

Assessing the Efficacy of Wildlife Underpasses in Mitigating Amphibian Road Mortality: A Case Study from the Northeastern United States

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Abstract

Roads pose significant threats to wildlife populations worldwide, leading to habitat fragmentation and high mortality rates among various species. Mitigation strategies such as wildlife underpasses have been implemented to alleviate these impacts, yet few studies have assessed their effectiveness before and after implementation. We conducted a case study in the northeastern United States to evaluate the efficacy of a wildlife underpass complex in mitigating amphibian road mortality. The study area encompassed a 1.3 km stretch of road, where two underpasses were constructed to facilitate amphibian passage. Through a comprehensive survey spanning five years pre-construction and seven years post-construction, we collected data on amphibian mortality and environmental factors. Linear mixed-effects models were used to assess changes in mortality rates before and after underpass construction using a before-after control-impact design. Our findings indicate a substantial reduction in mortality across the entire amphibian community and for non-arboreal amphibians within treatment areas post-construction. While arboreal amphibian mortality decreased, the difference was not statistically significant. The underpasses effectively facilitated amphibian movement, with observed usage by various species, including arboreal individuals. Overall, our study provides empirical evidence of the effectiveness of wildlife underpasses in reducing amphibian road mortality, highlighting them as a potentially important conservation action. These findings underscore the significance of incorporating underpass structures into transportation planning and infrastructure development to mitigate negative impacts on wildlife populations. Moreover, our study contributes valuable insights for future research and informs policy initiatives aimed at enhancing habitat connectivity and safeguarding vulnerable amphibian populations in environments bisected by roadways.

1. Introduction

Human infrastructure tends to have unintended negative consequences on the natural world (Sun and Narins, 2005; Egea-Serrano et al., 2012; Woinarski et al., 2020; Niebuhr et al., 2022). The creation of human infrastructure, such as roads, often leads to habitat fragmentation, degradation, and destruction; which are the primary causes of biodiversity loss globally (Andrén, 1994; Wilson et al., 2016; Crooks et al., 2017; de Lima Filho et al., 2021). As human populations continue to grow, more and more infrastructure will be built (McDonald et al., 2020). Human development and infrastructure may cause unintended negative impacts on many wildlife taxa (Andrén, 1994; Moore et al., 2021).

Roads can have detrimental impacts on wildlife species in multiple ways (Lodé, 2000; Lester, 2015). Roads may cause reduced genetic exchange between populations, change wildlife behavior, and can lead to local extirpations of wildlife populations (Riley et al., 2014; Schwartz et al., 2020; Driessen, 2021; Pokorny et al., 2022). Very few species are immune to the effects of roads. For example, large and mobile species, such as ungulates, are killed in large numbers by vehicular collisions, even at low traffic densities (Riley et al., 2014; Poulin et al., 2023). As human populations continue to grow, more vehicles will be used on roadways. Therefore, the negative impacts of roads on wildlife are likely to increase

without implementing mitigation strategies (Riley et al., 2014; Lester, 2015; Schwartz et al., 2020; Pokorny et al., 2022).

Many mitigation techniques have been implemented successfully across the globe to reduce wildlife road mortality. These techniques include drift fences, making bridges and culverts passable by wildlife, tunnels, overpasses, fencing, underpasses, and many more (Glista et al., 2009). All of these techniques provide opportunities for wildlife to safely cross roads (Bager and Fontoura, 2013; Bellis et al., 2013; Lester, 2015). Large wildlife underpass and overpass complexes, used in tandem with fencing, have been successful in reducing road mortality of large mammals in western North America (Sawaya et al., 2014; Sawyer et al., 2016; Simpson et al., 2016). Unfortunately, these techniques are harder to implement in the northeastern United States, where increased human densities and associated infrastructure (e.g., driveways) make it difficult for transportation agencies to install large overpass and underpass structures, especially in tandem with fencing.

Large crossing structures are expensive, ranging from \$500,000 to \$2.7 million for underpasses and \$2.7 million to \$6.2 million for overpasses; making them challenging for state and local governments to implement (Sugiarto, 2022). However, wildlife crossing structures designed for small wildlife, like amphibians, can be quite small and are relatively easy to install, especially when repairing roads (Fairbank, 2014; Huijser et al., 2016; Seiler et al., 2016). Wildlife underpasses are one of the most implemented infrastructural mitigation techniques used to prevent wildlife mortality on roads (Pimm et al., 2021). Despite this, very few studies have compared wildlife mortality levels before and after the installation of underpasses (Glista et al., 2008; Gagnon et al., 2011). Therefore, there is great importance in evaluating whether wildlife underpasses are an effective means of mitigating road mortality, especially considering the high cost of building them.

Amphibians are the most vulnerable group of vertebrates, with approximately 40.7% of amphibian species being susceptible to extinction (Wren et al., 2015; Churko, 2020; Luedtke et al., 2023). Roads negatively impact multiple life stages of amphibians (i.e., waterbody salinification via road salt, traffic noise impacting frog mating calls, etc.) (Taylor and Goldingay, 2010; Coelho et al., 2012; Beebee, 2014; Kioko et al., 2015). Temperate amphibians generally have two distinct life stages: an aquatic egg/larval stage and a terrestrial (or semi-terrestrial) adult stage (Gibbs and Shriver, 2005). Therefore, most amphibians require a habitat matrix that includes both wetlands and upland habitats to persist (Bradford, 1983; Lamoureux and Madison, 1999; Joly et al., 2003). The loss and fragmentation of these critical habitats is the most immediate threat to amphibians in the northeastern United States and across the globe (Gibbs, 1998; Cushman, 2006; Guilherme et al., 2007; Dutta, 2018).

Throughout the northeastern United States, many wetlands are separated from upland habitats due to roads. Fragmentation due to roads can lead to extreme mortality events when amphibians cross roads to breed and return to the uplands on rainy nights in the spring, summer and fall (Mazerolle, 2004; Sterrett et al., 2018). This mortality can be severe enough to cause local extirpations (Gibbs and Shriver, 2005). Conservationists have attempted to reduce road-induced mortality by building amphibian/wildlife

underpasses on roads that intersect important habitats for amphibians (Schmidt and Zumbach, 2008; Woltz et al., 2008; Pomezanski, 2018). Very few studies have investigated amphibian mortality before and after the installation of underpasses (Fahrig and Rytwinski, 2009). However, there is some evidence that they are effective at reducing mortality (Jackson, 2000).

In this study, we used linear mixed effects models to analyze amphibian mortality data collected prior to and after the installation of a wildlife underpass complex in Monkton, Vermont. The objectives of this study were 1) to determine if there was a significant decrease in amphibian mortality in the treatment areas after the construction of the underpasses; 2) to understand whether underpasses are equally effective for all amphibian species; and 3) to determine whether the underpass wing walls in so-called “buffer” areas impacted mortality more like treatment or control areas. We hypothesized that the total amphibian mortality would be significantly less than in control areas after underpass construction, and that reductions in mortality would be most pronounced for non-arboreal species, because arboreal species may climb over the structure. We also hypothesized that buffer areas would act more like control areas than treatment areas. Our research provides resource managers with valuable information about the effectiveness of underpasses for amphibians, which is essential given the concerning impacts of road-induced wildlife mortality and the high cost of these structures.

2. Methods

2.1. Background

In 1997, biologists in Vermont (Abenaki place name, N’dakinna) identified a segment of Monkton Road in the town of Monkton, as one of the State’s most important and vulnerable amphibian crossings. During just two nights in the spring of 2006, more than 1,000 amphibians were estimated to have been killed by automobiles (unpublished data). This prompted the Monkton Conservation Commission and partners to apply for grants, which allowed them to design and construct the underpasses on Monkton Road. The structures were built in 2015 and cost \$342,397. Data were collected for five years prior to construction (from 2011-15) and for seven years post construction (2016-22).

2.2. Study Area

The underpasses are located on a 1.3 km stretch of Monkton Road that runs north to south. There is a wetland to the west of the underpasses and upland habitat to the east of the underpasses. The underpass complex is composed of two separate crossing structures about 0.5 km apart, with wing walls on both the upland and wetland sides of the road that are intended to funnel amphibians to the tunnel openings (Fig. 1). The crossing structures are made of solid concrete. The northern crossing site covers 220.7 linear meters of the road, and the southern crossing site covers 243.3 linear meters of the road. Each of the eight wing walls end with hard plastic oriented in semi-circles designed to turn amphibians back toward the tunnel crossing area. When the crossing structures were installed, issues with rock ledge resulted in some design changes to the wing walls that were intended to guide

amphibians to the tunnel openings, making wing walls on the wetland and upland side unmatched in length and less angled than initially planned.

For the purposes of our study, the crossing site was divided up into two crossing structures (north and south), each with controls, treatments, and buffers (which were located at both ends of the treatment areas) (Figs. 1 and 2). The control areas were referred to as the northern control area (NC) and the southern control area (SC) and were separated from the treatment areas by more than 50 meters. The treatment areas were referred to as the northern treatment area (NTR) and the southern treatment area (STR). Treatment areas included both walls and tunnels. The buffer areas were referred to as the southern buffer 1 (SB1), the southern buffer 2 (SB2), the northern buffer 1 (NB1), and the northern buffer 2 (NB2). NB1 and SB1 were located on the northern side of their respective treatment areas, while NB2 and SB2 were located on the southern side of their respective treatment areas. The purpose of the buffer areas is described in detail below. In total, the walls and tunnels (the “treatment” and “buffer” areas) account for 18.5% of the total crossing area (Fig. 1). The tunnels form an upside down “U” shape and were partially buried in the substrate. The northern tunnel is ~ 1.5 meters tall, while the southern tunnel is ~ 0.9 meters tall. Both tunnels are ~ 1.5 meters wide.

The buffers were created to account for the possibility that some animals would turn away from the tunnel openings when encountering the wing walls rather than turning toward the openings and eventually through the tunnels; especially considering that the wing walls ultimately angled only slightly and were largely parallel to the road due in part to the design change. If amphibian mortality is less in the treatment areas than in the controls, and amphibian mortality in the buffer areas is not greater than in the controls, we can conclude that the underpass complex was successful. Further, evaluating the buffers separately will allow us to understand if the wing walls effectively extend the size of the treatment area.

The habitat to the west of the underpasses is characterized by a marsh with emergent vegetation that drains into a tributary of Little Otter Creek, VT. The habitat to the east of the underpasses is characterized by upland northern hardwood forest that is primarily composed of maple (*Acer spp.*), American beech (*Fagus grandifolia*), birch (*Betula spp.*), and rocky ledges. Monkton Road is a relatively busy, paved two-lane road, with a speed limit of 64 kilometers per hour.

2.3. Study Species

We anticipated encountering mole salamanders (*Ambystoma spp.*), eastern newts (*Notophthalmus viridescens*), wood frogs (*Lithobates sylvaticus*), green frogs (*Lithobates clamitans*), pickerel frogs (*Lithobates palustris*), American toads (*Anaxyrus americanus*), gray treefrogs (*Hyla versicolor*) and spring peepers (*Pseudacris crucifer*). Spring peepers and gray treefrogs are arboreal species that we expected may be able to climb over the underpass structures, thus bypassing their benefit. Animals were handled in accordance with the approved guidelines of the Institutional Animal Care and Use Committee at the University of Vermont.

2.4. Survey Methods

The Monkton Road amphibian crossing was monitored for five years prior to the construction of the underpasses (2011–2015) and seven years after the construction of the underpasses (2016–2022). Surveys were completed in the same manner prior to and after the construction of the Monkton Road underpasses. The monitoring primarily occurred between 20:30 and 22:30 when nighttime, rain, road traffic, and amphibian movement occurred in conjunction. Four to five surveys were conducted each year between late March and early May on nights that it rained, and where temperatures were above 1.67 °C. During the surveys, between one and eight people were involved with data collection. The crossing area was divided into the eight transects described above (Fig. 2). Data collected during each transect included the date, how many minutes were spent completing the surveys, how many cars went by during the surveys, what species were encountered, and whether encountered individuals were dead or alive. We also acquired the minimum and maximum temperature for the survey day, and the total amount of precipitation (rain or snow) that occurred on the survey day from the National Oceanic Atmospheric Administration (NOAA) (Table 1).

Table 1
Covariates used to predict amphibian mortality at the Monkton Road underpass complex in Monkton, Vermont.

Covariate	Code	Description	Data Source
Transect	Tran	Transect type (treatment, buffer, control).	Field data
Day of year	DOY	The day of year starting from January 1st (day 1) and ending on December 31st (day 365).	Field data
Year	Year	The year of the survey.	Field data
Period	Period	Interaction between period the survey took place (i.e., pre- or post-construction) and transect type.	Field data
Minimum Temperature	TempMn	The minimum temperature of the given survey measured in Celsius.	NOAA*
Maximum Temperature	TempMx	The maximum temperature of the given survey measured in Celsius.	NOAA*
Precipitation	Precip	The total amount of precipitation (rain or snow) of the given survey. Measured in centimeters.	NOAA*
<i>Note: Transect, day of year, year, and period are categorical variables, while minimum temperature, maximum temperature, and precipitation are continuous variables.</i>			
<i>*National Centers for Environmental Information. (Accessed on February 8, 2024). NOAA Climate Data Online (CDO) – Datasets. Retrieved from ncei.noaa.gov/cdo-web/datasets</i>			

2.5. Statistical Analysis

We transformed the amphibian mortality count data to the number of amphibians killed per meter of road in each transect type, due to the differences in linear sizes among transects. We hypothesized that there may have been a difference in mortality between arboreal and non-arboreal amphibians because

arboreal amphibians could climb over the crossing structure rather than pass through it. Therefore, we conducted all analyses on three subsets of data: total amphibian mortality per meter of road; non-arboreal amphibian (all species except spring peepers and gray treefrogs) mortality per meter of road; and arboreal amphibian (only spring peepers and gray treefrogs) mortality per meter of road. We predicted stronger differences between treatment and control areas for all amphibian and non-arboreal amphibian analyses.

We used the lme4 package in R to perform repeated-measures linear mixed-effects modeling with a Gaussian response variable (Bates et al., 2015; R Core Team, 2021). We were primarily interested in evaluating an interaction between transect type (i.e., control, treatment, or buffer) and period (interaction between pre- and post-construction and transect type), based on our before-after control-impact (BACI) design. However, we also explored covariates including minimum temperature, maximum temperature, and precipitation as fixed effects and day of year (DOY) and year as random effects. DOY and year were treated as random effects to account for the variability in amphibian migration intensity that was associated with each specific day and each specific year that we collected data. We scaled the minimum temperature, maximum temperature, and precipitation covariates. We pooled transect types across the northern and southern crossing structures (i.e., treatment = NTR + STR, control = NC + SC, etc.) during the modeling process. We hypothesized that the treatments would reduce mortality rates, while temperature and precipitation would have a positive relationship with mortality. In other words, we expected that both higher maximum and minimum temperatures, as well as increased precipitation, would correspond to increased mortality rates. We also hypothesized that there would not be an increase in mortality in the buffers after the construction of the underpass complex.

2.6. Model Selection

We fit 40 models for all combinations of our covariates for each of the three subsets of data (all amphibians, arboreal amphibians only, non-arboreal amphibians only). We included single random effects and additive and interactive fixed effects of up to six covariates for each model (Table A1). We calculated the Akaike Information Criterion Corrected (AICc) for each model, which is a measure that balances goodness-of-fit and model complexity. Lower AICc values indicate more parsimonious models (Burnham and Anderson 2004). We compared AICc values by calculating the difference in AICc values (ΔAICc) and assessing AICc weights. A difference of two or more in AICc suggests substantial evidence in favor of the model with the lower AICc value (Akaike, 1974). We applied the Bonferroni correction to lower the family-wise error rate and avoid false significant results.

3. Results

3.1. Species Encountered

During the course of the study, 5,273 amphibians were encountered; 3,053 anurans, 2,110 salamanders, and 110 which could not be identified due to mortality. In total, 62.9% of all encountered amphibians

were dead (69.4% of encountered frogs and 51.6% of encountered salamanders) (Fig. 3). The highest mortality recorded on a single survey night was 167 amphibians across the 464 meters of transects.

We encountered 12 amphibian species during our surveys. These species included: spotted salamanders (*Ambystoma maculatum*), blue spotted salamander complex (*Ambystoma laterale x jeffersonianum*), eastern newts (*Notophthalmus viridescens*), four-toed salamanders (*Hemidactylium scutatum*), northern two-lined salamanders (*Eurycea bislineata*), wood frogs (*Lithobates sylvaticus*), spring peepers (*Pseudacris crucifer*), northern leopard frogs (*Lithobates pipiens*), pickerel frogs (*Lithobates palustris*), American toads (*Anaxyrus americanus*), green frogs (*Lithobates clamitans*), and gray treefrogs (*Hyla versicolor*) (Table 2). Blue spotted salamander complex and four-toed salamanders are classified as uncommon and rare, respectively, in Vermont. We classified spring peepers and gray treefrogs as arboreal amphibians, while the other 10 species were classified as non-arboreal amphibians.

Table 2
The total number (N) of amphibians encountered at the Monkton Road underpass complex in Monkton, Vermont between 2011–2022.

Species	N	% Dead
Caudata		
<i>Ambystoma laterale x jeffersonianum</i>	184	35.9%
<i>Ambystoma maculatum</i>	1,702	49.2%
<i>Eurycea bislineata</i>	3	33.0%
<i>Hemidactylium scutatum</i>	4	25.0%
<i>Notophthalmus viridescens</i>	217	84.3%
Anura		
<i>Anaxyrus americanus</i>	10	50.0%
<i>Hyla versicolor</i>	5	40.0%
<i>Lithobates clamitans</i>	13	69.2%
<i>Lithobates palustris</i>	7	28.6%
<i>Lithobates pipiens</i>	4	25.0%
<i>Lithobates sylvaticus</i>	469	72.3%
<i>Pseudacris crucifer</i>	2,545	69.2%

3.2. Mixed-effects Modeling

Our best total amphibian mortality model received 39% of the model weight and included the interaction between transect type and period and maximum temperature as fixed effects; and DOY as a random effect (Table 3). This model suggests that there was no difference in total amphibian mortality between transect types during the pre-construction period, and that there was no difference in pre- and post-construction mortality for the control and buffer areas. However, there was a significant decrease in total amphibian mortality in the treatments during the post-construction period (Table 4). The results of our Bonferroni correction also support that there was a significant decrease in total amphibian mortality between periods (Fig. 4). The second-best supported model received 32% of the model weight and was identical to the top model except that it included minimum temperature instead of maximum temperature. All top 4 models included a transect by period interaction, supporting the idea that the amount of mortality per transect varied by period.

Table 3

Top 5 models for predicting the total, non-arboreal, and arboreal amphibian mortality at the Monkton Road underpass complex in Monkton, Vermont.

Fixed Effects	Random Effects	N Parameters	AICc	Δ AICc	Weight
Total Mortality					
Tran + MaxTemp + Tran * Period	DOY	9	3454.90	0	0.39
Tran + MinTemp + Tran * Period	DOY	9	3455.29	0.39	0.32
Tran + Precip + MaxTemp + Tran * Period	DOY	10	3456.96	2.07	0.14
Tran + Precip + MinTemp + Tran * Period	DOY	10	3457.35	2.45	0.11
Tran + MaxTemp	DOY	6	3462.15	7.25	0.01
Non-arboreal Mortality					
Tran + Precip + MaxTemp + Tran * Period	DOY	10	2915.41	0	0.49
Tran + Precip + MinTemp + Tran * Period	DOY	10	2916.98	1.57	0.22
Tran + MaxTemp + Tran * Period	DOY	9	2917.43	2.02	0.18
Tran + MinTemp + Tran * Period	DOY	9	2918.94	3.53	0.08
Tran + Precip + Tran * Period	DOY	9	2922.52	7.10	0.01
Arboreal Mortality					
Tran + Precip + MaxTemp	Year	7	3187.27	0	0.48
Precip + MaxTemp	Year	5	3188.81	1.54	0.22
Tran + Precip + MinTemp	Year	7	3190.54	3.27	0.09
Tran + Precip + MaxTemp + Tran * Period	Year	10	3190.60	3.33	0.09
Precip + MinTemp	Year	5	3192.03	4.76	0.04

Table 4

Top model results for total, non-arboreal, and arboreal mortality at the Monkton Road underpass complex in Monkton, Vermont. The "Estimate" column contains the regression coefficient estimate while the "95% CI" column includes the 95% confidence interval for that coefficient.

	Estimate	95% CI	t-value	p-value
Total Mortality				
TransTypeControl (Intercept)	11.86	4.58–19.18	3.21	0.003
TransTypeBuffer	0.65	-6.28–7.58	0.18	0.854
TransTypeTreatment	-3.13	-11.13–4.87	-0.77	0.442
TempMxScale	4.30	1.57–7.02	3.12	0.002
PeriodPost	7.99	-0.23–16.21	1.91	0.057
TranstTypeBuffer: PeriodPost	1.27	-7.73–10.28	0.28	0.782
TransTypeTreatment: PeriodPost	-12.30	-22.69 – -1.90	-2.32	0.021
Non-arboreal Mortality				
TransTypeControl (Intercept)	4.26	0.68–7.82	2.35	0.0200
TransTypeBuffer	1.66	-1.83–5.15	0.93	0.351
TransTypeTreatment	-1.70	-5.73–2.33	-0.83	0.408
PrecipScale	-1.24	-2.45 – -0.04	-2.05	0.041
PeriodPost	6.97	2.85–11.09	3.33	< 0.001
TempMxScale	2.11	0.76–3.46	3.09	0.002
TransTypeBuffer: PeriodPost	-0.60	-5.14–3.94	-0.26	0.796
TransTypeTreatment: PeriodPost	-8.47	-13.71 – -3.22	-3.17	0.002
Arboreal Mortality				
TransTypeControl (Intercept)	8.92	4.28–13.56	3.95	0.001
TransTypeBuffer	0.10	-3.17–3.36	0.58	0.954
TransTypeTreatment	-3.68	-7.45–0.09	-1.92	0.056
PrecipScale	2.48	1.03–3.94	3.36	< 0.001
TempMxScale	2.69	1.19–4.20	3.52	< 0.001

Our best non-arboreal amphibian mortality model received 49% of the model weight and included the interaction between transect type and period, precipitation, and maximum temperature as fixed effects, and DOY as a random effect (Table 3). This model suggests that there was no difference in non-arboreal

amphibian mortality between transect types during the pre-construction period, and that there was no difference in mortality in the control and buffer areas in the post-construction period. However, there was a significant decrease in non-arboreal amphibian mortality in the treatment areas from the pre-construction to the post-construction period (Table 4). The results of our Bonferroni correction further support that there was a significant decrease in total amphibian mortality between periods (Fig. 4). The second-best supported model received 22% of the model weight and was identical to the top model except that included minimum temperature instead of maximum temperature. All top 5 models included a transect by period interaction, supporting the idea that the amount of mortality per transect for non-arboreal amphibians varied by period.

Our best arboreal amphibian mortality model received 48% of the model weight and included transect type, precipitation, and maximum temperature as fixed effects, and year as a random effect (Table 3). This model suggests that there was not a statistically significant reduction in arboreal amphibian mortality between the two study periods (Table 4). The results of our Bonferroni correction also supports that transect type did not significantly reduce arboreal amphibian mortality (Fig. 4). However, the reduction in mortality was almost significant ($p\text{-value} = 0.056$, $CI = -7.45-0.09$), meaning that treatment areas almost differed from control areas significantly due to a large proportion of arboreal amphibians using the underpasses. The second-best supported model received 22% of the model weight, but did not include transect type. However, 3 of the top 4 models included transect type, supporting the idea that the treatment areas are having somewhat of a negative effect on arboreal amphibian mortality (i.e., reducing mortality).

4. Discussion

Amphibian populations are extremely susceptible to extirpation due to roads intersecting their critical habitat (Fahrig et al., 1995). Biologists and concerned citizens have attempted to reduce road induced mortality on amphibian populations by building wildlife underpasses. However, despite being one of the most implemented mitigation techniques used, very few studies have used before-after-control-impact (BACI) design to evaluate whether these structures are effective at reducing amphibian mortality (Pimm et al., 2021). The question of whether or not structures are the right choice depends on many factors – cost, species impacted, traffic mortality, etc., but should also include consideration of the potential for structure efficacy.

We found that the underpass structures significantly reduced the amount of amphibian mortality on the road, which suggests that amphibian underpasses are an effective mitigation strategy for combating road-related amphibian declines. We saw an 80.2% decrease in mortality in the treatment areas among all amphibians after the crossing structures were constructed, and a 94.3% decrease in mortality among non-arboreal amphibians. However, the crossing structures were not as effective at reducing mortality for arboreal amphibians, the majority of which in our study were spring peepers. Despite this, we still observed a 73.6% decrease in mortality among arboreal amphibians in the treatment areas after the construction of the underpass complex. Arboreal amphibian mortality may be further reduced by

modifying the crossing structure design. Hughes et al. (2021) found that fences with aluminum flashing and a 10 cm horizontal lip at the top were effective at preventing Pacific treefrogs (*Hyla regilla*) from getting into agricultural fields. So, perhaps this methodology could be adapted for crossing structures.

There was some concern that amphibians would work their way to the ends of the walls and onto the road surface, negating the positive impact of the treatment areas and leading to unintended consequences (Meshaka et al., 2007; Gilhooly et al., 2019). However, if this were the case, we would expect to see a significant increase in amphibian mortality in the buffer areas between the two time periods. Instead, we found that the lengths and angles of the walls in our study caused the buffer area to function similarly to a control. While the buffers did not appear to increase road mortality, they also did not extend the benefits of the treatment area. This implies that project managers should ensure that wing walls are angled away from the road as much as is feasible given other constraints to funnel amphibians towards tunnel openings in future construction projects focused on reducing amphibian mortality, especially if there are unexpected design changes during the construction phase of the project. Furthermore, project managers should also ensure that wing walls are matched on both sides of the road to safeguard the passage of amphibians moving both to the breeding pools and to the uplands. Future wildlife underpass studies could look at the effectiveness of “amphibian turnarounds” and what modifications would prevent arboreal amphibians from getting over the underpass wing walls.

Another concern would be whether crossing structures are effective at reducing fall migration mortality, especially considering that many migrating amphibian populations are recruitment-driven (Sterrett et al., 2018). However, Pagnucco (2010) observed that long-toed salamander (*Ambystoma macrodactylum*) mortality was substantially reduced during their fall migration after installing fencing and a tunnel. Therefore, we suspect that crossing structures would be effective for fall migrants, but we only collected data in the spring so further research would need to be conducted to confirm or deny their effectiveness in the fall.

Very few studies have compared wildlife mortality before and after the implementation of wildlife structures (Jackson, 2000; Fahrig and Rytwinski, 2009; Fitzsimmon and Breisch, 2015). Despite this, most studies focus on the effectiveness of crossing structures after they are built. For example: Allaback and Laabs (2003) focused on how far salamanders will travel along a wall before they give up on crossing the road; while Mata (2004) focused on what kind of structures are most used by wildlife. Even though there are a multitude of empirical studies regarding the effectiveness of wildlife crossing structures after construction (Huijser et al., 2016; Simpson et al., 2016; Denneboom et al., 2021), resource managers could greatly benefit from more BACI design projects focusing on comparing before and after mortality at wildlife crossing structures.

Our BACI study design (Smith et al., 1993) adds strength to the evidence we present that the crossing structures reduced amphibian mortality. Observations were made before and after the underpass construction and we had unimpacted control sites that we compared to impacted treatment sites. This should have accounted for any natural or preexisting differences between the transects, meaning that we

should have been able to estimate the “true” effect that the treatments had on mortality (Smith et al., 1993). Based on the assumption of this type of experimental design, the trajectories of the control and treatment areas should have been exactly parallel in the absence of the intervention (Smith et al., 1993).

Crossing structures may also benefit many other wildlife taxa (Caldwell and Klip, 2019). At our study location, wildlife cameras observed many mammal species, including American black bear (*Ursus americanus*), bobcats (*Lynx rufus*), racoons (*Procyon lotor*), and North American porcupines (*Erethizon dorsatum*) using the underpass complex to safely cross the road. We also observed a few bird species and common gartersnakes (*Thamnophis sirtalis*) using the underpass structures. Additionally, the Lewis Creek Association counted 2,208 amphibians using one of the underpasses via wildlife cameras between March 10th – May 3rd, 2016 (unpublished data).

Because underpasses may attract a large density of amphibians and many species use underpasses, there is a risk of increased predation (Matta et al., 2020). However, mortality from predations should be much less significant than the mortality caused by impacts with vehicles (Edwards et al., 2022). Furthermore, there is still a high degree of mortality in nearby areas where there are no crossing structures. So, theoretically the predators would still scavenge on animals that have already been killed (Muszynska et al., 2022). Additionally, predation could be reduced by installing “anti-predation” devices inside of the underpass tunnels (Tissier et al., 2016). In our study area, the underpass complex had large slate slabs in each tunnel to provide cover for amphibians.

In 2021 the U.S. Senate passed the Infrastructure Investment and Jobs Act that will provide \$350 million to municipalities, states, tribal governments, federal agencies, and nonprofit organizations to fund wildlife underpasses, as well as improving habitat connectivity (Bies, 2021). This study provides valuable information to resource managers and transportation agencies considering the construction of wildlife underpasses in important amphibian crossing areas.

5. Conclusion

This study evaluated the efficacy of wildlife underpasses in mitigating amphibian road mortality along a 1.3 km stretch of Monkton Road in Vermont. The construction of underpasses led to an 80.2% decrease in total amphibian mortality and 94.3% decrease in non-arboreal amphibian mortality in treatment areas. Although the reduction in arboreal amphibian mortality was not statistically significant, a 73.6% decrease was observed. The underpasses were effective for various amphibian species, including non-arboreal and arboreal species. However, modifications to the design could further reduce arboreal amphibian mortality. The buffer areas did not increase mortality, indicating that the wing walls effectively funneled amphibians towards the underpasses without causing unintended consequences.

These findings support the incorporation of wildlife underpasses into transportation planning and infrastructure development to enhance habitat connectivity and reduce wildlife mortality. The study provides empirical evidence that can inform policy initiatives aimed at protecting vulnerable amphibian populations. Overall, this study highlights the importance of wildlife underpasses as a conservation

strategy to mitigate the negative impacts of roads on wildlife populations and underscores the need for continued research and policy support to enhance their effectiveness.

Declarations

Author Contribution

S.G.P. designed the study's data collection and experimental design. M.R.M. and B.A.M. were responsible for the data analyses. M.R.M. wrote the main manuscript text and prepared figures and tables. All authors reviewed the manuscript.

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Data Availability

Data will be made available on request.

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Figures

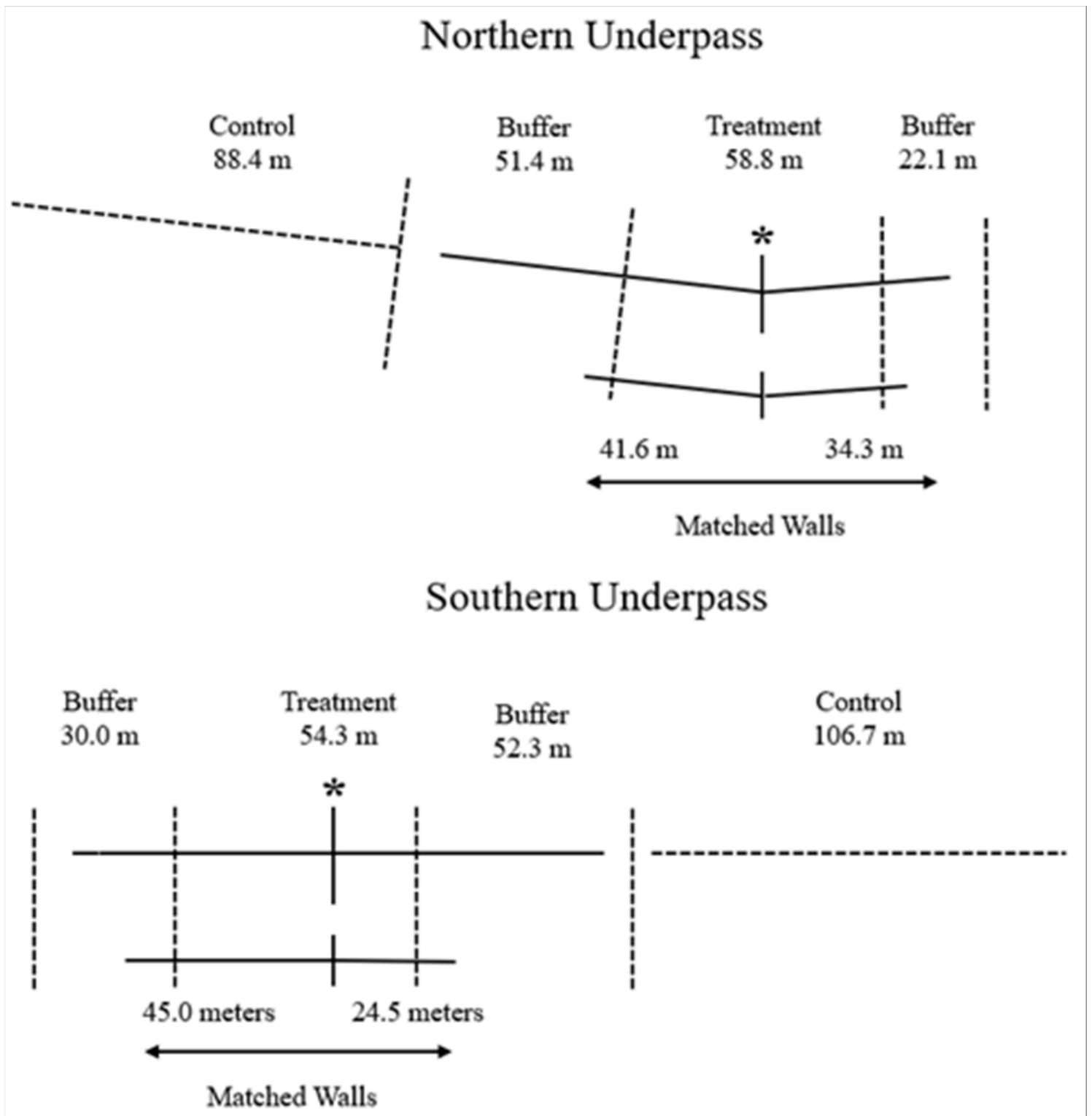


Figure 1

Schematic showing the configuration of the Monkton Road, Vermont amphibian underpasses. Solid horizontal lines represent walls, while dashed lines indicate the location of the buffer and control areas. *Indicates the location of the tunnel openings.

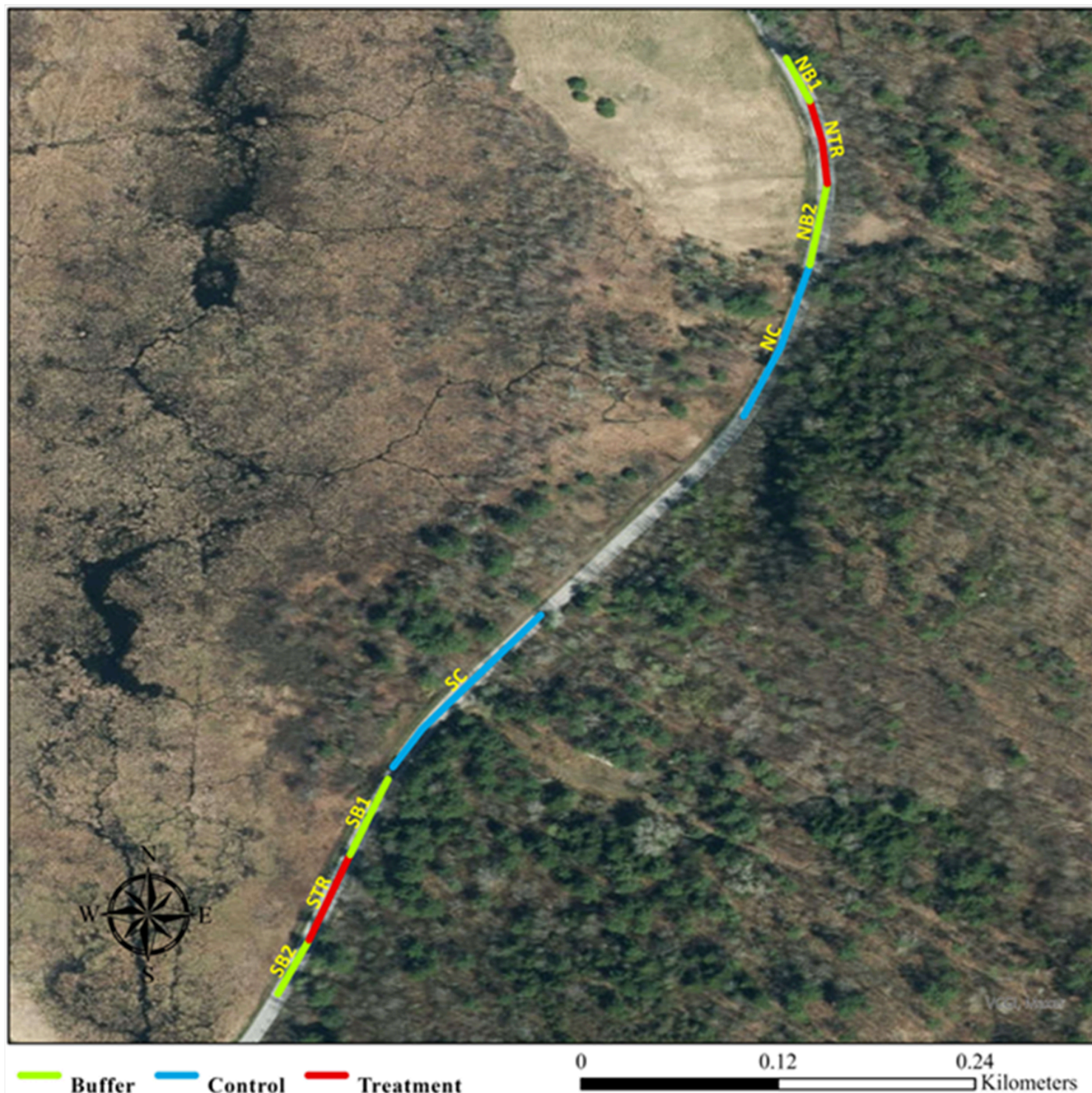


Figure 2

Control, treatment, and buffer areas at the Monkton Road, Vermont amphibian underpasses.

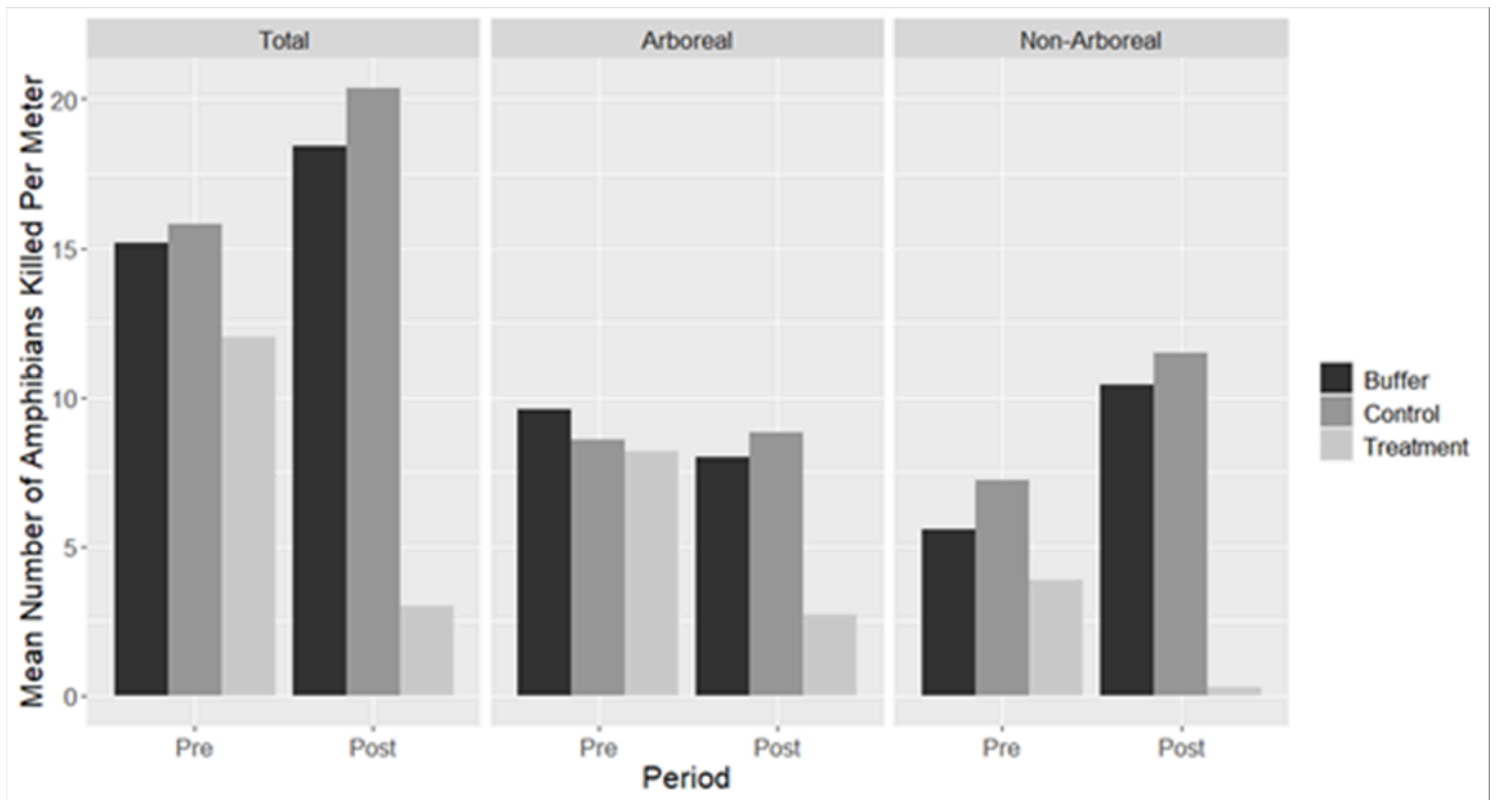


Figure 3

Mean number of amphibians killed per meter of road pre- and post-construction at the Monkton, Vermont amphibian underpasses.

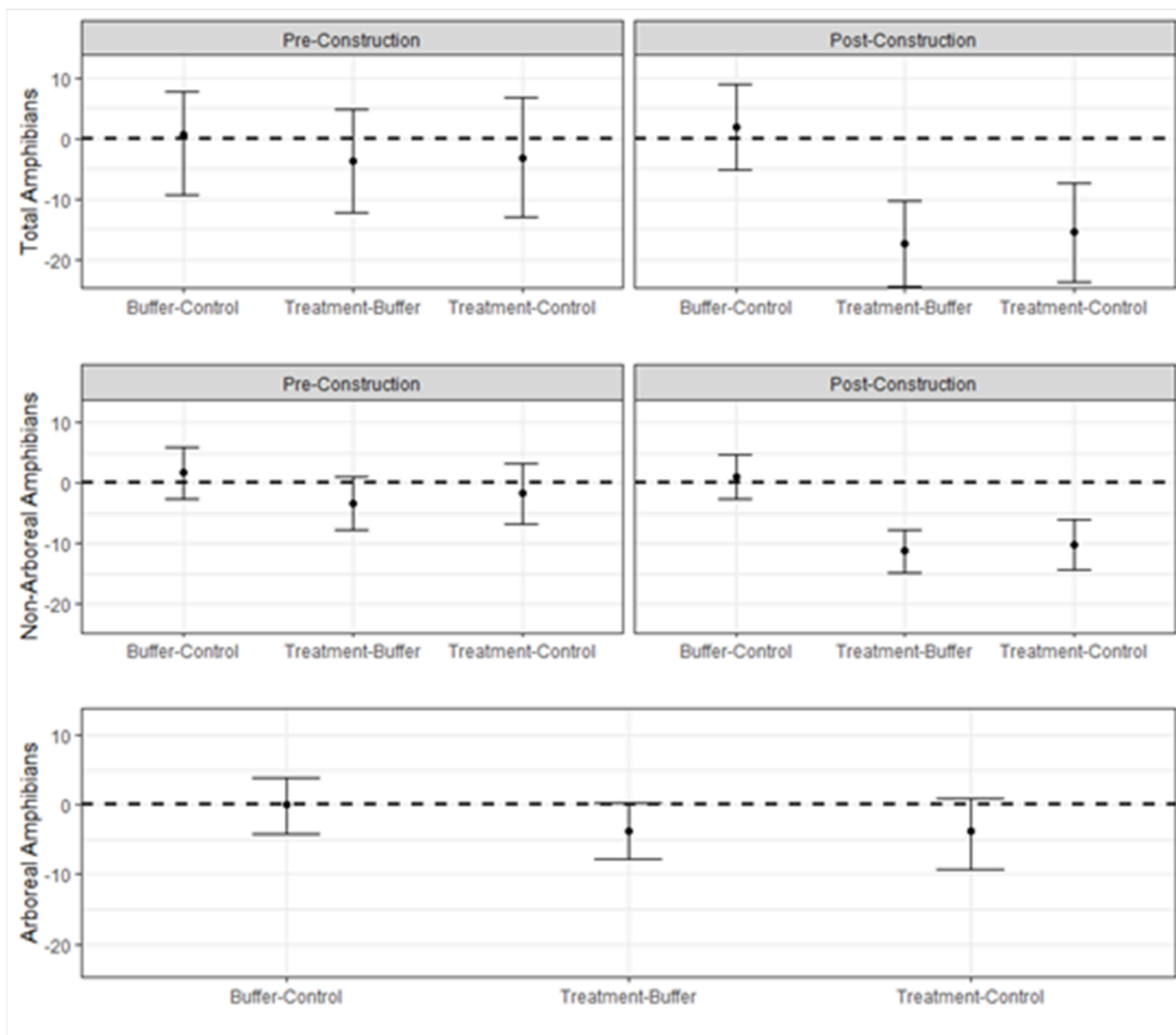


Figure 4

Difference in means of top model results after Bonferroni correction for amphibians killed per meter of road at the Monkton, Vermont amphibian underpass. Error bars are 95% confidence intervals for the model estimate.

Supplementary Files

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