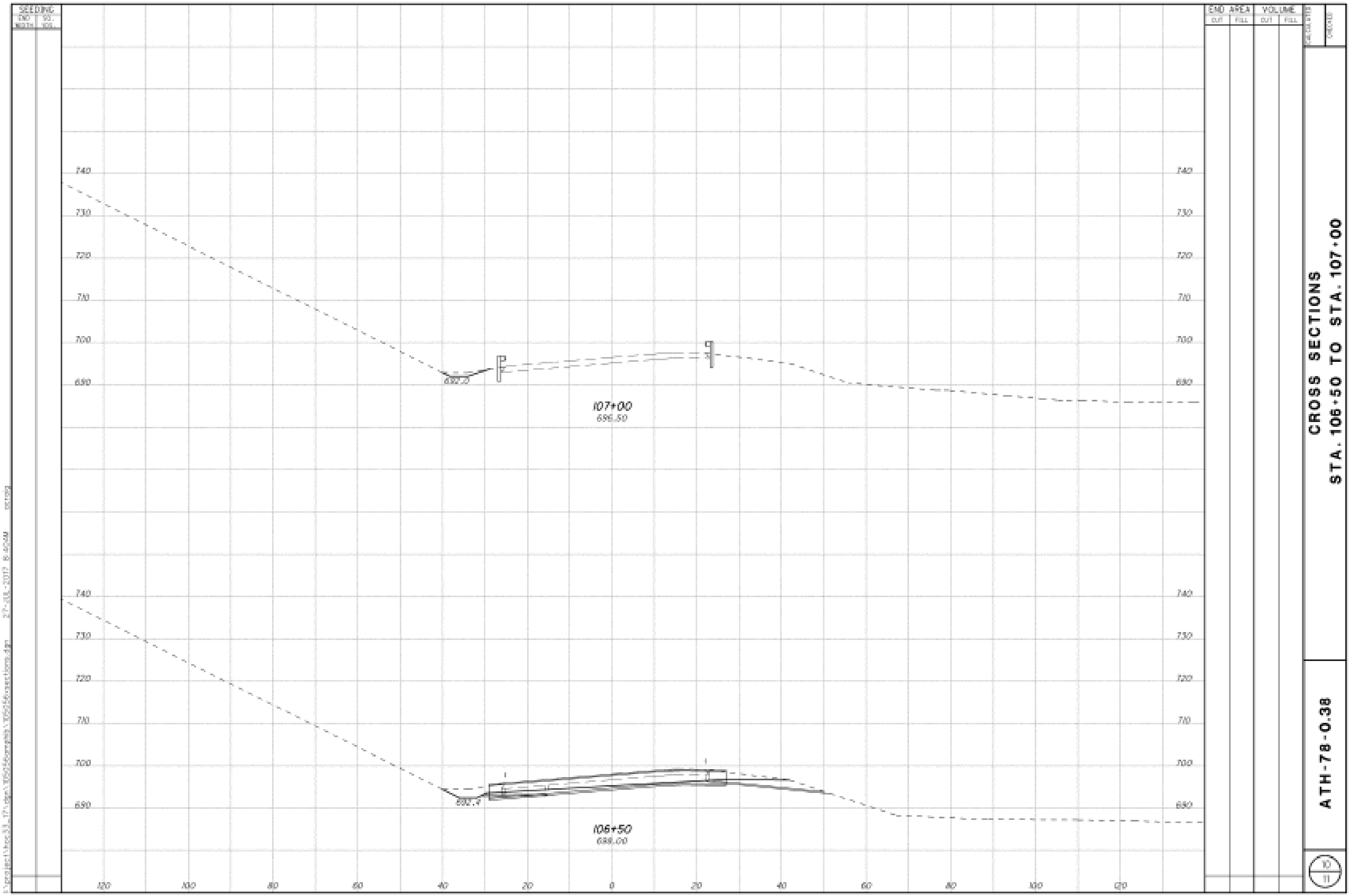

Figure A9-7. Design plan cross section for ecopassage 5 (north-most) of the 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio (ecopassages are labeled in Figure A9-2). Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

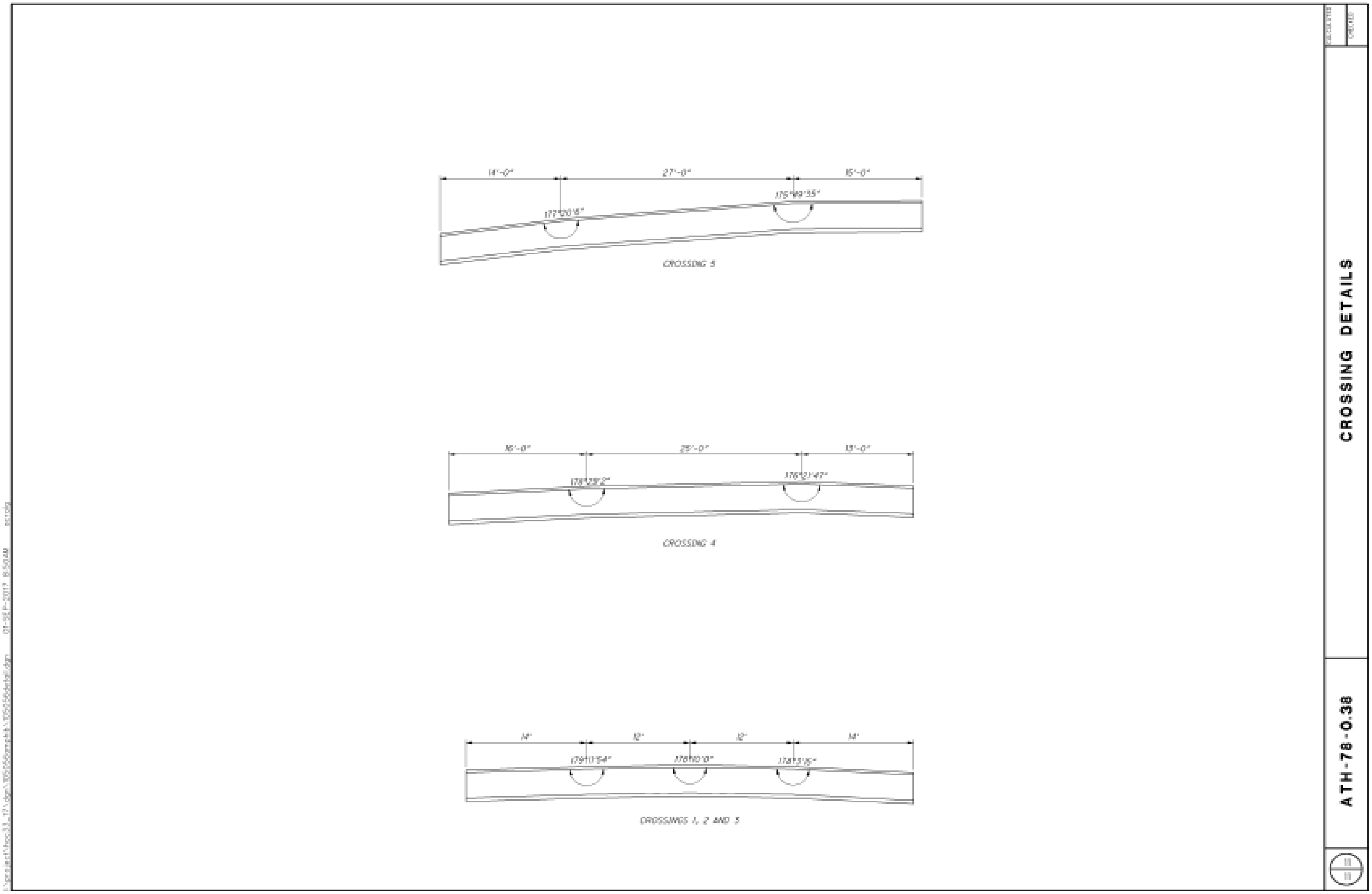

Figure A9-8. Design plans for ecopassages 1-3 (bottom), ecopassage 4 (center), and ecopassage 5 (top) of the 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio (ecopassages are labeled in Figure A9-2). Created by Alan Craig, District 10 Planning Engineer, Ohio Department of Transportation District 10.

Figure A9-9. Roadway that has been painted and sawed through (left), excavated road cut (center), and concrete ecopassage placed into an excavated road cut (right).

Figure A9-10. Ecopassage with layer of soil on the bottom (left), ecopassage with grated top in place (center), and rock bank and hay on biodegradable mesh used to prevent erosion (right).

Figure A9-11. Ecopassage headwalls being created at an angle on the east side of the road (left) and completed headwalls that are straight on the west side of the road (right).

Figure A9-12. Ecopassage barrier fencing not buried in the ground (top left), barrier fencing top lip not folded properly (top center), barrier fencing not properly interfacing with the ecopassage headwall to prevent animal trespass (top right), bare headwall being climbed by a Red-spotted Newt (bottom left), barrier fencing not properly attached to fence post (bottom center), and steep ditch with no erosion control measures (bottom right).

Figure A9-13. Barrier fencing attached to ecopassage headwalls (left) and grasses growing after the seeding of bare soils to reduce erosion (right).

Figure A9-14. Area of erosion along fence before (left) and after (right) a rock bank was installed to reduce erosion.

Figure A9-15. Area of erosion along barrier fence (left), area of erosion near an ecopassage headwall (center), and erosion on hillside (right) all after rains in spring 2019.

Figure A9-16. Flooding of ecopassage 3 in spring 2019 (left) and ecopassage 3 after an earthen dam was constructed to prevent further flooding (right) (ecopassages are labeled in Figure A9-2).


Figure A9-17. Before (left) and after (right) vegetation removal in front of one of the ecopassage entrances on August 23, 2019.


Figure A9-18. Before (left) and after (right) vegetation removal along the barrier fence on August 23, 2019.

Figure A9-19. Area where fencing had separated from the headwall (left), area along a headwall where two pieces of fence had separated (center), and a persistent eroded area along the southwest headwall of ecopassage 5 (right) (ecopassages are labeled in Figure A9-2).

Appendix 10: Monitoring of 2018 Barrier-Ecopassage System (2018-2019)

METHODS -- We monitored the 2018 barrier-ecopassage system implemented along State Route 78 near Nelsonville in Athens County, Ohio for one year (July 5, 2018 through July 5, 2019). The 2018 barrier-ecopassage structure had five ecopassages that were connected by 900 ft. of barrier fencing on each side of the road (Figure 4, Figure A9-1, Figure A9-2). The ecopassages were 48 in. wide by 36 in. tall concrete utility trenches with grated tops that were each about 54 ft. long (Figure 3, Figures A9-3 through A9-8). Four to eight inches of soil was placed on the bottom of each passage. Ecopassages were spaced about 150 ft. apart (Figure 4, Figure A9-1). The barrier fencing was 32-in. tall flexible plastic fencing (Figure 3). The plastic fencing had a lip at the top to prevent climbing animals from going up over the fence. On the east side of SR 78 the barrier fence was angled to encourage amphibians to travel toward the ecopassages (Figure 4, Figure A10-1). While monitoring the 2018 barrier-ecopassage system we assisted in barrier fence installation, maintained the fence, made repairs to the fence, and documented impairments to the fence or ecopassages that we could not mend (see Appendix 9: Implementation of 2018 Barrier-ecopassage System).

To monitor the effectiveness of the barrier fencing, two pitfall traps were placed at each end of the plastic barrier fence, one on each side of the fencing (Mata et al. 2005, Baxter-Gilbert et al. 2015; Figure A10-2; see Appendix 6: Population Monitoring for more about pitfall traps). Pitfall traps were installed on September 21, 2018. Traps were checked daily throughout the fall and spring. Each animal captured was measured, weighed, sexed, and batch marked (United States Geological Survey 2001, Ferner 2007, Dodd 2010).

We quantified the use of the ecopassages by herpetofauna using camera traps (Pagnucco et al. 2011, Leeb et al. 2013, Hobbs & Brehme 2017). The cameras were Spypoint Tiny-W3 cameras placed on the sides of each ecopassage near the entrance and angled so that the cameras viewed at least half (> 24 in.) of the ecopassage floor; together, both cameras photographed the entire floor of the ecopassage (Figure A10-3). We also installed 6-in. tall ramps that were buried into the soil 2 in. to get clear views of amphibians entering the passages; these ramps included the break-beam sensors that triggered the cameras (Leeb et al. 2013, Hobbs & Brehme 2017; Figure A10-3). In the fall of 2018, we explored the use of cameras with Jokie2 break-beam sensors in two ecopassages and compared them to cameras running on 30-second time lapse photography in three ecopassages. We conducted this comparison to determine how well the break-beam sensor would work over a 4-ft. opening with plants and debris that may regularly break the beam and trigger the camera to take photos (Hobbs & Brehme 2017). A second consideration was that, when triggered by a break-beam sensor, the Spypoint Tiny-W3 cameras take a maximum of one photo per minute, while the cameras can take two photos per minute using time lapse photography. Pagnucco

(2010, et al. 2011) recommended that ecopassage entrance cameras should take more than one photo per minute to monitor amphibians. Break-beam sensor cameras were examined and the batteries were replaced every two to five days, while time lapse cameras were examined and replacement batteries were needed every 36-48 hours. Break-beam sensor cameras were in place October 24-Novemebr 12, 2018 and time lapse cameras were in place October 26-November 12, 2018 (Table A10-1). We decided after this exploration period that the time-lapse cameras were more effective. We chose not to use the break-beam sensors in spring 2019 because the time lapse photography images gave us data on the length of time individuals spent approaching the passage entrance and near the passage entrance, which we could not obtain from cameras triggered by break-beam sensors. We also had higher failure rates of break-beam sensor cameras than time-lapse cameras. Spring 2019 ecopassage entrance time-lapse cameras were in place for 72 days between February 6, 2019 and June 26, 2019. We also placed two additional cameras facing straight down (each viewing about 13 in. of the passage entrance) in the west side entrances of the ecopassages to get dorsal views of amphibians for better identification (Figure A10-4).

We assessed the effectiveness of the 2018 barrier-ecopassage system by tabulating the number of amphibians captured in the pitfall traps and the number detected by the ecopassage camera traps. In late-fall 2018, we quantified travel through a passage by individual amphibians. However, in spring 2019, while we were able to determine travel through a passage by individual for some animals (Figure A10-5), there were often too many individuals to effectively track (Figure A10-6). Thus, we counted the total number of individuals that appeared to go in each entrance, then took the number of individuals at the entrance with the lower count as the amount that went through. We then subtracted the number of individuals from the entrance with the lower count from the entrance with the higher count and added those individuals to the number of individuals only seen at one entrance.

RESULTS -- Frogs and salamanders were detected in the 2018 ecopassages (Figure A10-5, Figure A10-6). In late-fall 2018, we found that the average amphibian was in view of at least one entrance camera for an average of 5.3 pictures or more than 2.5 minutes. In addition to animals that entered the passages, we observed animals that walked by the entrance without going into the passage, animals that entered and walked along the ramp before exiting again, and animals that travelled over the ramp only to turn and exit the passage.

We detected a total of 4,564 amphibians at the ecopassage entrances, of which 2,521 were detected at both ends of an ecopassage (Table A10-1, Table A10-2). In other words, 55.24% of amphibians detected at the ecopassage entrances went through the passages. Twelve amphibian species (Jefferson Salamander, Spotted Salamander, Marbled Salamander, Red-backed Salamander, Red-spotted Newt, American Toad, American Bullfrog, Green Frog, Pickerel Frog, Wood Frog, Gray Treefrog, and Spring Peeper) were detected by the 2018 ecopassage camera traps (Table 1, Figure A10-7 through A10-9). Red-backed Salamanders (which are non-migratory and do not breed in wetlands) were the only species that did not successfully cross through an ecopassage (Figure A10-7, Figure A10-9).

We did detect some amphibians, particularly Spring Peepers and Gray Treefrogs, scaling the ecopassage walls (Figure A10-6). We also captured animals coming up out of the grated tops during our spotlight surveys (see Appendix 6: Population Monitoring) and detected carcasses of amphibians right near or on the grated tops of the ecopassages (Figure A10-10).

We captured 11 species of frogs and salamanders in the pitfall traps at the ends of the 2018 barrier fence; the only species not captured in the pitfall traps that was detected in the ecopassages was Gray Treefrog (Figure A10-7 through A10-9), which is able to climb out of pitfall traps. A total of 1,284 individual amphibians were trapped in the fence end pitfall traps (Figure A10-7 through A10-9).

We detected 3.55 times more animals in the ecopassages than we captured at the barrier fence ends, even though the pitfall traps were open for 57 days more than the ecopassage camera traps (pitfall traps were open 20 days during the fall 2018 ecopassage camera trapping period and 124 days during the spring 2019 ecopassage camera trapping period). In addition, 1.96 times more animals were detected traveling through the ecopassages than were captured at the fence ends, indicating that the barrier fencing was guiding individuals and the ecopassages were providing a safe migration corridor to many amphibians.

Figure A10-1. The 2018 barrier fencing angled toward ecopassages from the center on the east side of State Route 78 near Nelsonville in Athens County, Ohio.

Figure A10-2. Pitfall traps on either side of the end of the 2018 barrier fencing along State Route 78 near Nelsonville in Athens County, Ohio (left) and several American Toads, a Pickerel Frog, and a Spotted Salamander trapped in a pitfall trap (right).

Figure A10-3. Spypoint Tiny-W3 cameras and viewing ramps set up to take photographs of the ecopassage entrances. Right photo shows some erosion from under the ramp after a rain, which was repaired later that day.

Figure A10-4. Spypoint Tiny-W3 cameras and viewing ramps set up on a west passage entrance (left) and an east passage entrance (right) in the spring of 2019.

Table A10-1. The camera type used, number of days camera trapped, the number of amphibians only detected at the west entrance, the number of amphibians only detected at the east entrance, and the number of amphibians detected at both entrances (travel through) for each of the 2018 ecopassages along State Route 78 near Nelsonville in Athens County, Ohio, October 24-November 12, 2018. Ecopassages are labeled in Figure A9-2.

ECOPASSAGE	1 (S)	2	3	4	5 (N)
CAMERA TYPE	TIME LAPSE	SENSOR	TIME LAPSE	SENSOR	TIME LAPSE
NUMBER OF DAYS CAMERA TRAPPED	8	15	8	15	8
WEST ENTRANCE ONLY	2	5	4	6	11
EAST ENTRANCE ONLY	4	9	5	7	14
TRAVEL THROUGH ECOPASSAGE	11	21	13	15	17
TOTAL ANIMALS PER ECOPASSAGE	17	35	22	28	42

Figure A10-5. A Spotted Salamander detected by a north facing ecopassage entrance camera on the west side of the road (top left and right), then detected by the south facing camera on the west side of the road (center left and right), and then detected by the south facing camera on the east side of the road (bottom left and right).

Figure A10-6. Examples of amphibians seen in the 2018 ecopassages along State Route 78 near Nelsonville in Athens County, Ohio. Jefferson Salamander (top left), two Spring Peepers with one climbing the ecopassage wall (top right), a Red-spotted Newt and an American Toad (bottom left), and eight juvenile American Toads (bottom right).

Table A10-2. The number of amphibians only detected at the west entrance, the number of amphibians only detected at the east entrance, and the number of amphibians detected at both entrances (travel through) for each of the 2018 ecopassages along State Route 78 near Nelsonville in Athens County, Ohio, February 6-June 26, 2019. All cameras used 30-second time lapse photography and were in place for 72 days. Ecopassages are labeled in Figure A9-2.

ECOPASSAGE	1 (S)	2	3	4	5 (N)
WEST ENTRANCE ONLY	183	211	235	188	236
EAST ENTRANCE ONLY	171	184	163	197	208
TRAVEL THROUGH ECOPASSAGE	417	510	376	587	554
TOTAL ANIMALS PER ECOPASSAGE	771	905	774	972	998

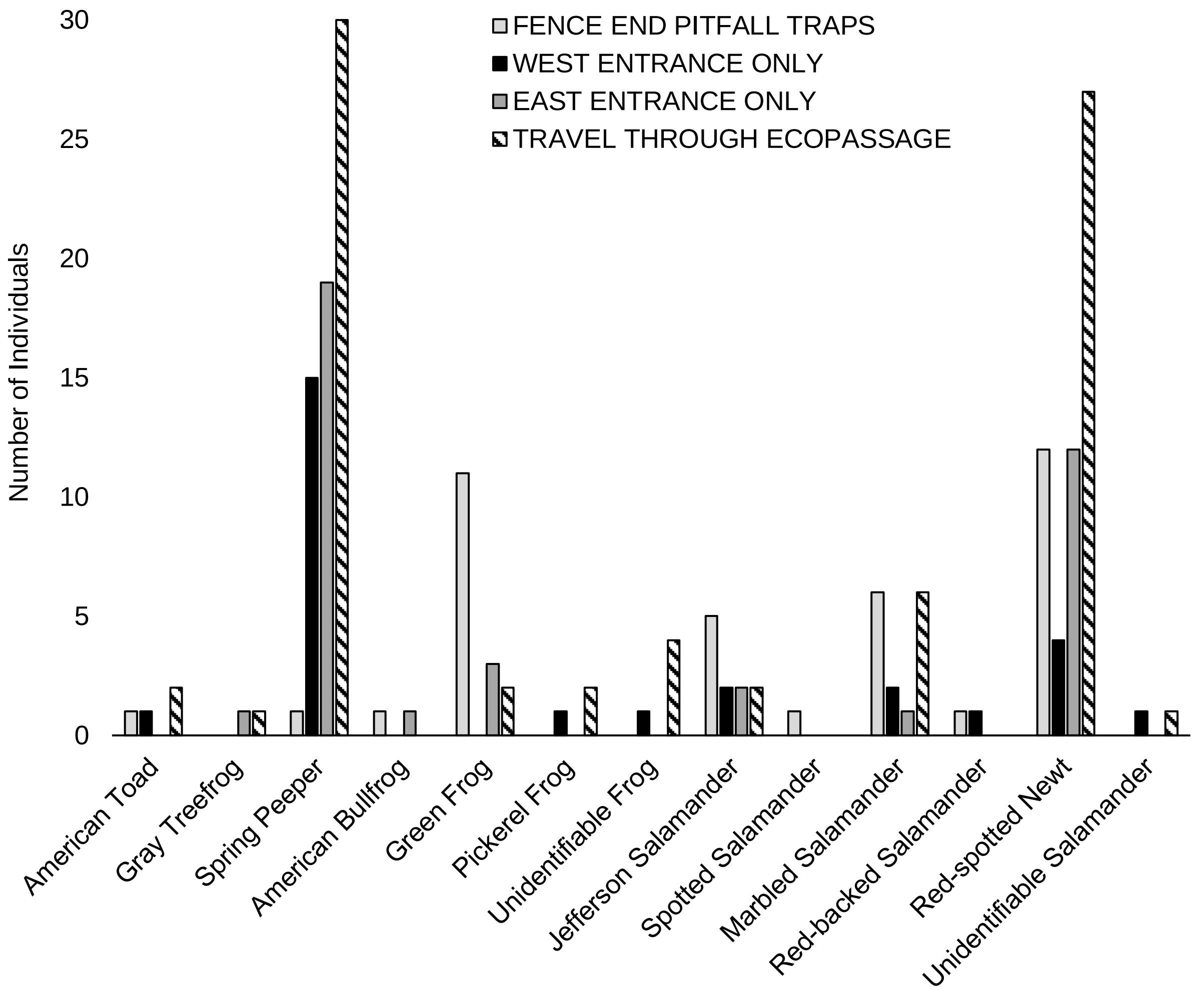

Figure A10-7. The number of individuals of each amphibian species captured in a pitfall trap at the end of the 2018 barrier fence, only detected at a west entrance, only detected at an east entrance, and detected at both entrances (travel through) for all five 2018 ecopassages along State Route 78 near Nelsonville in Athens County, Ohio, October 24-November 12, 2018.

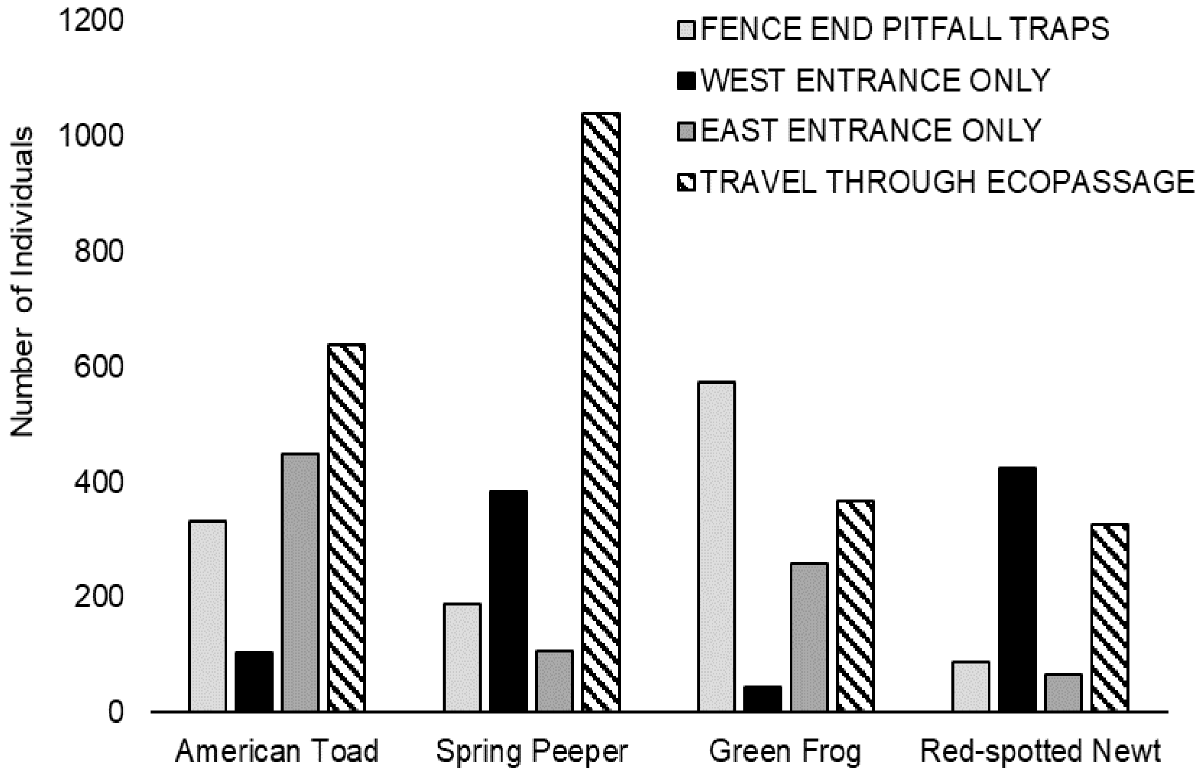

Figure A10-8. The number of individuals of each amphibian species captured in a pitfall trap at the end of the 2018 barrier fence, only detected at a west entrance, only detected at an east entrance, and detected at both entrances (travel through) for all five 2018 ecopassages along State Route 78 near Nelsonville in Athens County, Ohio, February 6-June 26, 2019.

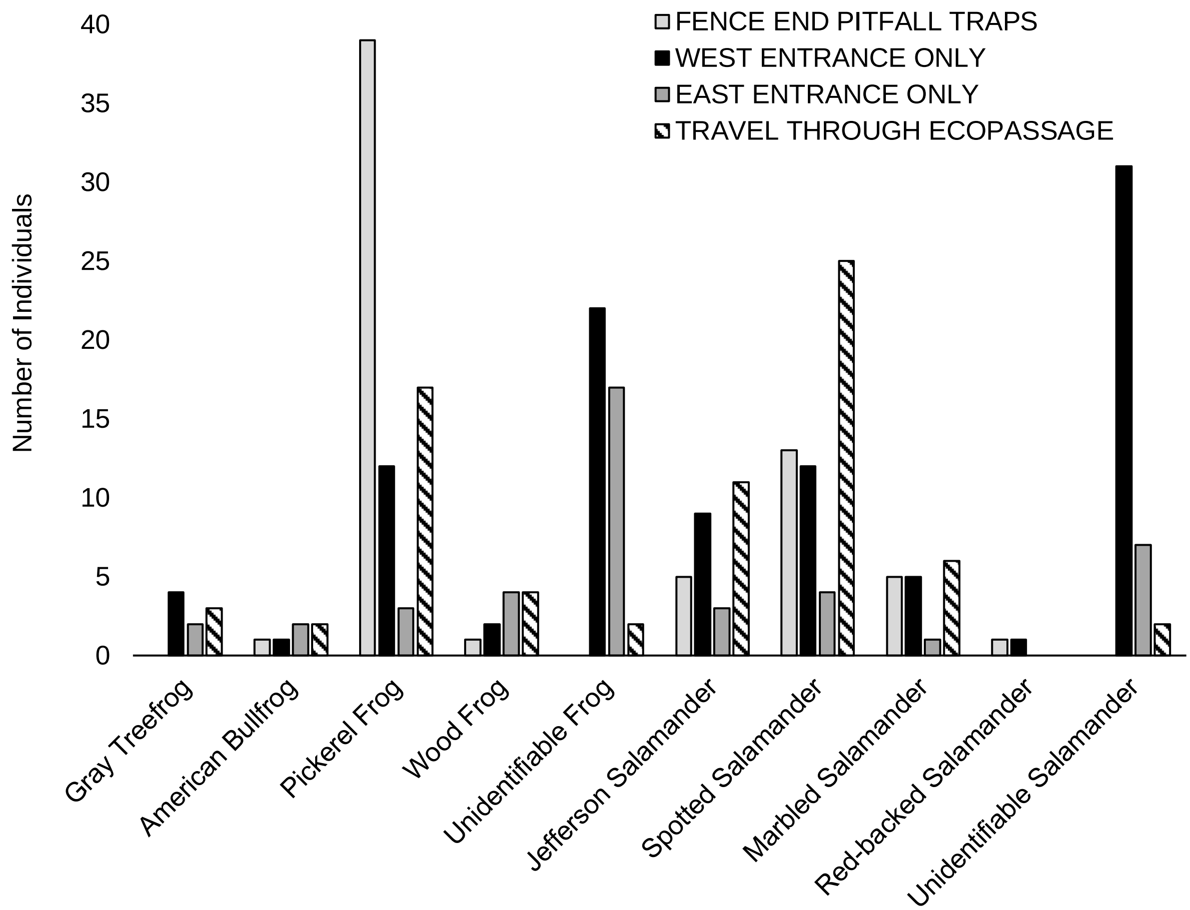

Figure A10-9. The number of individuals of each amphibian species captured in a pitfall trap at the end of the 2018 barrier fence, only detected at a west entrance, only detected at an east entrance, and detected at both entrances (travel through) for all five 2018 ecopassages along State Route 78 near Nelsonville in Athens County, Ohio, February 6-June 26, 2019.

Figure A10-10. Examples of roadkilled amphibians recovered on ecopassage tops. An American Toad (left) and two Spring Peepers (right).

Appendix 11: Comparing Effectiveness of 2013 and 2018 Barrier-Ecopassage Systems

We studied two barrier-ecopassage systems that are about 1,100 ft. apart along State Route 78 (SR 78) near Nelsonville in Athens County, Ohio (Figure 4). We monitored the 2013 barrier-ecopassage mitigation structure from spring 2015 through spring 2018 and the 2018 barrier-ecopassage mitigation structure from late-fall 2018 through spring 2019 (see Appendix 7: Monitoring of 2013 Barrier-ecopassage System and Appendix 10: Monitoring of 2018 Barrier-ecopassage System). The 2013 and 2018 barrier-ecopassage systems were not in the same location, nor were we able to monitor them during the same time period. Thus, we cannot make a quantitative comparison of their relative effectiveness. However, we provide a qualitative comparison of them.

The 2018 barrier-ecopassage system consists of three more ecopassages and 650 ft. more barrier fencing that the 2013 barrier-ecopassage system (Figure 4, Table A11-1); accordingly, this system should provide greater connectivity. We detected many more amphibians in the 2018 ecopassages than in the 2013 ecopassages: 4,564 vs. 35 amphibians, respectively. Amphibians detected in the 2018 ecopassages were 20.95% more likely to travel through an ecopassage than those detected in the 2013 ecopassages (Table A11-1). The ecopassage use rate, or number of amphibians traveling through an ecopassage per camera trap day, for the 2018 mitigation system was 28.97 amphibians per camera trap day, whereas the 2013 mitigation system ecopassage use rate was 0.02 amphibians per camera trap day (Table A11-1). When the total number of individuals detected at ecopassage entrances is divided by the length of the fence, the 2018 system had 4.93 more amphibians detected in the passages per foot of fencing than the 2013 system (Table A11-1). In addition, we detected five more amphibian species in the 2018 ecopassages even though the 2013 passages were monitored 6.01 times longer.

We also captured animals at the ends of the barrier fencing for both mitigation structures using pitfall traps to measure the number of animals that potentially chose not to use an ecopassage. However, some portion of the animals captured at the fence ends probably followed the barrier fence directly to a fence end without encountering an ecopassage. There were 9.69 times more amphibians captured in the 2013 barrier fence pitfall traps than were detected in the ecopassages, while 0.28 amphibians were captured in the 2018 barrier fence pitfall traps for each amphibian detected in the ecopassages (Table A11-1). We captured the same number of species in the 2013 and 2018 barrier end pitfall traps (n=11).

The addition of the 2018 mitigation structure reduced mortality along the road stretch it was introduced in from 62.21% of the total mortality for the 1.2 mi. stretch of SR 78 surveyed to 3.98% of the total mortality for the SR 78 stretch surveyed (Table A5-2, Figure A5-6). However, there was still a lower percentage of total mortality along the stretch fenced by the 2013 structure than the stretch fenced by the 2018 structure (Table A11-1), because there were greater numbers of animals migrating at the 2018 structure location. We did detect some amphibians scaling the 2018 ecopassage walls (Figure A10-6) and see animals out on top of the grates (Figure A10-10), which was a phenomenon we had never encountered while monitoring the 2013 ecopassages; this may also contribute to the higher percentage of total mortality in the fenced area of the 2018 barrier-ecopassage system.

Overall, amphibians used both mitigation systems. Both barrier fences appeared to guide animals while they were fully implemented and maintained to be functional barriers. However, there were higher amphibian use, detection, and capture rates along the 2018 mitigation structure, as well as higher amphibian richness (Table A11-1).

Table A11-1. Comparison of 2013 barrier-ecopassage system and 2018 barrier-ecopassage system specifications, monitoring efforts, and amphibian levels.

Barrier-ecopassage system implementation year	2013	2018
Number of ecopassages	2	5
Dimensions of ecopassage entrances	20 in. wide x 16 in. tall	48 in. wide x 28-32 in. tall
Length of ecopassages	60 ft.	54 ft.
Travel surface in ecopassages	treated concrete	natural soil
Sky exposure design element	two rows of 1 in. x 1.5 in. holes in top	grated top
Ability to see across ecopassage	none	full line-of-sight
Length of barrier fencing on each side of the road	250 ft.	900 ft.
Height of barrier fencing	24 in.	32 in.
Number of days cameras in place	523	87
Number of amphibians detected in ecopassages	35	4564
Number of amphibians traveling through ecopassages	12	2521
Percentage of amphibians detected in ecopassages that traveled through	34.29%	55.24%
Amphibian ecopassage use rate (number traveling through divided by number of days the cameras were in place)	0.023	28.977
Amphibians detected per foot of barrier fencing	0.14	5.07
Number of amphibian species detected in ecopassages	7	12
Number of amphibian species traveling through ecopassages	4	11
Number of days pitfall traps open during ecopassage camera trapping time period	672	144
Number of amphibians captured in pitfall traps	339	1284
Number of amphibian species captured in pitfall traps	11	11
Ratio of amphibians captured in pitfall traps to those detected in ecopassages	9.69	0.28
Ratio of amphibians captured in pitfall traps to those traveling through ecopassages	28.25	0.51
Percentage of total roadkill survey mortality occurring in fenced area	0.58%	3.98%
Percentage of total roadkill survey mortality occurring in fenced area divided by length of fenced area	0.0023%	0.0044%

Appendix 12: Fence Behavioral Observations

METHODS – We conducted behavioral observations of amphibians moving along the 2018 barrier-ecopassage system barrier fencing along State Route 78 near Nelsonville in Athens County, Ohio (Figure 3, Figure 4, Figure A10-1). Our behavioral observations were conducted on rainy nights and mornings in order to observe animal behaviors along the fence. In particular, we wanted to observe four things: (1) if the curve of the barrier fence between passages directed amphibians toward the nearest ecopassage, (2) if amphibians were able to climb over the 2018 barrier fencing, (3) the distance animals moved along the fence before turning away and going back to the habitat they came from, and (4) how amphibians behaved at the fence ends. Curving the fence between passages, applying a lip to the top of the fence, having less than 150 ft. between ecopassages, and bending the fence ends have all been recommended as best management practices for amphibian fencing (Clevenger et al. 2001, Dodd et al. 2004, Aresco 2005b, Kruidering et al. 2005, Huijser et al. 2015, Huijser et al. 2016), but none have been empirically evaluated.

Between July 17, 2018 and June 20, 2019, we observed 204 individuals of nine amphibian species moving along the 2018 barrier fence (Figure A12-1, Figure A12-2). For each individual observed we recoded species, time seen, which side of the road the animal was on, direction of travel, the length of fence traveled, whether the animal encountered an ecopassage, and the behaviors exhibited at the ecopassage. We also noted if individuals climbed the fence, how they approached the fence, and their behavior at the fence ends. We were often unable to watch animals from the time they approached the fence until the time that they abandoned following the fence, entered an ecopassage, or tried to circumvent the fence end.

RESULTS – We observed six amphibians as they approached the barrier fence and all of them followed the curve of the fence toward the nearest ecopassage. Four amphibians (two Spring Peepers and two Gray Treefrog) climbed up the barrier fencing (Figure A12-3), but were unable to get over the fence due to the lip at the top. We also witnessed several Red-spotted Newts and one Jefferson Salamander trying to climb the fencing, but none succeeded in making it off the ground. We observed nine amphibians (six Red-spotted Newts and three Spring Peepers) climb the concrete headwalls before we attached the plastic fencing on September 20, 2018 (Figure A12-3, see Appendix 9: Implementation of 2018 Barrier-ecopassage System). We observed six Green Frogs and one American Toad abandon walking along the fence and move in a direction away from the fence. The farthest we saw a Green Frog travel along the fence was 360 ft. (1 hr. and 4 min.). The farthest we watched a Pickerel Frog travel was 80 ft. (12 min.), and we saw one juvenile American Toad walk 60 ft. (30 min.). The farthest we witnessed Spring Peepers, Gray Treefrogs, and Marbled Salamanders travelling along the fence was 40 ft. (average time = 21.67 min.). We observed three Green Frogs and one American Toad walk toward the fence end and into a pitfall trap (Appendix 10: Monitoring of 2018 Barrier-ecopassage System). We did not observe any animals walk away from the fence when they reached the curve at the fence end.

Figure A12-1. Animals seen moving along the 2018 barrier fencing and entering the 2018 ecopassages in place along State Route 78 near Nelsonville in Athens County, Ohio, 2018-2019. Red-spotted Newt (top left), Spring Peeper (top right), Green Frog (bottom left), juvenile American Toad (bottom right).

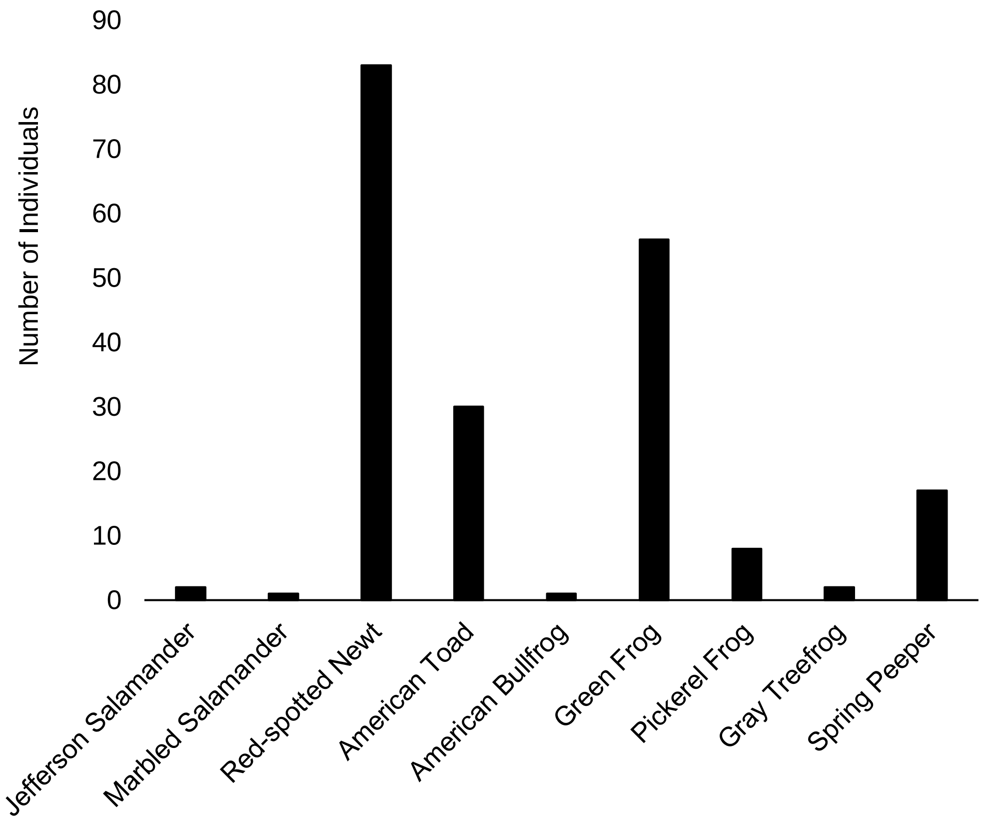

Figure A12-2. Number of individuals of each amphibian species observed moving along the 2018 barrier-ecopassage system fencing in place along State Route 78 near Nelsonville in Athens County, Ohio, 2018-2019.

Figure A12-3. A Spring Peeper climbing the 2018 barrier fence (left) and a Red-spotted Newt climbing a concrete ecopassage entrance headwall (right).

Appendix 13: Pseudofence Experiment

METHODS – Previous studies have found concentrated increases in animal-vehicle collisions beyond the edge of barrier fencing mitigation structures, which is known as the “fence-end effect.” This occurs when individuals trespass around the end of the fence and are then killed on the road (Clevenger et al. 2001, Dodd et al. 2004, Cserkész et al. 2013, Baxter-Gilbert 2014, Baxter-Gilbert 2015). To combat this issue, designing barrier fences that include fence-end treatments has been recommended (Clevenger et al. 2001, Dodd et al. 2004, Cserkész et al. 2013). The most commonly recommended treatment is to bend the end of the fence away from the road to redirect individuals towards the associated ecopassage structure; this approach is often recommended for amphibians (Clevenger et al. 2001, Dodd et al. 2004, Aresco 2005b, Kruidering et al. 2005, Huijser et al. 2015, Huijser et al. 2016). Although fence-end treatments have been frequently recommended as an important component of barrier fence design there has not yet been a study to test the effectiveness of a fence-end treatment on amphibian movement patterns.

To assess the effectiveness of a 45-degree angle fence-end treatment for amphibians, we deployed experimental “pseudofences” along State Route 78 near Nelsonville in Athens County, Ohio. The pseudofences were constructed of silt fencing held up by wooden posts. A 100 ft. roll of silt fencing was cut into 13 ft. long pseudofences, each with an attached 40 in. long fence-end treatment (Figure A13-1). To test the hypothesis that an angled fence-end will effectively turn amphibians back toward the ecopassages and prevent them from circumventing the barrier fence, one end of the fence was angled inwards at a 45-degree angle; the other end lacked any curvature. One funnel trap was placed at each end of every pseudofence array (Figure A13-2). Funnel traps were 10 in. x 10 in. x 17 in. mesh traps buried 5-7 in. into the ground so that the ground was level with the trap opening. We closed the back opening so that animals could only enter the trap by moving along the pseudofence. We placed wet sponges in each trap to prevent desiccation of amphibians. We designated the traps located on the linear side of the pseudofence array as the ‘control’ traps, whereas, the ‘treatment’ traps were adjacent to the angled fence-end treatment. In February 2019, we installed 13 pseudofence arrays adjacent to the wetland on the east side of the 2018 barrier-ecopassage system, as high levels of amphibian movement (62% of roadway mortality in 2015-2016) have been detected in this area (Figure 5, see Appendix 5: Roadway Mortality and Appendix 10: Monitoring of 2018 Barrier-ecopassage System). We used a random number generator to assign the fence-end treatment to left or right side of each pseudofence. We ran the experiment during optimal weather conditions in February-May 2019 for 47 nights. We recorded the trap each animal was captured in and all animals were identified to species, measured, weighed, and sexed (Heyer et al. 1994, Dodd 2010).

We used a paired t-test to analyze the difference in amphibian captures between the treatment traps and control traps. Our null hypothesis was that there would be no difference in the number of amphibians captured between the control and treatment traps. A higher frequency of amphibian captures in the control traps would indicate that the 45-degree angle fence-end treatment is effective at preventing migratory amphibians from going around the end of the fence. All statistical analyses were carried out in the statistical programming language R (R Core Team 2016).

RESULTS – We captured 56 individual amphibians across five species (Spring Peepers, American Toad, Green Frog, Pickerel Frog, and Red-spotted Newt) in the pseudofence funnel traps. During this experiment, 77% (n=43) of the total captures were in the control traps compared to 23% (n=13) in the treatment traps. Spring Peepers comprised the majority of the amphibians captured (n=36). All amphibian species were more often captured in the control traps compared to the treatment traps (Figure A13-3), and a significantly higher number of individuals were captured in the control traps overall (paired t-test, t value=3.5, df=12, P-value <0.05).

Figure A13-1. An experimental pseudofence set up with the 45-degree angle fence-end treatment on the right side of the fence.

Figure A13-2. A side view of an experimental pseudofence array with funnel traps at each end. The treatment trap is located on fence-end treatment (top) and the control trap adjacent to the linear fence (bottom).

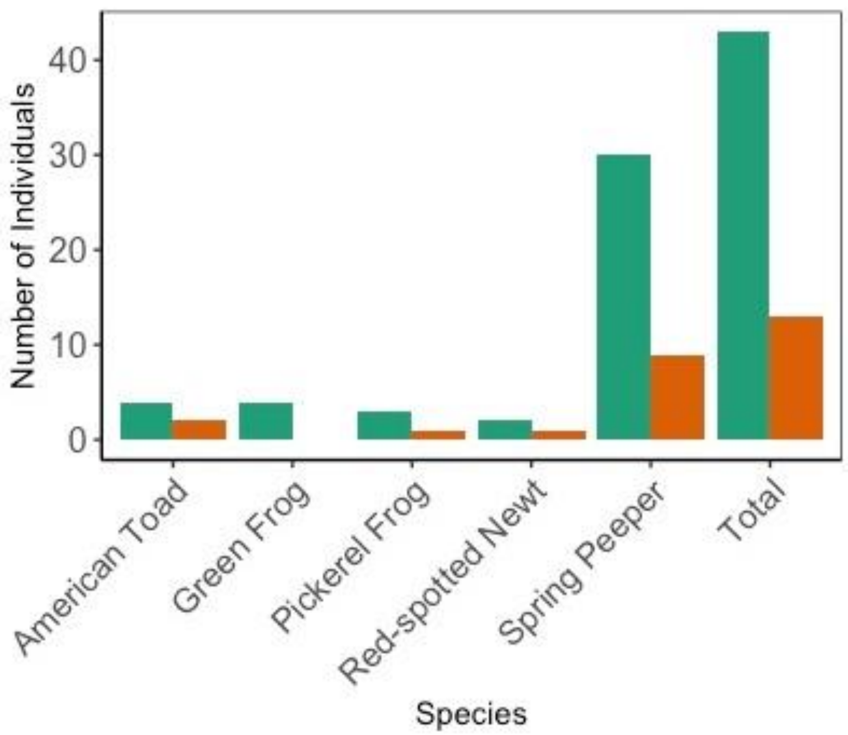

Figure A13-3. The number of individual amphibians captured in the control traps (green) and the treatment traps (orange) among all 13 of the experimental pseudofence arrays in place along State Route 78 near Nelsonville in Athens County, Ohio.

Appendix 14: Recommended 2018 Barrier-Ecopassage System Maintenance Plan

Maintenance of barrier-ecopassage mitigation structures is necessary throughout the life of the structure (Jacobson & Tonjes 2015). Maintenance of not only the ecopassages themselves, but of the barrier fencing is critical as the fencing is necessary to guide animals to the ecopassages (Cunnington et al. 2014, Baxter-Gilbert et al. 2015, Jacobson & Tonjes 2015, Markle et al. 2017). Below is a recommended timeline for annual 2018 barrier-ecopassage mitigation structure maintenance based on first year implementation and monitoring (see Appendix 9: Implementation of 2018 Barrier-ecopassage System), a literature review (Forman et al. 2003, Cushman 2006, Schueler et al. 2007, Mitchell et al. 2008, Beckmann et al. 2010, Clevenger & Huijser. 2011, Kingsbury & Gibson 2012, Cunnington et al. 2014, Andrews et al. 2015, Baxter-Gilbert et al. 2015, Jacobson & Tonjes 2015, Van der Ree et al. 2015, Gunson et al. 2016, Markle et al. 2017), and time and cost-effectiveness:

Early February – Full inspection of ecopassages and barrier fencing, noting any areas in need of repairs (ecopassage cracks or gaps, erosion of soil in ecopassages, holes under the fence, holes near ecopassage headwalls, cracks in the fence, areas where fencing is not attached to posts or headwalls, areas where top lip is not folded, etc.). In addition, investigation of the surrounding landscape for erosion or irregularities (ditch, west hillside, east hillside, etc.) is needed. Repairs could be implemented during the visit, or a follow up visit for implementation of repairs should be completed within two weeks of the inspection as amphibian migrations often begin in mid to late February.

Early April – Full fence inspection and implementation of repairs.

Mid-June – Full fence inspection, investigation of surrounding landscape, and implementation of repairs. In addition, mechanical removal of vegetation growing along the barrier fence and in front of ecopassage entrances.

Mid-July – Full fence inspection and mechanical removal of vegetation growing along the barrier fence and in front of ecopassage entrances. In addition, spraying of glyphosate-based herbicides with no surfactants to eliminate plant growth in the ecopassages while incurring minimal effects on animals using the passages and the nearby landscape (Hayes et al. 2002, Hayes et al. 2006, Govindarajulu, P. P. 2008. Dinehart et al. 2009, Edge et al. 2012, Wagner et al. 2013).

Late August – Full fence inspection, implementation of repairs, and mechanical removal of vegetation growing along the barrier fence and in front of ecopassage entrances.

Late October – Full fence inspection, investigation of surrounding landscape, and implementation of repairs.

In addition to the maintenance timeline above, we recommend that winter road salting be avoided along the mitigation structure (Sanzo & Hecnar 2006, Karraker et al. 2008, Mitchell et al. 2008). We also recommend that maintenance personnel, equipment, and vehicles be inspected for invasive species before entering the site to avoid the inoculation and spread of invasive species (Parendes & Jones 2000, Semlitsch 2003, Maerz et al. 2005, Kingsbury & Gibson 2012, Jacobson & Tonjes 2015), and we recommend the avoidance of mesh (including biodegradable mesh) in further erosion control measures as these materials entangle and kill wildlife, especially snakes (Mitchell et al. 2008, Kapfer & Paloski 2011; Figure A14-1).

Finally, the benchmark tasks below are required for the long-term maintenance of the 2018 barrier-ecopassage structure and should be carried out during the summer (late-June to mid-August) to avoid impacting amphibian movement (Russell et al. 2005, Jacobson & Tonjes 2015, Gunson et al. 2016; Figure A5-3):

- Trash cleanout of and soil addition to ecopassages every 5-7 years
- Flushing drainage pipes every 8-10 years
- Barrier fence replacement every 15-20 years (Animex 2016)
- Full inspection of ecopassage concrete interior every 15-20 years (Trenwa 2013)

Figure A14-1. Images of a Black Racer (left) and a Northern Watersnake (right) that were entangled and killed in the biodegradable mesh used along the 2018 barrier fencing.

Appendix 15: Full Literature Review

List of the literature used throughout the course of our research and used in the creation of this document.

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