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Tunnel and Fencing Options for Reducing Road Mortalities of Freshwater Turtles

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16. Abstract

This study was undertaken to investigate the relative effectiveness of experimental under-road passages and barriers for freshwater turtles as solutions to the problem of roadway mortality through a series of designed behavioral experiments. At outdoor laboratories, movements in response to varying light levels, and barrier opacity were examined for painted turtles (*Chrysemys picta*), Blanding's turtles (*Emydoidea blandingii*), and spotted turtles (*Clemmys guttata*). Additionally, tunnel size and tunnel entrance design were examined for painted turtles only. A total of 886 painted turtles, 53 Blanding's turtles, and 50 spotted turtles were used in factorial experimental designs to test for effects of (1) tunnel lighting and size, (2) artificial lighting, and (3) guidance structure characteristics. Behaviors of turtles were quantified both as binomial responses (success/fail), and continuous responses. As the amount of natural light transmitted through the tops of tunnels increased, successful completion of the trials increased. This relationship was also maintained when the lighting was artificial. All three tested species responded poorly to the 0% available light treatment. Guidance structure characteristics did not affect the willingness of turtles to enter passages. Turtles moved at a slower rate when traveling along a translucent barrier, compared to an opaque one. Our results indicate the importance of designing road passage structures for freshwater turtles that provide adequate tunnel lighting in combination with opaque and/or translucent barriers, as determined through evaluation of the wetland/roadway context in regards to protect objectives.

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Tunnel and Fencing Options for Reducing Road Mortalities of Freshwater Turtles

Final Report

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Disclaimer

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Executive Summary

The Tunnel and Fencing Options for Reducing Road Mortalities of Freshwater Turtles study was undertaken as part of the Massachusetts Department of Transportation (MassDOT) Research Program. The program is funded with Federal Highway Administration (FHWA) Statewide Planning and Research (SPR) funds. Through this program, applied research is conducted on topics of importance to the Commonwealth of Massachusetts transportation agencies.

Scope and Study Objectives

This research was conducted with the purpose of addressing concerns about the design of under-road passage systems for turtles. Specifically, a series of designed behavioral experiments were used to determine what the best recommendations are regarding passage width, length, and design type. The movements of turtles were examined in response to a variety of light levels, tunnel sizes, tunnel entrance designs, and barrier opacities in outdoor laboratories. This report presents the results of the study, with the aim of informing the design of passage systems that are both effective and cost efficient. The implementation of these findings will allow for public resources to be used wisely, while also meeting regulatory requirements regarding endangered turtle species.

The goal of this three-year study was to examine the effectiveness of road passage structures for freshwater turtles in Massachusetts, with an emphasis on identification of cost-effective structures that allow rare species of turtles to safely move between habitats bisected by two-lane and four-lane roadways.

Stemming from this goal, specific objectives include:

1) Evaluate variations of tunnel height, width, length, openness, and light level with regard to their influence on the movement behavior of painted turtles;
2) Evaluate variations of fence opacity, length, angle, and septum use with regard to their effectiveness in directing painted turtles through road passage structures. The septa were two sections of fence that formed a roughly wedge-shaped configuration intended to direct turtles into the tunnel entrance that might otherwise be bypassed; and
3) Evaluate select passage and fencing designs using two additional turtle species, the uncommon spotted turtle, and the state-listed Blanding's turtle.

Synopsis of the Research Issue

Increasingly, under-road passages are being employed to allow a wide range of wildlife species, including turtles to move safely between habitat patches that are bisected by roadways. Roadways have become a pervasive feature of the landscape and can be a significant source of mortality for turtles. Direct effects of roadways include injury, mortality, alteration/restriction of movement/behavior, and loss of habitat. Indirect effects include habitat fragmentation and degradation, isolation of turtle populations, disruption of gene flow and meta-population dynamics.

Turtle populations are extremely vulnerable to road mortality because their life history includes low annual recruitment, high adult survival, and delayed sexual maturity. If the additive mortality resulting from roadways is too great, then local turtle populations are at risk of decline. Even a seemingly modest 2-3% additive mortality caused directly by vehicles is suspected to be more than most turtle populations can withstand and still maintain positive population growth rates.

Significance of the Research

MassDOT frequently receives requests from regulatory agencies to include wildlife crossings in roadway designs in order to reduce mortality of rare and endangered turtle species and maintain their habitat continuity. However, due to limited empirical evidence, it has been unclear what the best recommendations are regarding passage width, length, and design type.

Few studies have evaluated the effectiveness of roadway passage structures for common turtle species as well as uncommon species, such as the spotted turtle (*Clemmys guttata*), and state-listed species such as the Blanding's turtle (*Emydoidea blandingii*). Tunnels and culverts that are not designed with the needs of turtles specifically in mind may be inadequate to meet their needs and can lead to failure in meeting the Commonwealth of Massachusetts goals of accommodating the safe passage of these species across roadways. Additionally, tunnels and culverts that are far larger than is required can lead to unnecessary design and construction costs.

Methods

Testing occurred over the course of three field seasons from 2009 through 2011. Field laboratories of various designs to test the response of turtles to passage system variables were constructed and utilized at three different locations. Hundreds of individual turtles encompassing three freshwater turtle species collected from a total of seven wetland sites were tested.

Sites harboring populations of each species of turtles used in the experiments within a reasonable distance of the field laboratory were identified prior to commencing field work. All turtle species were captured using large collapsible minnow traps baited with sardines packed in soybean oil. Table ES-1 provides a useful summary of field laboratory information including year used, field laboratory name, and trial group name.

Table ES – 1: Field laboratories and trial groups.

Year	Field Laboratory Name	Trial Group Name
2009	Tillson Tunnel Lab	Tunnel Trial Group 1
		Tunnel Trial Group 2
		Median Lighting Trial Group
	Tillson Exclusion Gate Lab	Exclusion Gate Trial Group
2010	Leverett Barrier and Tunnel Entrance Lab	Barrier and Tunnel Entrance Trial Group
	Tillson Artificial Lighting Lab	Artificial Lighting Trial Group
2011	Assabet Tunnel Lab	Assabet Tunnel Blanding's Turtle Trial Group
		Assabet Tunnel Spotted Turtle Trial Group
		Assabet Tunnel Painted Turtle Trial Group
	Assabet Barrier Lab	Assabet Barrier Blanding's Turtle Trial Group
		Assabet Barrier Spotted Turtle Trial Group
		Assabet Barrier Painted Turtle Trial Group

Results

A number of informative findings regarding the design of effective road passages for freshwater turtles were made during this study. Among the most noteworthy are those that relate to tunnel lighting level, tunnel aperture, tunnel length, and barrier opacity.

Light Level

In our experiments, rates of successful passage differed dramatically between the "bright" (pooled 100% and 75% available overhead light) and "dark" (0% available overhead light) treatments. For bright tunnels, successful passage rates were high, ranging from 80% to 100%. In the dark treatment, successful passage rates ranged between 31% and 70% and were more variable.

Openness Ratio (OR)

In experimental culverts, rates of successful passage increased as the openness ratio (OR) increased. For this study, OR was defined as a culvert's cross-sectional area divided by its length (OR = x-sec area/length). OR was a significant predictor of passage only at the 0% available overhead light level.

Light Level and Additional Species

All three tested turtle species responded poorly to the 0% available light treatment. Only 30% of painted turtles successfully passed through the tunnel given this treatment and both Blanding's and spotted turtles were either extremely reluctant or unwilling to pass through the dark tunnel, with only 8% and 0% passage rates, respectively. The majority of painted and Blanding's turtles were willing to use the tunnel with the 100% available light treatment but passage rates for spotted turtles were less favorable.

Artificial Lighting in Tunnels

Our results indicate that artificial lighting may be as effective as 100% available light in encouraging painted turtles to pass through tunnels. The artificial lighting treatment was paired with the poorest performing previously identified combination of tunnel length, opening size, and lighting level so that a "rescue effect" might be observed. The fluorescent lighting treatment consisted of one compact fluorescent light bulb per foot strung along a closed-top tunnel. Forty-five percent successfully completed trials in the 0% lighting treatment and 78% successfully completed trials in the artificial lighting treatment.

Tunnel Entrance and Barrier Laboratory

The following conclusions were drawn from the results of tests in the tunnel entrance and barrier laboratory:

1) Varying the angle of entrance had no effect on turtles and is probably not an important design element;
2) Using septa had no effect on turtles; and
3) Barrier opacity was not a significant predictor of time from beginning of trial to entrance into tunnel.

Assabet Barrier Laboratory

The results of tests in the Assabet Barrier Laboratory indicate that barrier opacity is a significant predictor of rate of travel in feet per minute (fpm) for painted turtles and spotted turtles. These two species moved at faster rates over the course one-hour behavioral trials when an opaque visual barrier was attached to the fence. Interestingly, tests did not show it to be a significant predictor for Blanding's turtles.

These results suggest that it may be possible to use either an opaque or translucent barrier to influence the behavior of turtles in different ways for different situations. For example, an opaque barrier could be used to swiftly direct turtles into a tunnel. Conversely, a translucent barrier could be used to dissuade turtles from moving beyond a certain point, such as where the barrier ends and access to a road surface is possible.

One-way Exclusion Gate

The following conclusion was drawn from the results of tests in the Tillson Exclusion Gate Laboratory:

- The exclusion gate that was tested appears to work well as it is intended and is a simple and straightforward means of allowing one-way passage through a barrier fence.

Tunnel Position

Tests were conducted using 40' tunnels of a single length, apertures of 2' x 2', 4' x 4', and 4' x 8', and at and below grade positions. Only a small effect of tunnel position on the behavior of turtles was observed. The total success for at-grade tunnels was slightly higher than that of below-grade tunnels at 56% and 46%, respectively.

Entrance Angle, Septum Use, and Barrier Opacity

Additional passage system variables, including entrance angle, septum, and median lighting, were tested and did not significantly affect the movement behavior of turtles in any way. It was hypothesized that there would be an increase in the number of turtles successfully completing trials when a 45° entrance was used, in contrast to a 90° entrance, because it effectively made the tunnel entrance area much wider. The septum was a wedge-shaped fence that extended from the center of the tunnel, with the function of guiding turtles into the tunnel that might otherwise bypass the opening.

Median Lighting

Tunnels were tested with a simulated roadway median lighting treatment. This was identical to the 0% treatment except for a 2' x 4' area at the midpoint of the tunnel top through which 75% of available light was transmitted. This additional treatment was intended to be analogous to a tunnel under a roadway where storm grates allow light into the tunnel's midpoint in the roadway median strip. Median lighting had no significant effect relative to 0% transmittance.

Conclusions and Recommendations

Light Level

Tunnels with the “bright” treatment performed markedly better than their closed-top counterparts or “dark” treatment. This trend was observed irrespective of aperture and length indicating that adequate lighting was critical to successful turtle passage in our experiments.

Openness Ratio (OR)

Among tunnels with the 0% available ambient lighting treatment we saw an increase in rates of successful passage as OR increased. It is important to note that a minimum OR of 0.82'or 0.25 meters is recommended in the MassDOT Stream Crossing Handbook for a box culvert, and the New England District Army Corps of Engineers require new permanent stream crossing to have an OR greater or equal to 0.82'or 0.25 meters. Although tunnels with an OR of 0.25 were not tested, tunnels with ORs of 0.2 and 0.4 were tested and successful passage rates of approximately 55% were observed.

Light Level and Additional Species

Overall, these results indicate that a tunnel with ample overhead light throughout is likely adequate to facilitate passage of tested turtles while a tunnel of the same dimensions which lacks overhead light would be ineffective. Spotted turtles were significantly more hesitant than the other two species to enter tunnels under either lighting treatment indicating that they may be inhibited by the width or length of the passage itself.

Artificial Lighting in Tunnels

Painted turtles responded favorably to the artificial lighting treatment and successfully navigated passages at rates comparable to those observed for tunnels with the 100% available ambient light treatment. This suggests that artificial lighting may be a viable means of: a) retrofitting existing tunnels and culverts that are dark and b) bringing ample light levels to small aperture closed-top tunnels. However, possible drawbacks of this technique are that it is unknown how other wildlife species might react to artificial lighting, and the maintenance of lighting may be logistically difficult.

Additional research on artificial lighting should collect data on the reliability, intensity, and timing of lighting as well as its effects on the willingness of other types of wildlife to use tunnels, since most passage systems will likely be serving many species in addition to turtles.

Barrier Opacity

The results from tests of barrier opacity suggest that it may be possible to use either an opaque or translucent barrier to influence the behavior of turtles in different ways for different situations. For example, an opaque barrier could be used to swiftly direct turtles into a culvert. Conversely, a translucent barrier could be used to dissuade turtles from moving beyond a certain point, such as where the barrier ends and access to a road surface is possible.

One-way Exclusion Gate

In tests using painted turtles, the turtle exclusion gate functioned as it was designed. All turtles tested were willing to pass through the gate, and most did so very quickly with no observed hesitancy. When facilitating turtles with a range of body sizes, care must be taken not to make a drop-off so high that smaller species or individuals are unwilling to use it.

Tunnel Position

Even though painted turtles were more hesitant to enter tunnels that were below grade, there might be situations where it is necessary to place a tunnel below grade due to the surrounding landscape topography. In these cases, it is likely that using larger tunnels or an open-top design, either of which would increase light levels, can mitigate the negative effect of embedded tunnels.

Entrance Angle and Septum

Because varying the angle of entrance from a single 90° turn to two 45° turns did not significantly affect the rate of successful trial completion, or the willingness of turtles to enter the culvert, it appears that this is not an important design element.

Median Lighting

It was hypothesized that allowing light to enter at the center of the tunnel, analogous to storm grates in a roadway median strip, might result in a higher rate of successful passage than the 0% transmitted light treatment test. However, median lighting had no significant effect relative to the 0% transmittance test. The overall mean scores of these tests across all dimensions ranged from 45% for 0% lighting treatment up to 50% for the simulated roadway median lighting. Therefore, it appears median lighting does not result in a higher rate of successful passage relative to no lighting.

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1.0 Introduction

The Tunnel and Fencing Options for Reducing Road Mortalities of Freshwater Turtles study was undertaken as part of the Massachusetts Department of Transportation (MassDOT) Research Program. The program is funded with Federal Highway Administration (FHWA) Statewide Planning and Research (SPR) funds. Through this program, applied research is conducted on topics of importance to the Commonwealth of Massachusetts transportation agencies.

Under-road passages are being increasingly employed to allow a wide range of wildlife species to move safely between habitat patches that are bisected by roadways. Passage systems are tools that have the potential to be of critical importance because roadways have become a pervasive feature of the landscape and can be a significant source of mortality for turtles.

MassDOT frequently receives requests from regulatory agencies to include wildlife crossings in roadway designs in order to reduce mortality of rare and endangered turtle species and maintain their habitat continuity. However, due to limited empirical evidence, it has been unclear what the best recommendations are regarding passage width, length, and design type.

Few studies have evaluated the effectiveness of roadway passage structures for common turtle species as well as uncommon species such as the spotted turtle (*Clemmys guttata*), and state-listed species such as the Blanding's turtle (*Emydoidea blandingii*). Tunnels and culverts that are not designed with the needs of turtles specifically in mind may be inadequate to their needs and can lead to failure in meeting the conservation goals of accommodating the safe passage of these species across roadways. Conversely, tunnels and culverts that are far larger than is required can lead to unnecessary design and construction costs.

This research was conducted with the purpose of addressing these concerns through a series of designed behavioral experiments. The movements of turtles were examined in response to a variety of light levels, tunnel sizes, tunnel entrance designs, and barrier opacities in outdoor laboratories. This report presents the results of the study, with the aim of informing the design of passage systems that are both effective and cost efficient. The implementation of these findings will allow for public resources to be used wisely, while also meeting regulatory requirements regarding endangered turtle species.

1.1 Objectives

The goal of this three-year study was to examine the effectiveness of road passage structures for freshwater turtles in Massachusetts, with an emphasis on identification of cost-effective structures that allow rare species of turtles to safely move between habitats bisected by two-lane and four-lane roadways.
Stemming from this goal, specific objectives include:

1) Evaluate variations of tunnel height, width, length, openness, and light level with regard to their influence on the movement behavior of painted turtles;
2) Evaluate variations of fence opacity, length, angle, and septum use with regard to their effectiveness in directing painted turtles through road passage structures. The septa were two sections of fence that formed a roughly wedge-shaped configuration intended to direct turtles into the tunnel entrance that might otherwise be bypassed; and
3) Evaluate select passage and fencing designs using two additional turtle species, the uncommon spotted turtle, and the state-listed Blanding's turtle.

2.0 Research Methodology

This chapter presents descriptions of the materials, methods, and procedures used in the research. Specific topics covered include: study animal source selection, collection of turtles, field laboratory site selection and characteristics, choice of experimental variables, experimental design, behavioral test procedure, behavioral analysis, and statistical methodology. Section 2.1 describes the laboratory sites and the collection of animals. Section 2.2 summarizes the design of tunnel laboratories in terms of dimensions such as length, aperture or opening size, and available overhead ambient light level and in terms of the trials performed. Section 2.3 describes the barrier and entrance configurations along with the trials conducted at the barrier testing laboratory. In total, 1,168 trials were conducted comprised of three turtle species, 12 trial groups, and six field laboratories located at three different sites.

2.1 Animals and Sites

Over the course of three field seasons from 2009 through 2011, hundreds of individual turtles encompassing three freshwater turtle species collected from a total of seven wetland sites were tested.

2.1.1 Study Animal Source Populations

Sites likely to support populations of painted turtles within a 15-mile radius of the field laboratory were identified prior to 2009, the first spring field season. As animals emerged from hibernation, accessible wetlands deemed likely to contain adequate numbers of turtles were briefly surveyed to determine the best sites for situating the field laboratories. Sites were chosen based upon accessibility, estimated size of turtle population and proximity to the field laboratory.

Populations of Blanding's turtles and spotted turtles used in passage experiments were located using the Massachusetts Division of Fisheries and Wildlife's Natural Heritage and Endangered Species Program (MDFW NHESP) database of elemental occurrences and through conversation with state biologists. Additionally, a contributing factor in selection of sites was the availability of a field laboratory site or sites in proximity to rare turtle populations.

Behavioral trials were conducted with Blanding's turtles and painted turtles captured on the Oxbow National Wildlife Refuge (NWR) in Harvard, Massachusetts. Spotted turtles were captured at the Hockomock Swamp State Wildlife Management Area in Bridgewater, Massachusetts. Following behavioral trials, turtles were returned to their points of origin.

2.1.2 Field Laboratory Sites

Field laboratories of various designs to test the response of turtles to passage system variables were constructed and utilized at three different locations. Table 1 provides a list of the field laboratory sites that were utilized, names the trial groups associated with each site, and identifies the years the sites were active. Trials were tested against each other and compared statistically within trial groups. Laboratory sites were selected in the towns of Amherst, Leverett and Sudbury.

Table 1: Field laboratories and trial groups.

Year	Field Laboratory Name	Trial Group Name
2009	Tillson Tunnel Lab	Tunnel Trial Group 1
		Tunnel Trial Group 2
		Median Lighting Trial Group
	Tillson Exclusion Gate Lab	Exclusion Gate Trial Group
2010	Leverett Barrier and Tunnel Entrance Lab	Barrier and Tunnel Entrance Trial Group
	Tillson Artificial Lighting Lab	Artificial Lighting Trial Group
2011	Assabet Tunnel Lab	Assabet Tunnel Blanding's Turtle Trial Group
		Assabet Tunnel Spotted Turtle Trial Group
		Assabet Tunnel Painted Turtle Trial Group
	Assabet Barrier Lab	Assabet Barrier Blanding's Turtle Trial Group
		Assabet Barrier Spotted Turtle Trial Group
		Assabet Barrier Painted Turtle Trial Group

With the exception of tests conducted in 2011, which involved Blanding's turtles and spotted turtles at the Assabet River NWR in Sudbury, Massachusetts, all tunnel tests and turtle exclusion gate tests on painted turtles were conducted at the Tillson Farm facility of the University of Massachusetts Amherst in Amherst, Massachusetts. The site was selected because it was easily accessible, located close to local populations of painted turtles, had ample storage space and provided the required electrical and water utilities. The Tillson Tunnel Lab, the Tillson Exclusion Gate Lab and the Tillson Artificial Lighting Lab were located at this site.

The Leverett Barrier and Tunnel Entrance Lab were located on a privately owned wooded lot in Leverett, Massachusetts. This site was selected because it is immediately adjacent to a wetland containing a painted turtle population, and the field laboratory could be oriented in a way to take advantage of the turtles' desire to escape the field laboratory and return to the wetland. The field laboratory was constructed onsite in an upland forested area close to the shore of the wetland.

The Assabet Tunnel Lab used for testing response to tunnel light level and the Assabet Barrier Lab for testing the response to barrier opacity were situated in a vacant gravel pit at the Assabet River NWR. These laboratories were used for tests on Blanding's turtles, spotted turtles, and painted turtles.

2.1.3 Collection of Turtles

All turtle species were captured using large collapsible minnow traps baited with sardines packed in soybean oil. Each year, trapping typically began in May and continued through July or early August. The trapping of Blanding's turtles began after the nesting period so as not to interfere with conservation and management of this species on the refuge. Traps were set and checked in the early morning. Bait was replaced on alternating days. Captured turtles were removed from the traps, checked for previously applied identifying shell notches and then transported to the experimental field site. Turtles were transported to the laboratory sites in 45-liter coolers to minimize the adverse effects of thermal stress and held for a maximum of 72 hours and most often less than 12 hours before being returned to the wetland in which they were captured.

On days when trials were not being run, traps were either removed from the wetland, or not activated. Each captured turtle was marked by being notched with a unique identification number (Ernst et al. 1974). This was done after they were exposed to the behavioral trials to ensure that individuals were not used for multiple trials.

All equipment that came into contact with the study animals was sanitized using a 10% bleach solution wash, given a detergent soak and a freshwater rinse and was allowed to sun dry. This procedure was intended to minimize the possibility of spreading pathogens among wetlands.

2.1.4 Covariates

In addition to the experimental variables, including data specific to the various tunnel laboratory trials (the Leverett Barrier and Tunnel Entrance Laboratory trials, and the Assabet Barrier trials) additional non-experimental covariates were tested to determine if they had any effect on the performance of the turtles in the experimental trials. The additional covariates are listed below. Additional predictors were a mixture of both categorical and continuous variables. None of the additional predictors were significant.

Categorical variables included:

1) Weather (Clear, Partly cloudy, Mostly cloudy, Overcast, Light rain, Heavy rain);
2) Age (Juvenile, Adult);
3) Sex (Male, Female, Unknown);
4) Gravid (Yes, No, Unknown); and
5) Tunnel start direction (North, South).

Continuous variables included:

1) Carapace length (millimeters);
2) Carapace width (millimeters);
3) Weight (grams);
4) Outside temperature (°Celsius);
5) Tunnel temperature (°Celsius); and
6) Trial start time (time of day in Julian time).

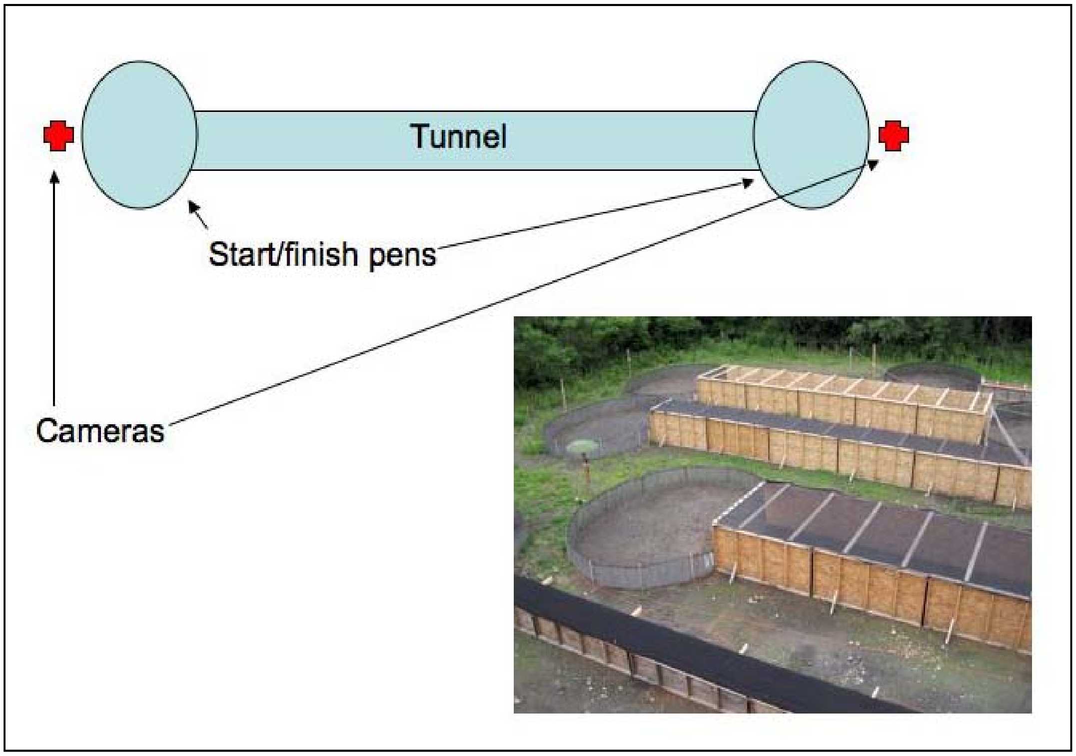
2.2 Experimental Tests of Tunnels

A number of experimental tunnels were constructed at field laboratory sites for use in a series of experiments examining the effects of tunnel variables on willingness of turtles to pass through these structures. A factorial design was used to examine the response of turtles to tunnel length, aperture or opening size, and available overhead ambient light level. The factorial experimental design was employed because it allows for the study of the effect of each factor or predictor variable on the response variable, as well as the effects of interactions between factors on the response variable.

2.2.1 Experimental Design for Tunnel Laboratory

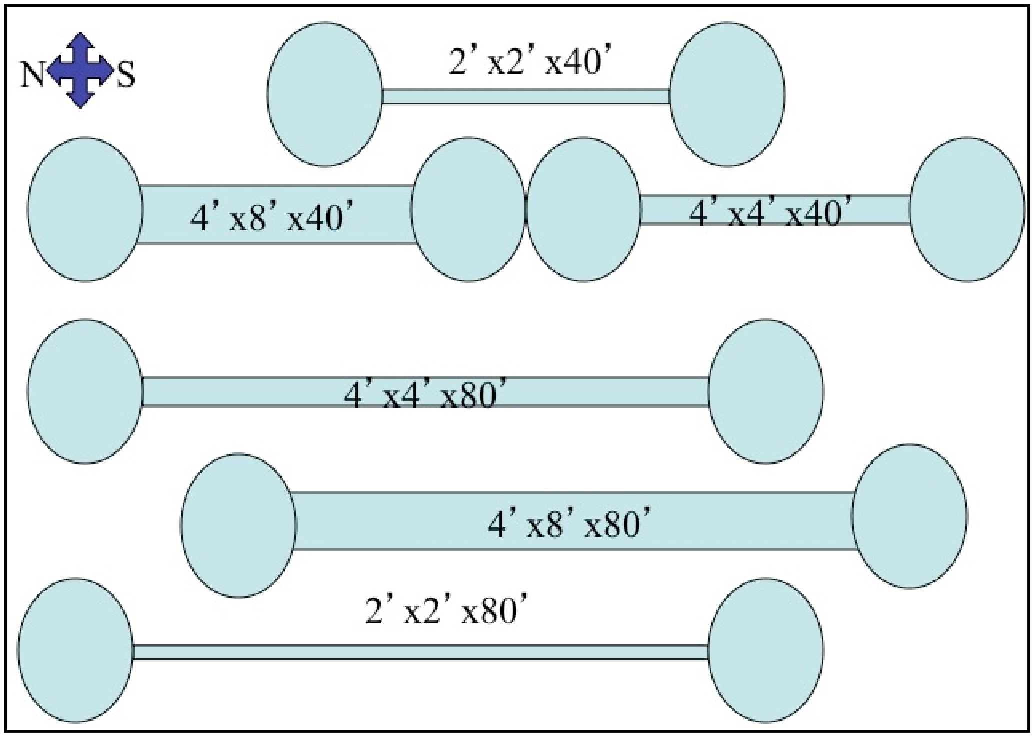
A factorial design was used to experimentally test tunnel size, length, and lighting on the passage of painted turtles. In a factorial design, a factor is the term used for a categorical predictor variable. All experimental tunnel trials used tunnel lengths of either 40' or 80', rectangular cross-section with a completely open top, with the exception of 2" x 4" cross beams placed at 4' intervals that could be covered with different materials to control the transmission of overhead light. Figure 1 depicts the experimental tunnel set up. Tunnels were always oriented north-south with the exception of the tunnel at Assabet River NWR which was oriented east-west due to site constraints. The sides of the tunnels consisted of plywood panels reinforced with 2" x 4" cross beams. The ground substrate of the tunnels was the natural soil or gravel at the site.

Figure 1: Schematic diagram of the experimental tunnel setup, and photograph depicting several of the test tunnels at the Tillson Tunnel Laboratory.



Tests encompassed three aperture or opening size treatments - 2' H x 2' W, 4' H x 4' W and 4' H x 8' W – that were crossed with two tunnel length treatments of 40' and 80', and four lighting treatments for the tops of the tunnels; including 100%, 75% and 0% available ambient light permitted, respectively, and simulated roadway-median storm drain with 75% ambient lighting. Changes were made from the initial scope of work by agreement with MassDOT regarding the size, position, and lighting level of tunnels tested in this laboratory. These changes were made to reflect what was learned as the study was being conducted so that the best combinations of variables could be examined in this research. Figure 2 is a schematic diagram of the six tunnel layouts used at the Tillson Tunnel Laboratory.

Figure 2: Schematic diagram of the tunnel laboratory layout with a total of 6 tunnels comprised of 3 size treatments.



Note: Treatments included: 2'H x 2'W, 4'H x 4'W and 4'H x 8'W, crossed with 2 length treatments of 40' and 80'.

The simulated roadway-median storm drain treatment was identical to the 0% available light treatment except that there was a 2' x 4' area at the center of the tunnel with the 75% light permitted treatment and it was only used on the three 80' tunnels. This additional treatment was intended to be analogous to a tunnel under a roadway where storm grates allow light into the tunnel at the midpoint in the roadway median strip. Tunnel sizes were selected based on the design recommendations found in the scientific literature (Jackson 2003, Woltz et al. 2008).

Enclosures attached on either end of the tunnels served as standardized start or exit pens for the trials. The enclosures were open-topped ellipses with 15' small diameters and 20' large diameters. The pen fencing was constructed of rabbit fencing 3' high and covered with landscape fabric. Rabbit fencing is made of 16 gauge welded, galvanized wire. At the bottom of the fencing, the mesh size is 4" H x 1" W, and becomes progressively larger toward the top of the fence where the maximum mesh size reaches 4" x 4". Landscape fabric is a partially opaque polyethylene fabric used in landscaping applications to block the growth of weeds in garden beds. The fabric encased fencing blocked most potential visual stressors and distractions from the surrounding environment. No food, water, or shelter was present inside the pens to ensure that turtles had some motivation for leaving the pen. The substrate of the pens was raked daily in order to remove vegetation and disrupt or eliminate any chemical trails left by turtles that were tested previously.

For tunnel tests of Blanding's turtles and spotted turtles, a single tunnel length of 80', with an opening size of 2' x 2', and two light levels (0% and 100% available ambient light transmitted from above) were selected. These two lighting treatments were paired because they represent both the poorest and best performing lighting treatments examined in the study (within a single combination of tunnel length and opening size). The great contrast in observed responses of painted turtles was used to determine whether the lighting levels were also important for the Blanding and spotted turtles. The design of this field laboratory is also a change from what was originally proposed in the scope of work. In the scope, it was anticipated that three passage structure designs would be tested on Blanding's turtles and eastern box turtles. The changes in design from three structures to just a single structure were made to reflect what was learned about the importance of light level as the study was ongoing. Spotted turtles were tested instead of box turtles because box turtles were not available due to circumstances beyond the control of the researchers. MassDOT approved all of these changes.

The decision to run single trials where only one tunnel option was available at a time to a turtle versus a choice experiment where a turtle had the choice between multiple tunnels was made because of:

1) Site limitations: A choice experiment would have only allowed the testing of four different tunnels at any given time, leading to a reduction in the number of tunnel sizes that could be tested;
2) Logistical constraints: With a choice experiment, tunnel start direction could not be randomized. Each tunnel would have had to be physically moved to another magnetic direction because there is documentation of turtles using magnetic fields to orient themselves; and
3) Experimental constraints: It would be difficult to compare one tunnel to each of the others, while maintaining a large and equal sample size. Turtles were randomly and evenly distributed across the treatments to ensure a balanced study.

The reactions of turtles to the experimental trials were assessed using three main response variables:

1) Total time to complete trial;
2) Total hesitations observed; and
3) Success.

Success was defined as completion of the trial in less than 60 minutes. Turtles that did not emerge from the tunnel after 60 minutes were not considered to have successfully completed the trial. Total time to complete a trial was the time from the start of the trial to either the turtle exiting the tunnel, or the trial being terminated due to a defined 60-minute limit.

Each of the following three behaviors was considered a hesitation:

1) Bypass - the turtle walked past the tunnel entrance without stopping;
2) Approach - the turtle walked up to the entrance, stopped, and then immediately turned around; and

3) False start - the turtle entered the tunnel, turned around, and came back out the entrance opening.

A factorial design was used to experimentally examine the effect of artificial lighting on movement behavior of painted turtles. Artificial illumination was tested in order to determine if there might be a viable alternative to an open-top design that also provides high light levels. The artificial lighting treatment was paired with the poorest performing combination of tunnel length, opening size, and lighting level so that a "rescue effect" might be observed. In the Tillson Tunnel Laboratory, a culvert with a 2' x 2' opening and a length of 80' was used to examine two overhead lighting options, 0% transmission, and fluorescent lighting. The fluorescent lighting treatment consisted of one compact fluorescent light bulb per foot strung along a closed-top tunnel. The bulbs used were 15 watt "soft white" compact fluorescent bulbs with a color temperature of approximately 2700 degrees Kelvin which is not intended to match the color temperature of natural daylight. Figure 3 and Figure 4 show a plan layout of the Tillson Tunnel Laboratory, with the diagram in Figure 3 showing the basic layout of the tunnel, the start and finish pens, and the placement of cameras used to monitor turtles during experiments. Figure 4 shows a photograph of an artificially illuminated tunnel.

Figure 3: Schematic diagram depicting the tunnel laboratory used in tests of artificial lighting.

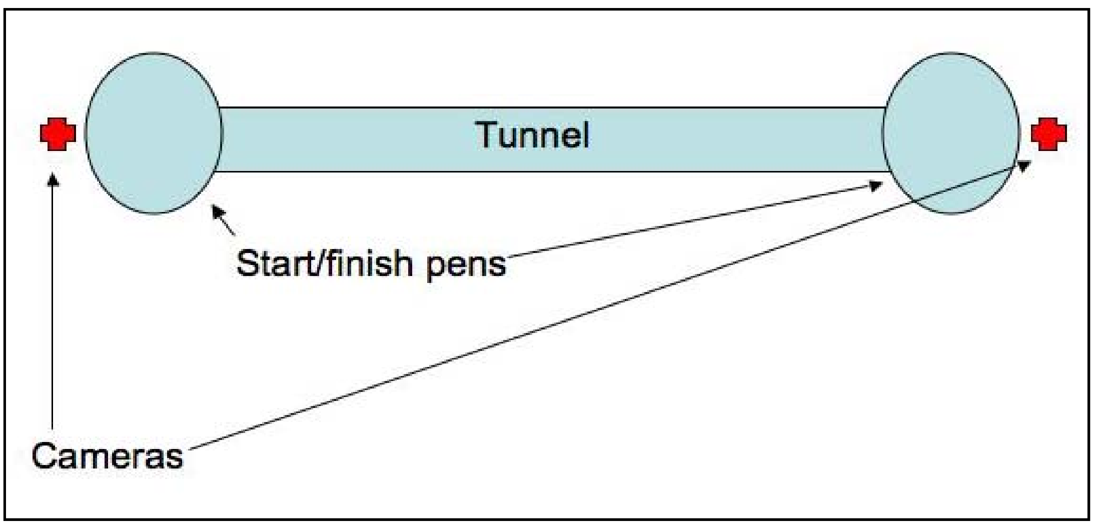


Figure 4: Photograph depicting the interior of the artificially illuminated tunnel used in experiments. In the image the fluorescent lighting is turned on.

2.2.2 Behavioral Trials in Tunnel Laboratory

Wild-caught turtles were brought to the Tillson Tunnel Laboratory and given a unique identification number. This number was written on tape that remained affixed to their carapace throughout the trials for identification purposes.

When turtles were at the Tillson Tunnel Laboratory, but not actively undergoing a trial, they were kept in holding pens. Holding pens for the turtles contained shade, water, and cover in the form of vegetation, leaf litter, and plywood hiding structures. No turtle in the experiments was allowed to go without water for more than two hours.

Randomization procedures were used to assign turtles to tunnels, determine the start direction, and select the light treatment. At the beginning of a trial, a turtle was placed in the start pen of one of six experimental tunnels. Once placed in the start pen, a turtle was given 60 minutes to complete the trial. Completion of the trial was defined as a turtle moving from the start pen through the tunnel and into the exit pen. Once the exit pen was reached or the 60-minute time limit exceeded, the turtle was removed from the trial. Turtles that did not successfully complete the trial were given a maximum time score of 60 minutes.

Behavior of the turtles in the start pen was recorded using a time-lapse trigger connected to a digital camera. The camera was elevated above the start pen and took a photo every 5 seconds for the duration of the trial. Closed-circuit video cameras were placed at the exit end of each tunnel in order to accurately determine the completion time for each turtle. Unless otherwise noted, individual turtles were only exposed to the test tunnel once in order to eliminate the effect of learning on movement rate through the tunnel.

Following a turtle's exposure to a trial, data on each individual was recorded including age, sex, gravidity (whether or not they were holding eggs), maximum carapace length, maximum carapace width, and weight. The carapace was notched using the Ernst (Ernst et al. 1974) notch code system. The notch code number was the turtle's identification number for the experimental trial. In addition to notches, photographs were taken of each turtle's carapace and plastron as an added means of identification. At the end of a day of trials, all turtles were released at their point of capture.

2.2.3 Behavioral Analysis of Tunnel Laboratory Trials

Total time to complete a trial, total hesitations, and success were used as response variables in evaluating turtles in the tunnel laboratory. Total time to complete a trial was the time from the start of the trial to either the turtle exiting the tunnel, or when the trial reached its limit of 60 minutes. Turtles that exited the tunnel in under 60 minutes were considered to have successfully completed the trial. This response was recorded in minutes and confirmed by comparing the start time and end time of each trial. A two-factor analysis of variance (ANOVA) was used as the statistical model. An ANOVA provides a statistical test of whether or not means of several groups are all equal and is a widely used test for comparing two, three, or more means.

Total hesitations were defined as the pooled number of bypasses, approaches, and false starts observed during the trial. These data were collected through review of the time-lapse photos generated from the camera positioned in the start pen. A two-factor ANOVA was used to model the total number of hesitations observed in the trial.

Success was measured using completion and hesitations data. Data were managed using Microsoft Excel software, and analyzed using R, a free software environment for statistical analysis and graphics. The most commonly used significance level, an alpha level of 0.05, was set for all statistical tests.

For analysis purposes, the 2009 painted turtle trials were divided into two groups. Tunnel Trial Group 1 included all combinations of passage opening size, length, and lighting as presented in Table 2 with the exception of the simulated roadway median lighting treatments. Tunnel Trial Group 2 utilized passages of just one length, 80', all three opening sizes, and simulated roadway median lighting or 0% ambient light transmission as presented in Table 3. The Artificial Lighting Trial Group was tested at the Tillson Artificial Lighting Lab in 2010. Sample sizes for this trial group are presented in Table 4.

Table 2: Tunnel Trial Group 1 – Number of turtles used in each treatment combination.

Tunnel Dimension	Percent Ambient Light Transmitted from Above		
Height (ft) x Width (ft) x Length (ft)	100%	75%	0%
2 x 2 x 40	25	25	27
4 x 4 x 40	26	26	25
4 x 8 x 40	25	25	27
2 x 2 x 80	25	25	24
4 x 4 x 80	25	27	24
4 x 8 x 80	28	30	25
All Dimensions	154	158	152
Total number of turtles		464	

Note: Treatment combinations encompassed three light levels, three tunnel apertures and two tunnel lengths.

Table 3: Tunnel Trial Group 2 – Number of turtles used in each treatment.

Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Simulated Roadway Median Lighting
2 x 2 x 80	28	24
4 x 4 x 80	27	29
4 x 8 x 80	28	25
All Dimensions	83	78
Total number of turtles	161	

Note: Treatment combinations encompassed three light levels, three tunnel apertures and two tunnel lengths.

Table 4: Artificial Lighting Trial Group – Number of turtles used in each treatment.

Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Artificial
2 x 2 x 80	31	40
Total number of turtles	71	

Akaike Information Criteria (AIC) were used to select the best models for both analysis groups. AIC is a measure of the relative goodness of fit of a statistical model and AIC values provide a means for model selection. The response variable was time to complete the trial, but because the trial involved comparing tunnels of two different lengths in Tunnel Trial Group 1, time in minutes to complete a trial was converted to a rate of feet per minute (fpm) to facilitate analysis.

Tukey's test was used to further examine factors deemed significant by the ANOVAs and determine which groups were significantly different from one another. Tukey's test is a single-step multiple comparison procedure and statistical test commonly used in conjunction with an ANOVA to determine what means are significantly different from one another. Data were managed using Microsoft Excel software, and analyzed using R, a free software environment for statistical analysis and graphics. The most commonly used significance level, an alpha of 0.05, was set for all statistical tests.

2.3 Experimental Tests of Barriers and Tunnel Entrance Variables

Two experimental arenas were constructed at two different sites for use in a series of experiments examining the effects of barrier opacity and tunnel entrance variables on turtle movement and willingness of turtles to enter a tunnel. In the Leverett Barrier and Tunnel Entrance Lab, the response of painted turtles to tunnel entrance angle, septum presence, and barrier opacity was examined. In the Assabet Barrier Laboratory, the effect of barrier opacity on the movement behavior of spotted turtles, Blanding's turtles, and painted turtles was examined.

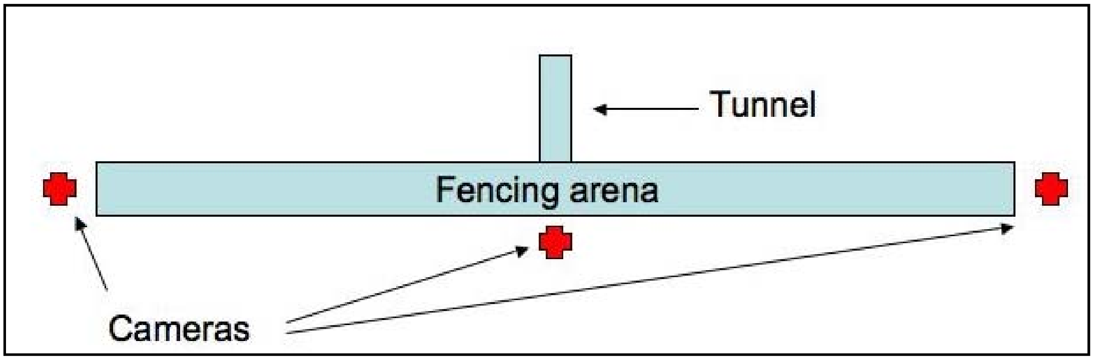
2.3.1 Experimental Design for Tunnel Entrance and Barrier Laboratory

A factorial design was used to examine the effect of tunnel entrance characteristics and barrier opacity on movement behavior of 170 painted turtles. For these trials, eight unique combinations of tunnel entrance angle, septum presence, and barrier opacity were evaluated with respect to the movement behavior of painted turtles.

The field laboratory consisted of a single tunnel and a large rectangular pen as shown in Figure 5. The tunnel was rectangular in cross section, with an open top that could be covered with different materials to change the lighting treatment. The tunnel measured 4' H x 4' W x

36' L and was identical to what was described for the artificial lighting laboratory except it differed in dimensions and entrance/exit design.

Figure 5: Schematic diagram depicting the layout of the barrier and tunnel entrance field laboratory.



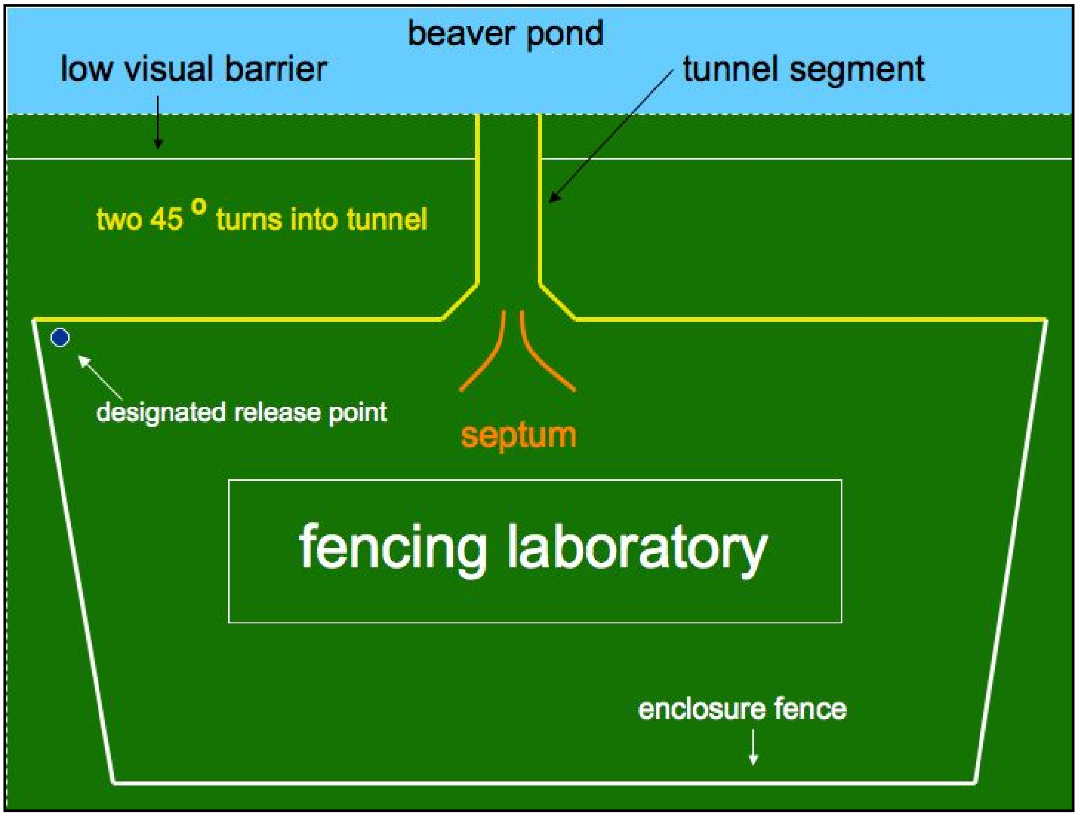
Note: The diagram shows the basic layout of the fencing pen, tunnel and the placement of cameras used to monitor turtles during experiments.

The rectangular-shaped pen measured 180' x 16' and was oriented so that its longest side was parallel to the wetland and oriented approximately north to south. The fence was supported by wooden stakes and was made of chicken wire fence with a 1" x 1" mesh size on the north, east, and south sides and a silt fence of the type commonly used to control sediment runoff on construction sites on the fourth side to block visibility. The ground substrate of the pen and tunnel consisted of existing leaf litter and soil found at the site. Large woody debris and vegetation that may create an obstacle to movement were removed. The remaining substrate was primarily pine needles.

It was possible to modify three sides of the fence in terms of how much visibility turtles potentially had into the area outside of the pen. These three sides of the pen had a view of the wetland, or were influenced by the open area of the wetland. A removable 1' visual barrier, designed to prevent turtles from being able to see directly through the chicken wire barrier, but still allowing for light from the open area of the wetland to influence their movement, could be added to those three sides. The visual barrier was constructed of tarpaper and was mounted against the fence on the outside of the pen to avoid physical contact with the turtles.

At the entrance end, the angle of entrance relative to a turtle's path along the barrier could be alternated between one of two options: 1) two 45° turns, or 2) a single 90° turn as depicted in Figure 6. Entrance angles could be changed by using removable tunnel wall panels, which were mounted against the stationary main tunnel by means of wooden stakes attached to the panels that fit into vertical pipes sunk into the earth. With either set of entrance angle panels in place, the total length of the tunnel was 40'. The easily removable septa were mounted into the ground using the same wooden stake/pipe method and were placed at the mouth of the tunnel entrance. The panels used for the 45° entrance angle were only 1' tall so as not to alter the amount of light the turtles perceived to be coming from the direction of the wetland.

Figure 6: Schematic diagram of fencing field laboratory in the context of its location immediately adjacent to a wetland.



Note: In this figure, the designated release point for test subjects is clearly labeled. Additionally, the laboratory is depicted with the 45° angled entrance and the septum in place.

Figure 7: Photograph of the tunnel entrance area of the barrier and entrance field laboratory. It is shown here with the opaque visual barrier, entrance, and the septa installed.

The septa were constructed of 3' H chicken wire attached to 4' L wooden stakes. Chicken wire is a 20 gauge galvanized wire fence with a one-inch mesh size. Each septum was arc-shaped and spaced at a distance of 14" apart at the tunnel entrance and 8' apart at their farthest point from the tunnel entrance. The septa extended 1' beyond the barrier fence into the “mouth” of the tunnel and another 6.5' from the barrier fence into the center of the pen. When viewed together the septa formed a roughly wedge-shaped configuration. This configuration was designed to direct turtles into the tunnel entrance that might otherwise bypass it, by forcing them to reorient at the entrance to the tunnel.

The exit end of the tunnel featured a platform that extended out over the water another 4' beyond the tunnel itself with rabbit fence to prevent turtles from escaping into the wetland upon exiting the tunnel. The same natural substrate that was in the tunnel was used on this platform as well.

2.3.2 Behavioral Trials in Tunnel Entrance and Barrier Laboratory

At the beginning of a trial, a turtle was placed in either the northeast or southeast corner of the experimental fencing arena. Once a turtle was placed in the arena, it was given 60 minutes to complete the trial. Completion of the trial was defined as a turtle navigating through the tunnel and into the exit pen. Once a turtle reached the exit pen or exceeded the

60-minute time limit, it was removed from the trial. If a turtle did not successfully complete the trial, it was given a maximum time score of 60 minutes. Behavior of the turtles in the vicinity of the tunnel entrance was recorded using a digital camera connected to time-lapse trigger. The camera was elevated above the start pen and took a photo every 5 seconds for the duration of the trial. Closed-circuit video cameras were placed at various locations around the laboratory and were used in conjunction with direct visual observation to document and record the locations of turtles at short intervals throughout the trial. No food, water or shelter was present inside the pens to ensure that turtles had motivation to leave the pen. Substrate of the pens was raked daily in order to remove vegetation and reduce any chemical trails left by turtles that were tested previously.

2.3.3 Behavioral Analysis of Tunnel Entrance and Barrier Laboratory Trials

Behavioral responses of turtles were assessed as:

1) Success/failure to complete trial in 60 minutes;
2) Time to complete the trial; and
3) Mean times from trial beginning to when turtles entered the tunnel.

Data were collected by direct observation and by reviewing the time-lapse photos generated from the camera positioned in the start pen. The camera in the start pen was positioned in such a way that turtles could be seen at either end of the tunnel.

Success was measured using completion data. Turtles that exited the tunnel in under 60 minutes were considered to have successfully completed the trial. This response was recorded in minutes and confirmed by comparing the start time and end time of each trial. A two-factor ANOVA was used as the statistical model. Data was managed in a Microsoft Excel spreadsheet. Statistical analyses were conducted using R. An Alpha level of 0.05 was set for all statistical tests.

Total time to complete a trial was the time from the start of the trial to either the turtle exiting the tunnel, or when the trial reached its allowed completion time of 60 minutes. Turtles that navigated the fencing laboratory and exited the tunnel in under 60 minutes were considered to have successfully completed the trial. This response was recorded in minutes and confirmed by comparing the start time and end time of each trial. An ANOVA was used as the statistical model.

Mean times from trial beginning to when turtles entered the tunnel were recorded by direct observation of turtle locations at regular intervals during trials. A T-test was used to examine whether there was a significant effect of barrier opacity on the mean times from trial beginning to when turtles entered the tunnel. The T-test is a commonly used statistical measure to determine if a mean is significantly different from the null or normally distributed mean. This analysis included only finished trials where the turtles entered the opening and completed the trial.

2.3.4 Experimental Design for Barrier-only Laboratory

A large square pen was used to experimentally examine the effects of barrier opacity on the movement behavior of spotted turtles, Blanding's turtles, and painted turtles. The pen measured 50' x 50' with a chicken wire fence perimeter of the same specifications as the tunnel entrance and barrier lab including the same type of opaque visual barrier to create two levels of fence opacity, 0% and 100%. Figure 8 depicts a schematic of the barrier-only laboratory and photographs of the laboratory both with, and without the opaque visual barrier in place.

Figure 8: Schematic of barrier-only laboratory and photographs illustrating the two fence treatments, which were with and without an opaque visual barrier.

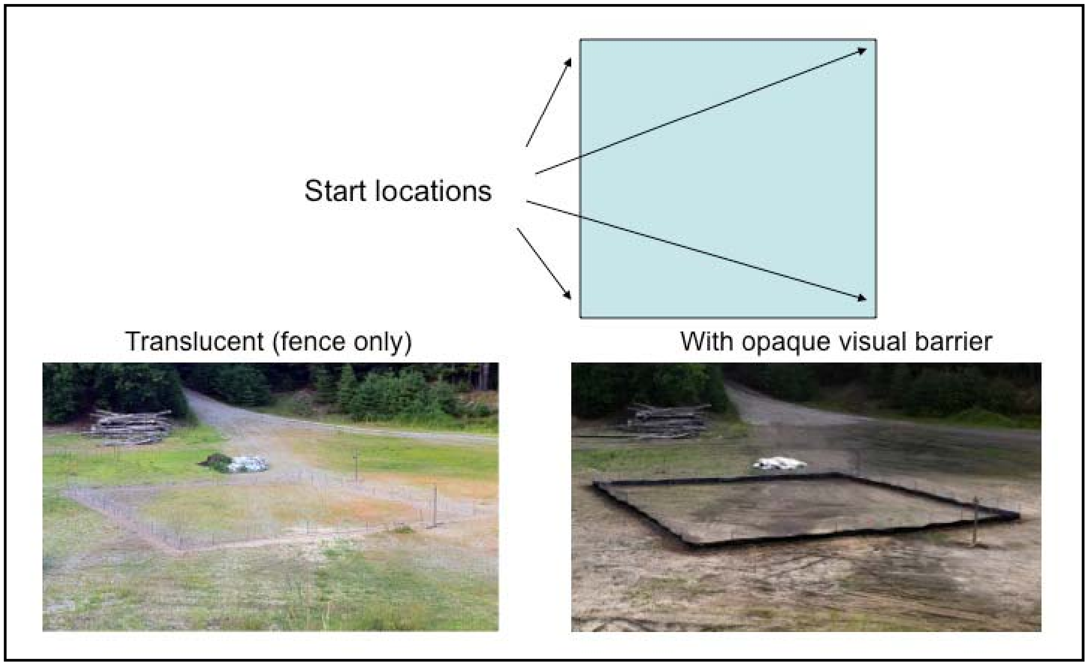


Table 5: Assabet Barrier Lab – Number of turtles used in each treatment combination.

		Visual Barrier	
		Y	N
Species	Painted turtle	18	14
	Blanding's turtle	27	23
	Spotted turtle	24	25
Total number of turtles		131	

Note: The treatment combinations encompassed three species and two visual barrier treatments.

2.3.5 Behavioral Trials in Barrier-only Lab

The experimental procedure for the barrier only laboratory was the same as the procedure for the barrier and tunnel entrance laboratory with the following exceptions:

1) Barrier opacity was the only variable under manipulation;
2) In this laboratory turtles were randomly placed in one of the four corners at the start of the trial instead of just two corners; and
3) The trial length was always exactly 60 minutes and the locations of turtles during the trial were consistently recorded every two minutes by the experimental observer. The duration was consistently 60 minutes because there was no way for turtles to finish earlier in this field laboratory by exiting the arena or "completing" the trial in any way.

2.3.6 Behavioral Analysis of Barrier-only Lab Trials

Behavioral responses of all three turtle species tested in the barrier-only lab were assessed using an approach similar to that used in the tunnel entrance and barrier laboratory.

Response was assessed as: The rate of travel by turtles along the barrier.

Data were collected by direct observation at regular two-minute intervals and by remote monitoring using closed circuit video cameras strategically positioned around the pen.

A rate of travel in feet per minute (fpm) was calculated for each trial. To calculate this measure, the number of times a turtle visited one of the four sides of the pen during a trial was tallied. A visit to a side was tallied anytime a turtle left one of the four sides and went either directly to another side or went into one of the middle pen quadrants and then went back to a side again. Turtles that did not leave the start corner were excluded from analysis.

For each trial, the number of visits to the pen sides was multiplied by 50, because each pen wall was 50' long. This generated a rough measure of total distance traveled that is most likely an overestimate of total distance traveled during a trial but is useful nonetheless for analysis. To calculate fpm, the estimate of distance traveled was divided by the number of minutes sampled.

2.3.7 Experimental Design for Turtle Exclusion Gate

A subset of turtles from the artificial lighting experiment were used to test a one-way turtle exclusion gate designed to allow turtles to easily pass in or out of an area but only in one direction. Figure 9 is a photograph of a turtle exclusion gate of the same design with two minor exceptions. The fence material depicted here is chain-link rather that chicken wire, and the base of the gate is granite rather than wood.

Figure 9: Photograph depicting a turtle exclusion gate in Groton MA. The design of this gate is similar to that of the gate tested at the Tillson Exclusion Gate Field Laboratory.

Turtle behavior in the exclusion gate laboratory was assessed as:

1) Yes/no the animal crossed the gate in the intended direction; and
2) Yes/no the animal subsequently returned and crossed the gate in the unintended direction.

The exclusion gate laboratory was constructed at the Tillson Farm facility and consisted of a 10' x 10' silt fence pen divided into two sections by a chicken wire fence with a “gate” in the middle. The gate was a 2' break in the fence, with a 1' drop-off, made possible by situating the structure on a gently sloping hill with the lower portion excavated to produce the drop-off. Water and shade were provided on both sides of the chicken wire fence.

2.3.8 Behavioral Trials in Exclusion Gate Laboratory

At the beginning of a trial, a group of 10-12 painted turtles was placed together in the upper level of the experimental arena. Once placed in the arena, the turtles were given 60 minutes to complete the trial. Completion of the trial was defined as a turtle navigating through the gate and into the lower half of the arena. Turtles were allowed to remain in the arena for the full 60-minute duration even after all individuals had passed through the gate. This was done to ensure that the gate was indeed a one-way passage and turtles were not able to return to the upper level of the experimental arena through the gate. Behavior of the turtles in the vicinity of the gate was monitored with direct visual observation recording the locations of turtles at short intervals throughout the trial.

2.3.9 Behavioral Analysis of Exclusion Gate Trial

The success of the exclusion gate was analyzed by counting the number of turtles that went through the gate and were not able to return through it, in the allotted time. Exclusion gate trials were analyzed using a T-test with an Alpha of 0.05.

3.0 Results

A number of informative findings regarding the design of effective road passages for freshwater turtles were made during this study. Among the most noteworthy are those that relate to tunnel lighting level, tunnel aperture, tunnel length, and barrier opacity.

3.1 Results of Tunnel Laboratory Tests

The results from tunnel laboratory tests reported here provide indication as to the effects of tunnel variables on the willingness of turtles to pass through these structures.

3.1.1 Results of Tunnel Laboratory Tests of Size and Ambient Lighting

From May 22 to July 22, 2009, 625 painted turtles were tested in the Tillson Tunnel Laboratory. Tests encompassed three aperture or opening size treatments - 2' H x 2' W, 4' H x 4' W and 4' H x 8' W – that were crossed with two tunnel length treatments of 40' and 80', and four lighting treatments for the tops of the tunnels; including 100%, 75% and 0% available ambient light permitted, respectively, and simulated roadway-median storm drain with 75% ambient lighting.

The population of painted turtles involved in the trials was separated into two groups. Tunnel Trial Group 1 consisted of 464 turtles and included all of the aforementioned variables except for the simulated roadway-median lighting treatment, as is presented in Table 2. Tunnel Trial Group 2 consisted of 161 turtles which were tested in three aperture or opening size treatments - 2' H x 2' W, 4' H x 4' W, and 4' H x 8' W - a single length treatment of 80', and two lighting treatments, 0% available ambient light permitted and simulated roadway-median, as is presented in Table 3.

For Tunnel Trial Group 1, results from the ANOVA indicated that tunnel length and percent ambient light transmitted were significant predictors ($P<0.05$) of the movement rate of turtles through the tunnels. Tukey's test showed that turtles move faster through longer tunnels ($P<0.05$), and tunnels that had better lighting at 75% or 100% as compared to 0% ($P<0.05$). Of the 464 turtles in Tunnel Trial Group 1, 357 successfully completed the trial. Data on Tunnel Trial Group 1 are presented in Table 6, which lists the percent of successful trials out of the total for each combination of tunnel dimensions and lighting treatment. The highest rates of successful passage were observed for the tunnels subjected to the 100% available ambient lighting treatment. Among tunnels subjected to the 0% available ambient light treatment, passage rates were reduced with a decrease in tunnel aperture and an increase in tunnel length.

Table 6: Tunnel Trial Group 1 – Percentage based on the number of turtles that successfully completed the trial in 60 minutes for all 464 turtles tested.

Tunnel Dimension	Percent Ambient Light Transmitted from Above		
Height (ft) x Width (ft) x Length (ft)	100%	75%	0%
2 x 2 x 40	100%	100%	56%
4 x 4 x 40	92%	81%	60%
4 x 8 x 40	96%	96%	70%
2 x 2 x 80	88%	92%	31%
4 x 4 x 80	88%	81%	54%
4 x 8 x 80	79%	77%	52%
All Dimensions	90%	87%	53%
Overall		77%	

Note: Percentage success is shown for each treatment combination encompassing three light levels, three tunnel apertures and two tunnel lengths.

For turtles in Tunnel Trial Group 2, results from an ANOVA indicated that tunnel width had a significant positive effect on passage rate of turtles (P-value of <0.05 ($P<0.05$)), F-statistic of 4.77 ($F=4.77$), and 2 degrees of freedom ($df=2$), with turtles moving faster through 4' W and 8' W tunnels compared to 2' W tunnels ($P<0.05$). Of the 161 turtles in the Tunnel Trial Group 2, 76 successfully completed the trial. Data on the Tunnel Trial Group 2 are presented in Table 7, which lists the percent of successful trials out of the total for each combination of tunnel aperture and lighting treatment. Rates of successful passage did not differ significantly between the two lighting treatments and rates of successful passage for both lighting treatments increased as tunnel aperture was enlarged.

Table 7: Tunnel Trial Group 2 – Percentage success based on the number of turtles that successfully completed the trial in 60 minutes for all 161 turtles tested.

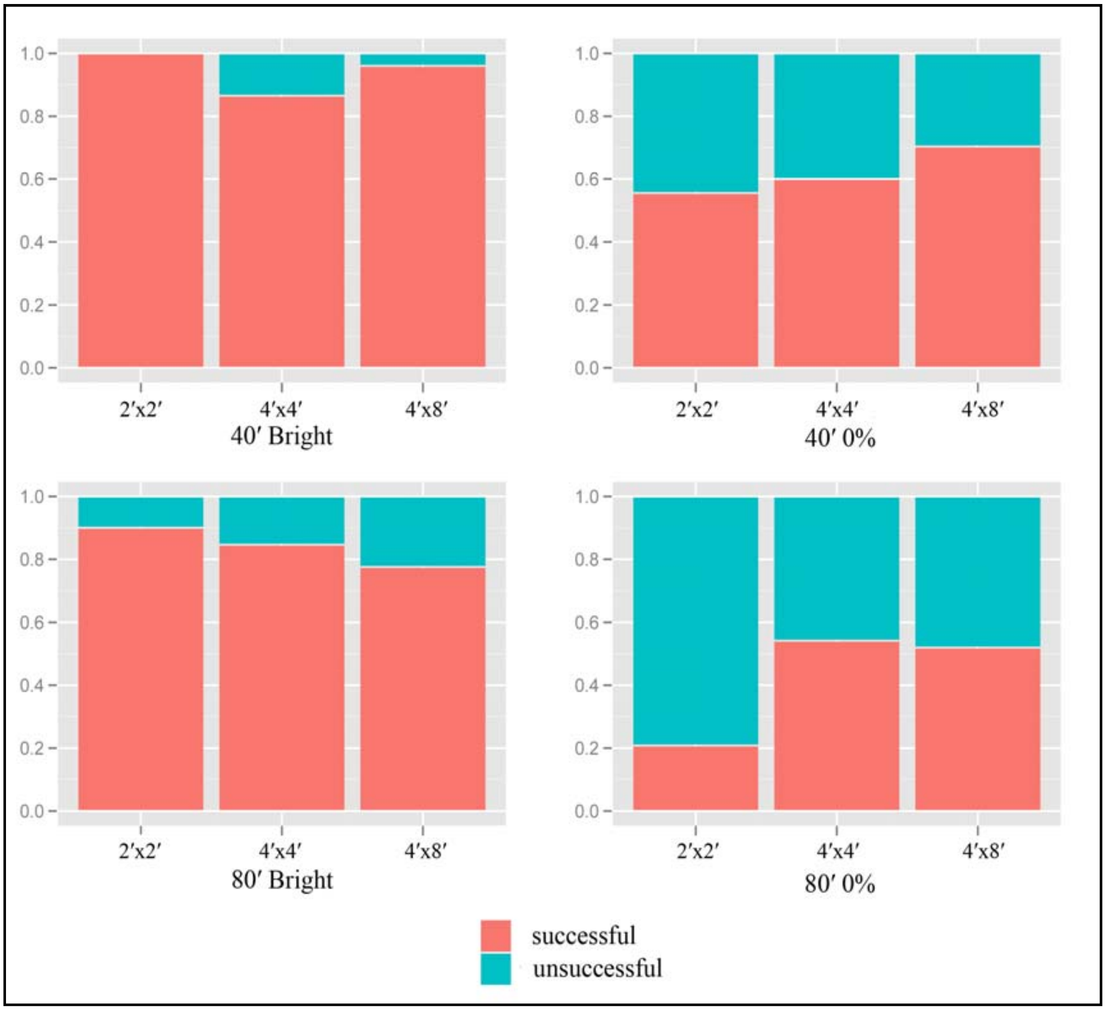
Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Simulated Roadway Median Lighting
2 x 2 x 80	29%	33%
4 x 4 x 80	52%	55%
4 x 8 x 80	54%	60%
All Dimensions	45%	50%
Overall	47%	

Note: Percentage success is shown for each treatment combination encompassing two light levels, three tunnel apertures and a single tunnel length.

Light level was an important predictor of passage and successful passage was positively associated with an increase in the level of available ambient light permitted to enter tunnel tops. In our experiments, rates of successful passage differed dramatically between the "bright" defined as pooled 100% and 75% available overhead light and "dark" defined as 0% available overhead light treatments.

Figure 10 graphically presents rates of passage grouped by available ambient light level and tunnel length amongst painted turtles. For bright tunnels, successful passage rates were high, ranging from 80% to 100%. For tunnels with the dark treatment, successful passage rates were more variable and ranged between 31% and 70%. Among the dark tunnels, the smallest aperture and longest length tunnel (and thereby darkest) performed the worst, while the largest aperture and shortest length tunnel (and thereby brightest) performed the best.

Figure 10: Tunnel Trial Group 1.

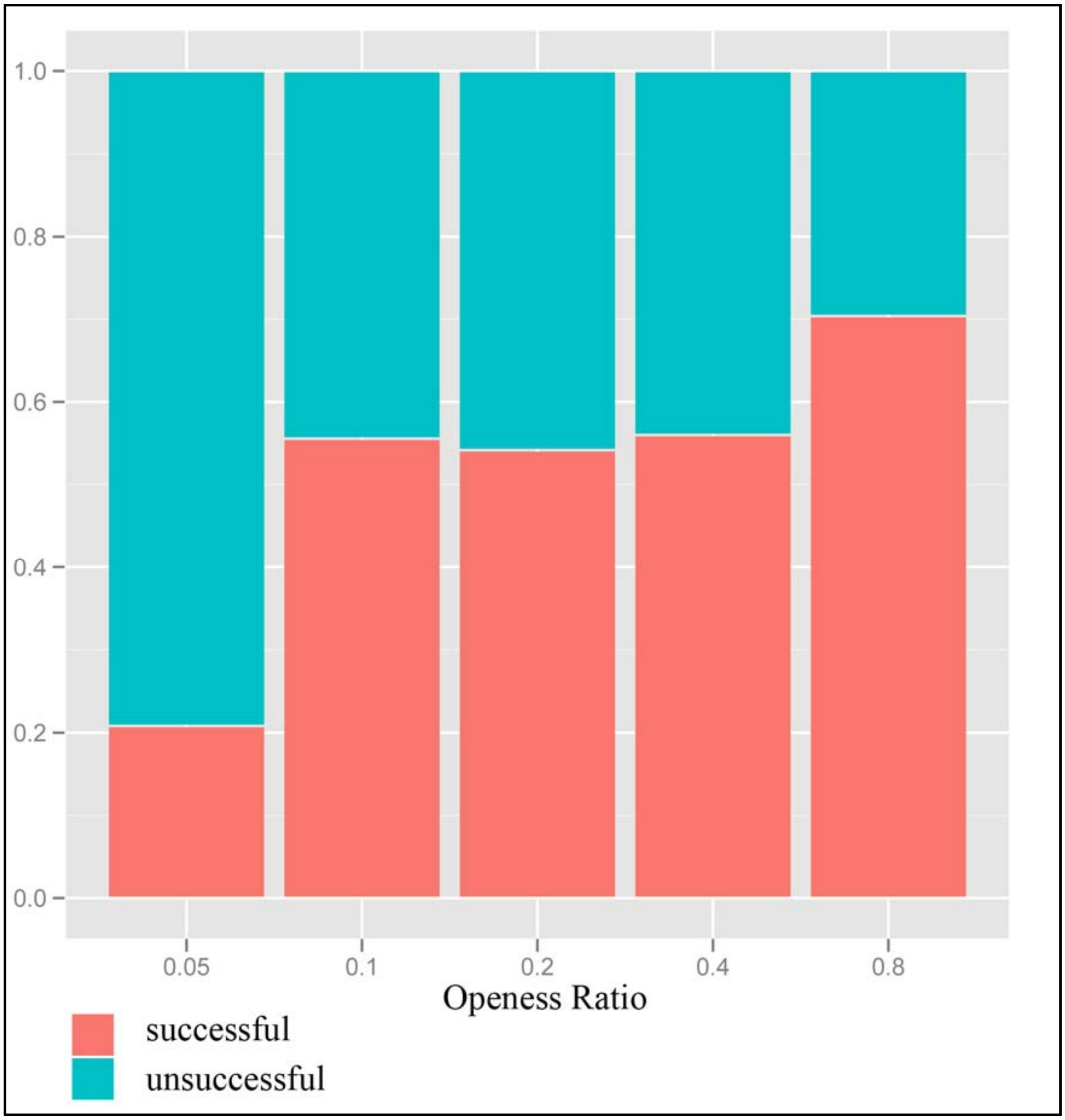


The bar graphs in Figure 10 give rates of passage success amongst painted turtles grouped by available ambient light level and tunnel length. Light levels are “Bright” (pooled 100% and 75%) and 0%. Tunnel lengths are 40' and 80'. The y-axis ranges from 0 – 1 and depicts the fraction out of the total number (n) for each combination of tunnel aperture, tunnel length, and lighting level that were either successful or unsuccessful trials. Sample sizes: (40' “Bright”: 2' x 2' n=50, 4' x 4' n=52, 4' x 8' n=50) (40' 0%: 2' x 2' n=27, 4' x 4' n=25, 4' x 8' n=27) (80' “Bright”: 2' x 2' n=50, 4' x 4' n=52, 4' x 8' n=58) (80' 0%: 2' x 2' n=24, 4' x 4' n=25, 4' x 8' n=25).

For turtles in Tunnel Trial Group 1, rates of successful passage increased as the openness ratio (OR) increased. The OR is defined as a culvert’s cross-sectional area divided by its length (OR = x-sec area/length) and is a commonly used measure in the wildlife passage literature used to give an indication of the degree to which a tunnel or culvert is more or less an enclosed or open space independent of the scale of the structure. Figure 11 graphically presents the rates of passage amongst painted turtles grouped by five levels of the OR encompassing all tested passage lengths and apertures. The OR was a significant predictor of passage only at the 0% available overhead light level. At the two extremes, for ORs of 0.05'

(80' x 2' x 2') and 0.8' (40' x 4' x 8' tunnel), successful passage rates ranged widely from approximately 20% to 65%, respectively. These results are useful because they provide some indication of how different degrees of openness influence the use of passage structures by turtles.

Figure 11: Tunnel Trial Group 1.



The bar graph in Figure 11 gives rates of passage success amongst painted turtles grouped by five levels of openness ratio (OR) in feet (0.05' n=24, 0.1' n=27, 0.2' n=25, 0.4' n=50, 0.8' n=27) encompassing all tested tunnel lengths and apertures. N is the number of turtles tested for each openness level. The y-axis ranges from 0 – 1 and depicts the fraction out of the total N for each OR tested that were either successful or unsuccessful trials.

3.1.2 Results of Tunnel Laboratory Tests of Artificial Lighting

From June 21 to August 6, 2010, 71 painted turtles were tested in the artificial lighting laboratory. This group of trials is referred to as the Artificial Lighting Trial Group. Of those, 45% successfully completed trials for the 0% lighting treatment and 78% successfully completed trials for the artificial lighting treatment. Results of the tests are presented in Tables 8, 9, 10, and 11. Table 8 gives the percentage of successfully completed trials, Table 9 gives the median times to complete trials, Table 10 gives the mean times to complete trials, and Table 11 gives the mean rates of travel for all successfully completed trials.

Successful completion of trials was analyzed using a Chi-square goodness of fit test. Results were found to be significantly different than random, meaning light level affected the likelihood of a turtle completing a trial ($\chi^2 = 6.54$, df = 1, $P = 0.011$). Table 8 displays the success rates for these trials and clearly illustrates the dramatically higher rates of successful passage observed for artificially illuminated tunnels compared to tunnels subject to the 0% lighting treatment.

Table 8: Artificial Lighting Trial Group – Percentage success based on the number of turtles that successfully completed the trial in 60 minutes or less for all 71 turtles tested.

Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Artificial
2 x 2 x 80	45%	78%
Overall	63%	

Note: Percentage success is shown for both the 0% and artificial lighting treatments.

The total trial time was analyzed with an ANOVA and results indicated that light level was a highly significant predictor of total trial time ($P<0.0001$, $F=16.74$, df=1). Table 9 gives median total trial times in minutes. Table 10 gives mean total trial times in minutes. Both tables illustrate the pattern of shorter total trial times observed for artificially illuminated tunnels in comparison to tunnels subject to the 0% lighting treatment.

Table 9: Artificial Lighting Trial Group – Median (SD) times in minutes to complete the trials for all 71 turtles tested.

Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Artificial
2 x 2 x 80	60 (17.40)	20.5 (19.68)
Overall	32 (20.72)	

Note: Median times are listed for each combination of variables followed by the standard deviation (SD) in parentheses. This is shown for both the 0% and artificial lighting treatments.

Table 10: Artificial Lighting Trial Group – Mean (SD) total times in minutes for turtles that completed the trial.

Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Artificial
2 x 2 x 80	29.07 (9.98)	18.32 (9.98)
Overall	21.67 (11.52)	

Note: Mean times are listed for both the 0% and artificial lighting treatments and are followed by the standard deviation (SD) in parentheses.

Hesitations were analyzed with an ANOVA and results indicated that total hesitations were significantly influenced by light level. Turtles were more hesitant to enter tunnels with the 0% lighting treatment. This was the case when successfully completed and unsuccessfully completed trials were examined as a pooled group ($P<0.001$, $F=21.19$, $df=1$) as well as for the subset of successfully completed trials only ($P<0.05$, $F=6.25$, $df=1$).

Among successfully completed trials, light level was not a significant predictor of rate of travel in the tunnel ($P<0.41$, $F=0.69$, $df=1$). Table 11 gives the mean rates of travel and shows that among turtles that completed trials, rate did not significantly differ between the two lighting treatment.

Table 11: Artificial Lighting Trial Group – Mean (SD) rates of travel in feet per minute (fpm) for all 43 turtles that successfully completed trials.

Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Artificial
2 x 2 x 80	12.38 (4.49)	11.43 (6.29)
Overall	11.43 (5.80)	

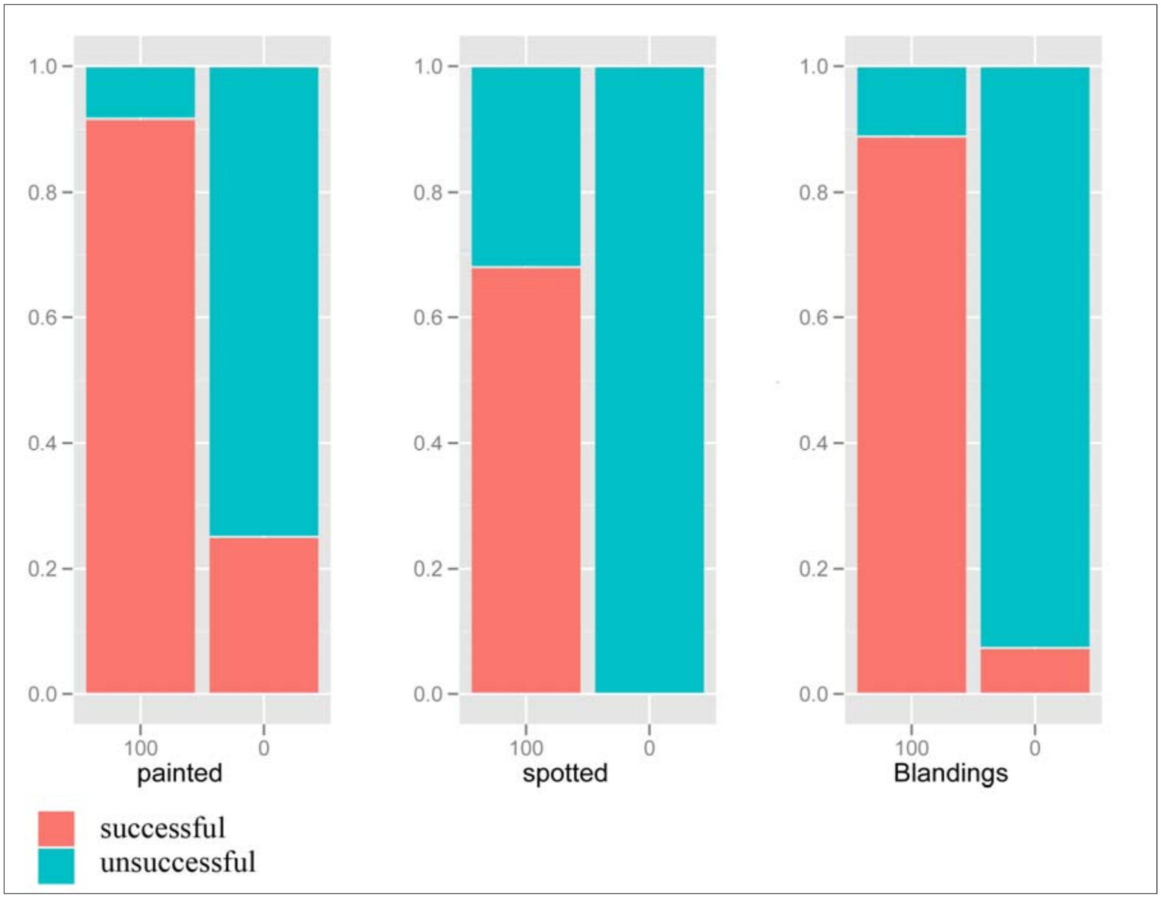
Note: Mean times are listed for both the 0% and artificial lighting treatments and are followed by the standard deviation (SD) in parentheses.

3.1.3 Results of Tunnel Laboratory Tests of Lighting Level on Additional Species

All three tested species responded poorly to the 0% available light treatment. These trial groups were the Assabet Tunnel Blanding's turtle Trial Group, Assabet Tunnel spotted turtle Trial Group, and the Assabet Tunnel painted turtle Trial Group. Figure 12 provides a visual depiction of success rates broken up by species for both lighting treatments that were tested. Only 30% of painted turtles successfully passed through the tunnel given this treatment and both Blanding's and spotted turtles were either extremely reluctant or just unwilling to pass through the dark tunnel with only 8% and 0% passage rates, respectively.

The majority of both painted and Blanding's turtles tested were willing to use the tunnel with the 100% available light treatment with an 89% successful rate of passage. However, passage rates for spotted turtles were not as favorable at a 68% successful rate of passage.

Figure 12: Assabet Tunnel Lab - Rates of passage success among 3 turtle species (painted turtles n=20, spotted turtles n=50, and Blanding's turtles n=53) grouped by available ambient light level (100% & 0%).



Note: N is the number of turtles of each species tested. The y-axis ranges from 0-1 and depicts the fraction out of the total N for each combination of species and lighting level that were either successful or unsuccessful trials.

3.2 Results of Barrier and Tunnel Entrance Variables

In this section, the results of barrier and tunnel entrance laboratory tests are reported. These results provide some understanding of the effects of barrier opacity on the movement behavior of turtles, as well as the effects of tunnel entrance variables on the willingness of turtles to enter and pass through these structures.

3.2.1 Results of Tunnel Entrance and Barrier Laboratory Tests

From June 21 to August 5, 2010, the 190 painted turtles in the Barrier and Tunnel Entrance Trial Group were tested in the Leverett Barrier and Tunnel Entrance Lab in a factorial experimental design to examine the effect of eight unique treatment combinations of tunnel entrance angle, septum use, and barrier opacity on movement behavior. The number of turtles tested in each treatment is presented in Table 12. Of the 190 individuals, 92 successfully navigated the arena to complete the trial, 27 were removed after 60 minutes, and 51 were disqualified before 60 minutes due to one or more reasons. Disqualified individuals were not included in the analyses.

Table 12: Barrier and Tunnel Entrance Trial Group – Number of turtles used in each treatment encompassing the field lab with two different entrance angles, with and without a septum and with and without a visual barrier in place.

		Entrance Angle	Visual Barrier Y	Visual Barrier N
Septum	N	45˚	47 (31)	3 (1)
		90˚	49 (32)	5 (4)
	Y	45˚	30 (24)	4 (2)
		90˚	47 (25)	5 (2)
Overall			190 (119)	

Note: The number of turtles used in each treatment, excluding disqualified individuals, is in parentheses. Under the Visual Barrier heading, Y indicates that the opaque visual barrier was in place and N indicates that it was not.

It was hypothesized that an increase in the number of turtles successfully completing trials would be observed with the 45° entrance as compared to the 90° entrance because it effectively made the tunnel entrance area much wider. The septa formed a roughly wedge-shaped configuration intended to direct turtles into the tunnel entrance that might otherwise be bypassed. Two entrance angles, one with two 45˚ turns and the second with a single 90˚ turn, were crossed with either septa or no septa in place, and two levels of barrier opacity, fully translucent and opaque.

Analyses were conducted excluding the trials for which an opaque visual barrier was not installed since this group had too small of a sample size for hypothesis testing. Results from an ANOVA indicate that neither entrance angle nor septum were significant predictors of success.

Referring to Table 13, it is clear that among the trials with the opaque barrier in place that there is no discernible relationship between success rates and entrance angle or septum. Neither entrance angle nor septum was a statistically significant predictor of total time to complete the trial, hesitations or time spent in tunnel.

Table 13: Barrier and Tunnel Entrance Trial Group – Percentage success based on the number of turtles that successfully completed the trial in 60 minutes for all 119 turtles that were not disqualified.

		Entrance Angle	Visual Barrier	
			Y	N
Septum	N	45˚	80.6%	0%
		90˚	68.5%	75%
	Y	45˚	79.2%	0%
		90˚	88%	50%
Overall			77.3%	

Note: The data in Table 13 encompasses the field laboratory set up with two different entrance angles, with and without a septum and with and without a visual barrier in place.

Table 14: Barrier and Tunnel Entrance Trial Group – Median (SD) times in minutes to complete trials for all turtles excluding disqualified turtles.

		Entrance Angle	Visual Barrier	
			Y	N
Septum	N	45˚	17 (24.34)	60 (NA)
		90˚	21 (22.30)	19 (24.34)
	Y	45˚	24 (19.17)	60 (0)
		90˚	11 (18.94)	44 (22.63)
Overall			17 (22.00)	

Note: Median times are listed for each combination of variables followed by the standard deviation (SD) in parentheses. NA indicates that there was no SD value for that particular combination of variables because all trials were 60 minutes long.

Table 15: Barrier and Tunnel Entrance Trial Group – Mean (SD) total times in minutes for turtles that completed the trial.

		Entrance Angle	Visual Barrier	
			Y	N
Septum	N	45˚	26.55 (24.34)	60 (NA)
		90˚	30.84 (22.30)	26.50 (23.34)
	Y	45˚	29.46 (19.17)	60 (0)
		90˚	21.12 (18.94)	44.00 (22.63)
Overall			27.82 (22.00)	

Note: Mean times are listed for each combination of variables followed by the standard deviation (SD) in parentheses. NA indicates that there was no SD value for that particular combination of variables because all trials were 60 minutes long.

Table 14 displays median times to complete trials and Table 15 displays mean times to complete trials, both are in minutes and both exclude disqualified trials.
Barrier opacity was not a significant predictor of time from beginning of trial to entrance into tunnel. A T-test indicated that there was not a significant difference between the mean total times (T-Test statistic = 0.44). It should be noted that the sample size for the successful trials without the barrier was only four trials, which was considerably smaller than the sample of 88 for those trials with the visual barrier in place. The smaller sample may have had some effect on these results.

3.2.2 Results of Barrier-only Laboratory Tests

The influence of barrier opacity on movement behavior of painted turtles as well as spotted turtles and Blanding's turtles was examined in a field laboratory independent of any tunnel entrance variables. These trial groups were the Assabet Barrier Blanding's turtle Trial Group, Assabet Barrier spotted turtle Trial Group, and the Assabet Barrier painted turtle Trial Group. In total, tests of barrier opacity were conducted on 32 painted turtles, 50 Blanding's turtles, and 49 spotted turtles. For each of the three species, half of the individuals were exposed to an opaque fence and the other half to a translucent fence in order to quantify the effect of fence opacity on their rate of movement. Table 16 provides the mean rates of travel in fpm for each species with and without the visual barrier attached to the fence as well as the results of T-tests between the two treatments. A visual barrier significantly increased the rate of travel in fpm for painted turtles and spotted turtles but not for Blanding's turtles. It is unclear why behavior in response to the barrier opacity differed from the other species. A statistically significant difference in fpm was not observed for Blanding's turtles.

Table 16: Assabet Barrier Lab – Mean rates of travel in feet per minute (fpm) for each species with (Y) and without (N) the visual barrier attached to the fence. Results of T-tests are given in the form of P-values.

		Visual Barrier		
		Y	N	T-test P-Value
Species	Painted turtle	8.40	5.15	P< 0.001
	Blanding's turtle	7.15	6.36	P< 0.001
	Spotted turtle	7.81	5.11	P = 0.132

3.2.3 Results of Turtle Exclusion Gate Tests

On June 16 and from August 5-6, 2010, the 28 painted turtles in the Exclusion Gate Trial Group were tested in the Tillson Exclusion Gate Lab, a one-way turtle exclusion gate laboratory designed to allow turtles to easily pass in or out of an area but only in one direction. All turtles successfully passed through the gate to the lower level and were unable to return to the upper level indicating the one-way gate functioned as intended.

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4.0 Conclusions

4.1 Conclusions from Tunnel Laboratory Tests

This chapter contains discussion and conclusions drawn from the results of this research. The interpretation of these data will be useful in informing the design of tunnel passage systems intended specifically for use by freshwater turtles in Massachusetts.

4.1.1 Discussion and Conclusions from Tunnel Laboratory Tests of Size and Lighting

The highest rates of successful passage were observed among treatments that allowed for the highest ambient light levels.

In Tunnel Trial Group 1, the mean success rate across all dimensions for each light level was 90% for 100% ambient lighting; 87% for 75% ambient lighting; and only 53% for 0% ambient lighting as presented in Table 6. Among the 100% and 75% lighting treatments, an interesting trend was observed. For both lengths of the 100% treatment and the 80' length of the 75% treatment, the success rate dropped as the tunnel opening size grew larger. This trend did not continue for the 0% treatment. Success most likely decreased with increasing width in the brightest light treatments because turtles may be more comfortable in these large bright spaces and thus were not driven to escape. There is likely a critical tradeoff between tunnel opening size and light levels, which is discussed in detail in this section.

In Tunnel Trial Group 2, success rates were generally quite low with the mean success rate across all dimensions ranging from 45% for the 0% lighting treatment to 50% for the simulated roadway median lighting as presented in Table 7. There was a clear pattern of success increasing as tunnel opening size increased. This result may be attributed to the increase in light levels that occur inside tunnels that occurs as tunnel aperture is increased.

Among trials for Tunnel Trial Group 1 the percent of available ambient light transmitted through the tops of tunnels, and tunnel length, were significant predictors of rate of travel. Across all tunnel dimensions and lengths the rate of travel in feet per minute (fpm) increased as the amount of ambient light increased, as indicated in Table 17. The mean rates for each lighting level in fpm were 2.7 for 0%, 5.1 for 75%, and 5.0 for 100%. Pronounced differences were observed between the 0% and 75% as well as the 0% and 100% lighting treatments. As a general trend, rates of travel were higher for the 80' tunnels than for the 40' tunnels. These results may be attributed to the tendency of turtles to move through tunnels at a relatively steady pace once they have entered.

Table 17: Mean (SD) rates of travel in feet per minute (fpm) for all turtles in Tunnel Trial Group 1.

Tunnel Dimension	Percent Ambient Light Transmitted from Above		
Height (ft) x Width (ft) x Length (ft)	100%	75%	0%
2 x 2 x 40	4.4 (3.1)	4.6 (2.4)	2.4 (2.4)
4 x 4 x 40	4.0 (2.7)	5.0 (3.6)	2.6 (2.9)
4 x 8 x 40	4.8 (2.8)	4.8 (2.8)	2.3 (2.4)
2 x 2 x 80	6.9 (3.8)	6.8 (4.3)	1.6 (0.9)
4 x 4 x 80	6.3 (4.4)	5.2 (3.5)	3.8 (3.6)
4 x 8 x 80	4.1 (2.8)	4.2 (2.8)	3.5 (3.9)
All Dimensions	5.0 (3.4)	5.1 (3.3)	2.7 (2.9)
Overall		4.3 (3.4)	

Note: Mean rates of travel are listed for each combination of variables followed by the standard deviation (SD) in parentheses.

Among trials for Tunnel Trial Group 2, tunnel width was a significant predictor of rate of travel as presented in Table 18. Unlike Tunnel Trial Group 1, lighting level was not a significant predictor. This result is most likely because there was little difference between the two treatments in ambient light levels for most of the tunnel. It was hypothesized that the simulated roadway median treatment where light was allowed to enter at the center of the tunnel might result in a faster of rate of travel than the 0% treatment. This hypothesis turned out not to be the case and fpm was essentially equal for the two lighting treatments for Tunnel Trial Group 2. In this case, tunnel width was a significant predictor of rate of travel. Significant differences between the 8' and 2' as well as the 4' and 2' W tunnels were detected. Under both lighting treatments light levels were much lower in the 2' x 2' tunnel compared to the others that lead to the very slow rates of travel observed**.**

Table 18: Mean (SD) rates of travel in feet per minute (fpm) in Tunnel Trial Group 2 for all turtles.

Tunnel Dimension Height (ft) x Width (ft) x Length (ft)	Percent Ambient Light Transmitted 0%	 Simulated Roadway Median Lighting
2 x 2 x 80	1.8 (1.3)	1.7 (1.2)
4 x 4 x 80	2.4 (1.8)	3.0 (2.5)
4 x 8 x 80	2.7 (2.3)	3.2 (2.7)
All Dimensions	2.3 (1.8)	2.7 (2.3)
Overall	2.5 (2.1)	

Note: Mean rates of travel are listed for each combination of variables followed by the standard deviation (SD) in parentheses.

For Tunnel Trial Group 1, the mean time for successful completion of trials was similar for the 100% and 75% treatments for all opening sizes and lengths at 15.9 minutes and 15.2 minutes, respectively. As expected, the mean time for all opening sizes and lengths with the 0% treatment was considerably longer at 22.1 minutes as presented in Table 19. In Table 20 for Tunnel Trial Group 2, a clear trend was observed of turtles moving through tunnels in less time as tunnel size increased.

Table 19: Mean (SD) total times in Tunnel Trial Group 1 for turtles that completed the trial.

Tunnel Dimension Height (ft) x Width (ft) x Length (ft)	Percent Ambient Light Transmitted from Above 100%	 75%	 0%
2 x 2 x 40	15.24 (11.12)	13.24 (11.69)	15.87 (9.64)
4 x 4 x 40	14.88 (11.26)	8.90 (5.13)	18.13 (15.53)
4 x 8 x 40	12.42 (10.13)	12.29 (10.05)	23.89 (16.82)
2 x 2 x 80	13.32 (8.28)	15.61 (10.58)	40.80 (18.29)
4 x 4 x 80	16.77 (11.01)	19.82 (12.38)	21.31 (16.38)
4 x 8 x 80	23.82 (15.65)	21.22 (14.04)	24.92 (17.86)
All Dimensions	15.99 (11.81)	15.19 (11.65)	22.11 (16.26)
Overall	17.05 (13.13)		

Note: Mean total times are listed for each combination of variables followed by the standard deviation (SD) in parentheses.

Table 20: Mean (SD) total times in Tunnel Trial Group 2 for turtles that completed the trial.

Tunnel Dimension	Percent Ambient Light Transmitted	
Height (ft) x Width (ft) x Length (ft)	0%	Simulated Roadway Median Lighting
2 x 2 x 80	34.38 (16.78)	44.63 (17.76)
4 x 4 x 80	32.71 (16.36)	24.75 (13.14)
4 x 8 x 80	29.67 (15.97	27.53 (17.76)
All Dimensions	31.84 (15.95)	29.90 (17.36)
Overall	30.84 (16.61)	

Note: Mean total times are listed for each combination of variables followed by the standard deviation (SD) in parentheses.

Turtles in tunnels with the "bright" treatment performed markedly better than turtles in closed-top treatments. This was observed irrespective of aperture and length indicating that adequate lighting was critical to successful turtle passage in the experiments. Among tunnels with the dark treatment, the extreme variance in performance is likely explained by the varying amount of light let in through the ends, a direct result of their respective ORs.

Interestingly, among tunnels with the bright treatment, those with the smallest aperture, 2' x 2', had higher rates of successful passage than larger aperture tunnels. Based on observations from many trials, this seemingly counterintuitive response does not appear to be due to a preference for smaller apertures. Rather, it may be the result of turtles being relatively comfortable once they have entered the larger aperture tunnels and thus lacking the motivation to escape the experimental arena. This situation is likely unique to the experimental environment. It should not present a problem for passage systems bisecting natural landscapes where the animals encountering them are motivated to reach important resources, and will, as a result, not linger in tunnels.

It was hypothesized that allowing light to enter at the center of the tunnel, analogous to storm grates in a roadway median strip, might result in a higher rate of successful passage than the 0% available ambient light treatment. However, median lighting had no significant effect relative to tunnels with the 0% available ambient light treatment, with the overall means across all dimensions ranging from 45% for the 0% lighting treatment to 50% for the simulated roadway median lighting. As observed among tunnels of varying sizes with the 0% available light treatment, there was a clear pattern of success rates increasing as tunnel opening size increased.

Among tunnels with the 0% available ambient lighting treatment, an increase in rates of successful passage of painted turtles was observed as the OR increased, shown in Figure 11. Under this treatment, for all 6 tunnel sizes comprising 5 different ORs, rates of successful passage for even the tunnel with the greatest OR did not match those observed for the 100%

and 75% lighting treatments at any opening size or length. Additionally, the tunnel with the smallest OR of 0.05' performed worse than tunnels of the remaining four ORs. This result may be an indication that a threshold in the OR has been reached that is outside what most turtles are willing to use.

It is important to note that a minimum OR of 0.82' (0.25 meters) is recommended in the *MassDOT Stream Crossing Handbook* for a box culvert. The embedded portion of a tunnel or culvert is not included in the calculation of cross-sectional area for determining the openness ratio.

Openness ratio is a measure that has some bearing on the design of stream crossings constructed in Massachusetts. To be eligible for the Category One provision of the Army Corps of Engineers Massachusetts General Permit (MGP) where no application is required, new stream crossings must be designed and constructed in accordance with the "Massachusetts River and Stream Crossing Standards for New Stream Crossings of the MassDOT Stream Crossing Handbook"; including the Openness Ratio of greater than 0.82' (0.25 meters). Replacement Crossings do not need to meet these standards. New crossings, which cannot meet the standard, are eligible for the Category II requiring an application review under the MGP, or are eligible for Individual Review.

In tests of a tunnel with an almost identical OR to that recommended in the stream crossing standards of 0.80', successful passage rates of approximately 70% were observed. If an open-top or "open grated" tunnel top cannot be incorporated into a design, then an appropriately sized "closed top" culvert with an OR greater than or equal to 0.80' should be used.

It is likely that acclimation to, or scent cues within, closed topped culverts in the field may result in increased passage of turtles through the structures as the individuals interact with the structure over time. Subsequently, the 70% passage for the OR value of 0.80' may underestimate what occurs outside of the experimental area, provided an adequate barrier system is in place to prevent roadway access. A high OR value closed top structure will still provide adequate passage for most of a population upon first interaction with the crossing structure. Over time, the rate of successful passage should increase due to acclimation. Further testing is needed on this hypothesis.

Conclusions from tunnel laboratory tests of size and lighting are as follows:

1) Results indicate the importance of designing road passage structures that provide adequate lighting for freshwater turtles;
2) Of the tunnel design variables examined, transmitted light outweighed tunnel opening size and length in promoting movement of painted turtles;
3) The smallest and darkest tunnels examined appear to be at the lower limit of acceptable use by turtles;
4) Success decreased with increasing width in the brightest light treatments most probably because turtles felt comfortable in these large bright spaces and thus were not driven to escape. There may be some critical tradeoff between tunnel opening size and light levels; and

5) A high OR value in closed top structures should be used where "open-top" tunnels are not feasible and "closed-top" tunnels are the only option. These structures should have an OR of greater than or equal to 0.80' to be effective for the turtle species tested.

4.1.2 Discussion and Conclusions from Tunnel Laboratory Tests of Artificial Lighting

Painted turtles responded very favorably to the artificial lighting treatment and constant tunnel dimensions. The turtles successfully navigated passages at rates comparable to those observed for tunnels with the 100% available ambient light treatment. This result suggests that artificial lighting may be a viable means of: 1) retrofitting existing tunnels and culverts that are prohibitively dark, and 2) bringing ample light levels to small aperture closed-top tunnels. However, reservations remain concerning this technique since it is unknown how other wildlife species might react to artificial lighting. The maintenance of lighting may be logistically difficult.

Light level was inversely related to total trial time, and turtles in trials with artificial lighting typically completed trials in much less than 60 minutes. Light level was positively correlated with the likelihood of a turtle finishing a trial. This result is consistent throughout this study where success of trials increased with an increase in light level.

Total hesitations were inversely related to light level. Turtles exhibited a greater number of hesitations in trials with 0% light than turtles in trials with artificial lighting. Turtle hesitations are likely the most conservative way of measuring the effectiveness of tunnels. A single hesitation indicates that a turtle may not use the tunnel in a realistic setting and thus be at a greater risk of road mortality.

Light level was not a significant predictor of rate of movement through experimental tunnels. Once a turtle entered a tunnel, it typically passed through without any further hesitancy regardless of the lighting treatment.

Additional research on artificial lighting and alternative means of providing light is needed. Data should be collected on the reliability, intensity, and timing of lighting as well as its effects on the willingness of other types of wildlife to use tunnels. Since most passage systems will most likely be serving many species in addition to turtles, research that explicitly investigates these concerns is well warranted and probably necessary in order to avoid installation of passage systems that do more harm than good.

In addition, an artificial lighting system in a culvert requires regular maintenance, adding complexity and potentially compromising the reliability of the entire passage system. Maintenance of a lighting system may be, or become, an unsustainable cost. Should a lighting system fail for any reason such as lack of maintenance, technical malfunction, etc., the passage may then be unusable to the target population and result in failure to achieve the goals of the passage system. The most serious problem that could result from this would be a major increase in road mortality and a reduction or loss of connectivity of habitat bisected by roads.

The following conclusion was drawn from the results of the artificial lighting tests:

1) The results indicate that artificial lighting may be as effective as 100% available light in encouraging turtles to pass through tunnels.

4.1.3 Discussion and Conclusions from Tunnel Laboratory Tests of Lighting Level on Additional Species

Overall the results from tunnel laboratory tests of available ambient lighting level on three turtle species indicate that a tunnel with ample overhead light throughout is likely adequate to facilitate passage of most turtles. Conversely, a tunnel of the same dimensions, which lacks overhead light, may be inadequate to facilitate passage as seen in Figure 12. Spotted turtles were significantly more hesitant than the other two species to enter tunnels under either lighting treatment indicating that they may be inhibited by the width or length of the passage itself.

4.2 Conclusions from Barrier and Tunnel Entrance Variables

A number of conclusions can be drawn from tests of barriers and tunnel entrance variables that are critical in the design of effective culvert systems.

4.2.1 Discussion and Conclusions from Tunnel Entrance and Barrier Laboratory

Varying the angle of entrance from a single 90° turn to two 45° turns does not appear to be an important design element because it did not significantly affect the rate of successful trial completion or the willingness of turtles to enter the culvert. This finding was a surprise because the original prediction was that a culvert entrance having 45° entrance angles would provide a wider entrance that would be more attractive to turtles. That being said, there are other reasons such as structural/load bearing concerns that the angle of entrance could be an angle other than 90°. Overall, it is unlikely that variations in angle of entrance will negatively impact the use of a passage system by turtles.

Similarly, placing septa at the entrance to a tunnel had no effect on the turtles. Because there are a limited number of other functions, it is advised that septa not be included in the design of passage systems.

Barrier opacity was not a significant predictor of elapsed time from the beginning of the turtle's trial to its entrance into the tunnel. This result does not mean that it does not affect the movements of turtles in any way. The discussion section for tests in the Assabet Barrier Lab provides additional insight regarding the effects of barrier opacity.

The following conclusions were drawn from the results of tests in the tunnel entrance and barrier laboratory:

1) Varying the angle of entrance had no effect on turtles and is probably not an important design element;
2) Using septa had no effect on turtles; and
3) Barrier opacity was not a significant predictor of elapsed time from the beginning of the turtle's trial to its entrance into the tunnel.

4.2.2 Discussion and Conclusions from the Barrier-only Laboratory

The results of tests in the Assabet Barrier Laboratory indicate that barrier opacity is a significant predictor of rate of travel in fpm for painted turtles and spotted turtles. These two species moved at faster rates over the course of one-hour behavioral trials when an opaque visual barrier was attached to the fence. Interestingly, these tests did not show opacity to be a significant predictor of the rate of travel for the Blanding's turtles.

These results suggest that it may be possible to use either an opaque or translucent barrier to influence the behavior of turtles in different ways for different situations. For example, an opaque barrier could be used to swiftly direct turtles into a tunnel. Conversely, a translucent barrier could be used to dissuade turtles from moving beyond a certain point such as where the barrier ends and access to a road surface is possible.

4.2.3 Discussion and Conclusions from Tests of a Turtle Exclusion Gate

In tests using painted turtles, the turtle exclusion gate functioned as it was designed. All turtles tested were willing to pass through the gate, and most did so very quickly with no observed hesitancy. During tests, no turtles were able to return through the gate despite the fact that many were observed trying to do so. The height of the drop-off may need to be modified for some species, especially larger species such as the snapping turtle where a 12" drop may not be high enough to prevent animals from returning through the gate. When facilitating turtles with a range of body sizes, care must be taken not to make a drop-off so high that smaller species or individuals are unwilling to use it. Perhaps a modest drop off, such as the 12" height tested, with a "no-grip" polished surface that prevents climbing, may be the best option.

The following conclusion was drawn from the results of tests in the Tillson Exclusion Gate Laboratory:

1) The exclusion gate, as tested, appears to work well as it is intended and is a simple and straightforward means of allowing one-way passage into and out of areas.

4.3 Implementation

This study was undertaken to inform the design of effective road passage systems for freshwater turtles through a series of designed behavioral experiments. Turtles were examined for their response to a variety of light levels, tunnel sizes, tunnel entrance designs, and barrier opacities in outdoor laboratories. This section on implementation explains how the results of this study could be applied within the context of MassDOT project development and design. These recommendations are intended to provide information to supplement *Chapter 14: Wildlife Accommodation of the Massachusetts Highway Project Development and Design Guide*, published in 2006 (Design Guide).

4.3.1 Project Development and Design

MassDOT recognizes the importance of reducing impacts to wildlife and improving habitat connectivity within the Design Guide, however emphasizing public safety is the first and foremost function of the Department. When wildlife versus transportation conflicts arise, safety must come first, and consequently exclusion techniques such as barrier fencing are typically appropriate measures to reduce the conflicts. However in areas of statewide or regional ecological importance, or in areas of high wildlife mortality, the Design Guide recommends coupling exclusion techniques with wildlife passage accommodations such as tunnels and culverts.

To determine whether a project should consider wildlife passage accommodations, MassDOT project managers, engineers, and environmental analysts should follow the flow chart shown on Figure 3-1 of the *Design of Bridges and Culverts for Wildlife Passage at Freshwater Streams, December 2012* (i.e. the MassDOT Stream Crossing Handbook: Appendix A). The chart helps determine when wildlife passage accommodation should be considered during project design, though best professional judgment should also be relied on when evaluating project sites for wildlife accommodation upgrades. Once it has been determined that wildlife passage accommodation should be considered in project design, the implementation steps summarized in Sections 4.3.2 through 4.3.7 are recommended. A team comprised of project managers, engineers and environmental analysts should evaluate these implementation steps and their associated evaluations.

The implementation evaluation should also be considered in the context of the broad categories of MassDOT activities: construction of new roadways and structures (bridges/culverts); reconstruction and replacement of roadways and structures; and maintenance of existing roadway infrastructure. For each of these activity types, there are different opportunities and constraints for the provision of turtle passage accommodation. For example, a lack of existing infrastructure constraints typically allows for flexible design during the planning of new construction projects. Conversely, existing infrastructure design often dictates the suite of practicable alternatives available during the development of reconstruction and maintenance projects.

4.3.2 Implementation Step 1: Develop Conservation Objectives

Conservation objectives are determined upon an evaluation of turtle population, landscape context, habitat features, life cycle needs, wetland/roadway context, site evaluation, and other factors. The conservation objectives may vary due to the site characteristics and turtle community composition. Consequently, biologists at MassDOT, the Massachusetts Division of Fisheries and Wildlife, the Natural Heritage and Endangered Species Program, or others knowledgeable with the ecology of the target species, should be consulted for expert opinion on developing site-specific conservation goals.

4.3.3 Implementation Step 2: Passage Structure Evaluation – Conduct Constraints Analysis

In addressing turtle passage accommodation the project designer, at a minimum, should consider the following applicable constraints, including, but not limited to the following:

1) The structure's initial construction cost and the cost of long-term structure maintenance;

2) Potential displacement of, or adverse effects on, other structures, land uses, or utilities in the vicinity of the crossing;

3) Possible impacts of a modification on the structure itself or other nearby structures having archaeological or historic significance;

4) The constructability and feasibility of maintaining of the structure;

5) Potential environmental impacts associated with the construction process to install the structure, specifically: impacts to wetlands, endangered species habitats or other ecologically sensitive areas. The mitigation requirements for such impacts should also be considered;

6) Potential impacts to areas important to the natural history of the local species, including habitats for foraging, basking, nesting, and migration. Impacts to these areas should be weighed against the project's potential to preserve, enhance, or restore connectivity for the population within the local ecosystem;

7) Site-specific constraints to connectivity for the target population. Physical boundaries of a project/site must be well defined in order to determine if connectivity might be hampered by property boundaries, etc;

8) Potential adverse effect of a passage and barrier system on non-target species; and

9) Other constraints associated with the project shall be determined and evaluated depending on the activity category the project falls within: a) new construction, b) reconstruction, or c) maintenance. Knowledge of construction plans, construction timetable, and the construction budget may all be useful to determine which of the possible conservation measures are feasible given any combination of opportunities and limitations presented by the aforementioned considerations.

In addition, many of the constraints discussed in Chapter 5 of the *MassDOT Stream Crossing Handbook* appear to be applicable to turtle passage structures.

4.3.4 Implementation Step 3: Passage Structure Evaluation – Order of Preferred Alternatives

The culmination of the site specific conservation goals and the constraints analysis results will determine the most ecologically beneficial turtle passage structure appropriate for the site. The Order of Preferred Alternatives cited below and further described in Table 21, demonstrates the range of turtle passage structure types tested within this study, from the most preferred structure type, full light / large tunnel, to the least preferred structure type, low light / small tunnel. Table 21 outlines the order of preference for alternative design measures for maximizing turtle passage including some remarks that should aid in the structure selection process.

Order of Preferred Alternatives:

1) Full Light Large Tunnel or Bridge;
2) Full Light Tunnel;
3) Low Light Large Tunnel;
4) Low Light Small Tunnel; and
5) Barrier-only.

Table 21: Order of preference for alternative design measures for maximizing turtle passage.

Order of Preference	Alternative Design Measures	Remarks
1	Full Light Large Tunnel or Bridge	Strive for ambient light level within structure that is equal to that outside of structure. Full light level achieved by either: 1) very large size 2) open-top design The large size may accommodate the full range of wildlife in the locale, including larger species such as deer.
2	Full Light Tunnel	Strive for ambient light level within structure that is equal to that outside of structure. Full light level is likely easiest to achieve with an open-top design. If site is constrained by the roadway profile in regards to culvert size, it is most important to have a culvert that provides full light which could make the difference between turtles using the structure or not. For turtle passage, a small full light tunnel is more optimal than a low light large tunnel.
3	Low Light Large Tunnel	Closed-top tunnels typically provide lower light levels. Consequently, large tunnels are needed to provide adequate light levels to facilitate turtle passage. When designing closed-top tunnels to maximize light within the structure, openness ratio should be used as a reference metric; higher openness ratios equate to higher light levels.
4	Low Light Small Tunnel	May facilitate enough of the passage of a turtle population to provide genetic connectivity and reduce the number of turtles killed on the road. Minimal aperture of 2' x 2' based on the lower bounds of what was tested and to accommodate adult snapping turtles.
5	Barrier-only	Does not allow for connectivity across the roadway but is still has ability to greatly reduce mortality. Important tool for maintaining current population levels and, over time, the transportation infrastructure could be upgraded to include a tunnel to facilitate connectivity and genetic exchange.

There is a wide range of available techniques that enhance connectivity between turtle populations. Alternative techniques for barriers may include simple barriers and barriers in combination with a tunnel, multiple tunnels, or overpasses. Passage options include tunnels of which there are different types including: corrugated metal and concrete structures, box, open-top, and closed top tunnels, as well as precast or prefabricated open bottom arches and spans. These types of structures can be used both as tunnels (structures which do not provide hydrologic connectivity between wetland resources) and as culverts (structures which provide hydrologic connectivity between wetland resources). Lastly, wildlife overpasses have been implemented successfully to provide passage for a wide range of wildlife species.

4.3.5 Implementation Step 4: Passage Structure Evaluation – Details on Preferred Alternatives

Each of the designs described below should be integrated with a barrier system to increase their effectiveness in safely conveying turtles into the structures allowing them to pass beneath the roadway. Although it is important for all tunnels to be implemented in conjunction with a barrier fence, it is of the utmost importance to utilize barrier fence with low light tunnels, as turtles will naturally chose to cross roadways which receive full light. By excluding the roadway crossing option through barrier fencing, the tunnel is the only avenue available for crossing the road.

For closed-top tunnels, the OR should be used as a general indication of how bright a structure will be relative to outside conditions. Based on the research results from this study an OR greater than or equal to 0.80' is recommended. Additionally, to be eligible for the Category One/"No Application" provision of the Army Corps of Engineers Massachusetts General Permit, new stream crossings must be designed and constructed in accordance with the *Massachusetts River and Stream Crossing Standards for New Stream Crossings*; including the OR of greater than 0.82' (0.25 meters). Minimally, a tunnel should have an aperture of 2' x 2' based on the lower bounds of what was tested. This size is also the smallest likely to accommodate an adult snapping turtle, the largest native freshwater turtle in the Northeast.

Full Light Large Tunnel or Bridge – This option is the most preferred option because it was found in experiments that turtles were most willing to use a tunnel with ample light levels and large aperture. Within this structure the ambient light level is equal to that outside of the structure. Its large size exceeds what is required to facilitate the passage of turtles and can accommodate larger wildlife species as well. The full light level in this design is achieved by either having a very large size or an open-top design where light is permitted to enter through grating at the top of the tunnel. Additionally a large tunnel or bridge should allow for at least some degree of ecological continuity where a particular habitat type may continue uninterrupted beneath the roadway.

Full Light Tunnel – This option is not as spacious as the Full Light Large Tunnel or Bridge, thus it may not accommodate as many additional wildlife species, but the ambient light level within this structure is equal to that outside of the structure. The full light level in this design may be achieved by having a very large size, but more commonly it will be achieved with an open-top design. It is significant to note that 2' x 2' Full Light Tunnels were documented to be preferred by turtles over this 4' x 8' Low Light Tunnels. Consequently, significant cost savings can be achieved through utilizing a smaller Full Light Tunnel structure than a larger closed-top structure.

Low Light Large Tunnel – This option should still allow for the passage of many turtles. The only measure taken to provide adequate light levels is the size of the tunnel. There may be a great deal of variation in the amount of light in this tunnel relative to conditions outside the tunnel. The OR of each particular tunnel should provide an indication of the light level.

Low Light Small Tunnel – This option is the least preferred tunnel option because it has been found in experiments that turtles rarely used small dark tunnels. This type of structure is still preferable to no crossing structure at all because it may facilitate enough passage of a population to provide genetic connectivity across the roadway and reduce the number of turtles killed on the road.

Barrier-only – This option is the least preferred of all alternatives because it does not allow for connectivity across the roadway. However, barriers are still valuable to prevent roadway mortality especially on moderate to high traffic roads where many turtles may be killed annually. Preventing annual mortality is important to maintaining current population levels, and, over time, the transportation infrastructure could be upgraded to include a tunnel to facilitate genetic exchange between isolated populations. Therefore, if it is determined that a tunnel is not feasible at a specific time, the installation of a barrier system could be of significant conservation benefit for the local population.

4.3.6 Implementation Step 5: Passage Structure Evaluation – Passage Structure Selection

The conservation objectives and constraints analysis will dictate where a project falls within the Order of Preferred Alternatives. Selecting the highest-ranking design measure that the constraints will allow shall result in the highest potential to meet the conservation objectives developed for the site. When a design measure is chosen, the design should maximize the structures ability to provide turtle passage, while minimizing the potential for roadway surface access by turtles. As the design process advances, the project team should work together to achieve a final product that meets the conservation objectives of the site to the maximum extent practicable.

4.3.7 Implementation Step 6: Situational Determination of Barrier Fencing Type and Barrier Fencing Selection

Experiments of barrier opacity on movement behavior of turtles suggest that it is possible to use either an opaque or translucent barrier to influence the behavior of turtles in different ways to achieve different objectives. For example, an opaque barrier could be used to swiftly direct turtles into a tunnel. Conversely, a translucent barrier could be used to dissuade turtles from moving beyond a certain point, such as where the barrier ends and access to a road surface is possible. The integration of an appropriate barrier design into a turtle passage structure is essential to ensure turtles are guided into the passage structure and to prevent turtle access to the roadway surface.

4.4 Recommendations for Further Research

The following recommendations for further research have been drawn from findings and observations made over the course of this study. Experimental research on the design of passage systems for turtles and other wildlife is still relatively uncommon. As a result, there are a number of unexplored areas specific to turtle passage systems and within this general field that warrant rigorous investigation.

1) Identify existing designs, or develop new open-top tunnel type designs, that meet safety concerns and standards for all road types in Massachusetts. The design of such structures should be a collaborative effort between engineers and road ecologists;

2) Examine the effects of vehicle noise on movement behavior of turtles and their willingness to use under-road passage systems of varying design. This observation may be accomplished experimentally, or, alternatively, by monitoring passage use with respect to vehicle noise in situ;

3) Conduct experimental tests of passage systems on additional species;

4) Conduct experimental tests to determine whether amphibian species will readily pass through a dry culvert. This test may be especially important for open-top tunnel designs that cause the substrate to rapidly lose moisture, and, thus, may act as a barrier to amphibians that require moist travel conditions;

5) Revise the *Design of Bridges and Culverts for Wildlife Passage at Freshwater Streams*, also referred to as the MassDOT Stream Crossing Handbook, to include a section on facilitating passage of freshwater turtles at stream crossings;

6) Determine spacing requirements for passages along a barrier by passively monitoring turtles along a barrier during a natural migration, for instance, by using electronic tags; and

7) Continue experiments to examine artificial lighting as an alternative to open-top tunnels for turtles. Much remains to be determined regarding the intensity and timing of light in culverts relative to the passage of turtles.

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5.0 References

Department of Fish and Game, Division of Ecological Restoration. June 2012. *Massachusetts Stream Crossing Handbook, 2nd Edition.* Amy Singler, Brian Graber and Carrie Banks.

Ernst, C.H., M.F. Hershey and R.W. Barbour. 1974. A new coding system for hard-shelled turtles. Transactions of the Kentucky Academy of Science, 35:27–28.

Jackson, S.D. 2003. Proposed design and considerations for use of amphibian and reptile tunnels in New England. Unpublished report. University of Massachusetts, Amherst, MA, USA.

Massachusetts Department of Transportation. December 2010. Design of Bridges and Culverts for Wildlife Passage at Freshwater Streams.

The River and Stream Continuity Partnership. March 2006; revised March 2011 and March 2012. *Massachusetts River and Stream Crossing Standards*.

Woltz, H.W., J.P. Gibbs, and P.K. Ducey. 2008. Road crossing structures for amphibians and reptiles: informing design through behavioral analysis. Biological Conservation 141: 2745-2750.

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6.0 Appendices

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6.1 Appendix A - MassDOT Project Wildlife Accommodation Scenario for New and Replacement Stream Crossings

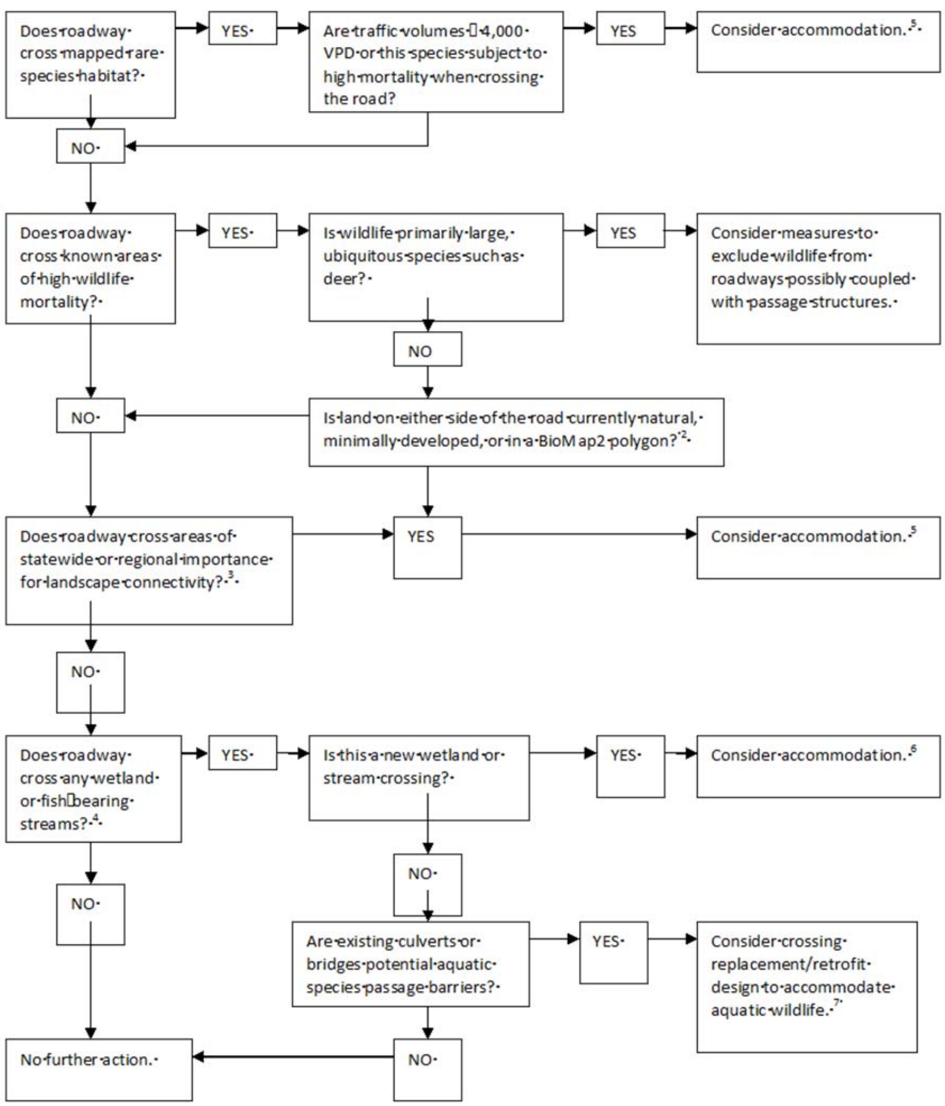

Figure 3.1: MassDOT Project Wildlife Accommodation Scenario for New and Replacement Stream Crossings

*Taken from page 42 of *Design of Bridges and Culverts for Wildlife Passage at Freshwater Streams*

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6.2 Appendix B – Selected Bibliography

The following is a selection of titles found useful during the conduct of this research. This selected bibliography was compiled as a stand-alone document and is by no means a complete record of the works and sources consulted for this Final Report.

1. ACO Wildlife. Information about amphibian and small wildlife tunnels. 2009. ACO Polymer Products, Inc. <ACO Wildlife Website>. Accessed 18 Nov 2009.

2. Andrews, K. M., J. W. Gibbons, and D. M. Jochimsen. 2006. Literature Synthesis of the Effects of Roads and Vehicles on Amphibians and Reptiles. Federal Highway Administration (FHWA), U.S. Department of Transportation, Report No. FHWA-HEP-08-005. Washington, D.C., USA.

3. Aresco MJ. 2004. Highway mortality of turtles and other herpetofauna at Lake Jackson, Florida, USA, and the efficacy of a temporary fence/culvert system to reduce roadkills. In Proceedings of the International Conference on Ecology and Transportation, Center for Transportation and the Environment, North Carolina State University, pp. 433-449. <http://repositories.cdlib.org/jmie/roadeco/Aresco2003a>. Accessed 17 Nov 2009.

4. Aresco, Matthew J. 2005. Mitigation measures to reduce highway mortality of turtles and other herpetofauna at a north Florida lake. Journal of Wildlife Management 69: 549-560.

5. Aresco, Matthew J. 2005. The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. Biological Conservation 123: 37-44.

6. Ascensao, F., and Mira, A. 2006. Factors affecting culvert use by vertebrates along two stretches of road in southern Portugal. Ecol Res (2007) 22: 57–66.

7. Ashley, E.P., A. Kosloski, and S.A. Petrie. 2007. Incidence of intentional vehicle-reptile collisions. Human Dimensions of Wildlife 12(3): 137-143.

8. Ashley, E.P., and J.T. Robinson. 1996. Road mortality of amphibians, reptiles, and other wildlife on the Long Point causeway, Lake Erie, Ontario. Canadian Field Naturalist 110 (3): 403-412.

9. Baldwin, E.A., M.N. Marchand, and J.A. Litvaitis. 2004. Terrestrial habitat use by nesting painted turtles in landscapes with different levels of fragmentation. Northeastern Naturalist 11(1): 41-48.

10. Beaudry, F., P.G. deMaynadier, and M.L. Hunter Jr. 2008. Identifying road mortality threat at multiple spatial scales for semi-aquatic turtles. Biological Conservation 141: 2550-2563.

11. Boarman, W.I., M.L. Biegel, and G.C. Goodlett. 1998. A passive integrated transponder system for tracking animal movements. Wildlife Society Bulletin 26: 886-891.

12. Boarman, W.I., and M. Sazaki. 1996. Highway mortality in desert tortoises and small vertebrates: success of barrier fences and culverts. Pp. 169-173 *In* G.L. Evink, P. Garrett, D. Zeigler and J. Berry (eds.) Trends in addressing transportation related wildlife mortality, proceedings of the transportation related mortality seminar. State of Florida Department of Transportation, Tallahassee, Florida. FL-ER-58-96.

13. Boarman, W.I., and M. Sazaki. 2006. A highway's road-effect zone for desert tortoises (*Gopherus agassizii*) Journal of Arid Environments 65: 94–101.

14. Brooks, R.J., G.P. Brown, and D.A. Galbraith. 1991. Effects of sudden increase in natural mortality of adults on a population of the common snapping turtle (*Chelydra serpentina*). Canadian Journal of Zoology 69: 1314-1320.

15. Brown, J.P. 2006. Eco-logical: An Ecosystem Approach to Developing Infrastructure Projects. U.S. Department of Transportation Research and Innovative Technology Administration Volpe National Transportation Systems Center. Cambridge, MA, USA.

16. Budischak, S.A., J.M. Hester, S.J. Price, and M.E. Dorcas. 2006. Natural history of *Terrapene carolina* (box turtles) in an urbanized landscape. Southeastern Naturalist 5(2): 191-204.

17. Burke, V.J., and Gibbons, W. 1995. Terrestrial Buffer Zones and Wetland Conservation: A Case Study of Freshwater Turtles in a Carolina Bay. Conservation Biology 9 (6): 1365-1369.

18. Cavallaro, L., K. Sanden, J. Schellhase, and M. Tanaka. 2005. Designing road crossings for safe wildlife passage: Ventura County guidelines. Thesis, University of California, Santa Barbara, USA.

19. Clevenger, A.P. 2005. Conservation Value of Wildlife Crossings: Measures of Performance and Research Directions. GAIA 14: 124-129.

20. Clevenger, A.P., B. Chruszcz, and K. Gunson. 2001. Drainage culverts as habitat linkages and factors affecting passage by mammals. Journal of Applied Ecology 38: 1340–1349.

21. Clevenger, A.P., B. Chruszczc, and K.E. Gunson. 2003. Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. Biological Conservation 109: 15–26.

22. Clevenger, A.P., and N. Waltho. 2000. Factors Influencing the Effectiveness of Wildlife Underpasses in Banff National Park, Alberta, Canada. Conservation Biology 14 (1): 47-56.

23. Clevenger, A.P., and N. Waltho. 2005. Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. Biological Conservation 121: 453–464

24. Code of Massachusetts Regulations (CMR), 321 CMR 10:00 Massachusetts Endangered Species Act Regulations. <CMR 10:00 >. Accessed 10 Mar 2009.

25. Code of Massachusetts Regulations (CMR), 321 CMR 10:12 Massachusetts Endangered Species Act Regulations, Delineation of Priority Habitat of State-listed species. <CMR 10:12>. Accessed 10 Mar 2009.

26. Code of Massachusetts Regulations (CMR), 321 CMR 10:23 Massachusetts Endangered Species Act Regulations, Conservation and Management Permit. <CMR 10:23>. Accessed 10 Mar 2009.

27. Compton, B. W., and P. R. Sievert. 2002. An evaluation of turtle tunnels and curbs at Towermarc Office Park. Unpublished report. University of Massachusetts, Amherst, MA, USA.

28. Congdon, J.D., A.E. Dunham, and R.C. van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. Conservation Biology 7: 826-833.

29. Congdon, J.D., A.E. Dunham, and R.C. van Loben Sels. 1994. Demographics of common snapping turtles (*Chelydra serpentina*): implications for conservation and management of long lived organism. American Zoologist 34 (3): 397-408.

30. Corlatti, L., Hackl, K., and Frey-Roos, F. 2009. Ability of Wildlife Overpasses to Provide Connectivity and Prevent Genetic Isolation. Conservation Biology, 23: 548–556

31. Cramer, P.C., and J.A. Bissonette. 2005. Wildlife Crossings in North America: the state of science and practice. Proceedings of International Conference on Ecology & Transportation 9: 442-447. 29 August-2 September 2005, San Diego, California.

32. Cuperus, R., Canters, K.J., Udo de Haes, H.A., Friedman, D.S. 1999. Guidelines for ecological compensation associated with highways. Biological Conservation 90: 41-51.

33. Dodd, K.C. Jr., Barichivich, W.J., and Smith, L.L. 2004. Effectiveness of barrier wall and culverts in reducing wildlife mortality on a heavily traveled highway in Florida. Biological Conservation 118: 619-631.

34. Ernst, C.H., M.F. Hershey and R.W. Barbour. 1974. A new coding system for hard-shelled turtles. Transactions of the Kentucky Academy of Science 35: 27–28.

35. Fahrig, L, J.H. Pedlar, S.E. Pope, P.D. Taylor and J.F. Wegner. 1995. Effect of road traffic on amphibian density. Biological Conservation 73: 177–183.

36. Festin, S.M. 1996. Summary of national and regional travel trends: 1970-1995. Office of Highway Information Management, Federal Highway Administration, U.S. Department of Transportation, Washington D.C. <Link to PDF>. Accessed February 2002.

37. Findlay, S.C., and Bourdages, J. 2000. Response Time of Wetland Biodiversity to Road Construction on Adjacent Lands. Conservation Biology 14: 86-94.

38. Ford, A.T., Clevenger, A.P., and Bennett, A. 2009. Comparison of Methods of Monitoring Wildlife Crossing-Structures on Highways. Journal of Wildlife Management. 73: 1213-1222.

39. Forman, R.T.T., and L.E. Alexander. 1998 Roads and Their Major Ecological Effects. Annual Review of Ecology and Systematics 29: 207-231+C2.

40. Forman, R.T.T. 2000. Estimate of the area affected ecologically by the road system in the United States. Conservation Biology 14: 86-94.

41. Forman, R.T.T. and Deblinger, R.D. 2000. The Ecological Road-Effect Zone of a Massachusetts (U.S.A) Suburban Highway. Conservation Biology 13: 36-46.

42. Forman, T.T., D. Sperling, J.A. Bissonette, A.P. Clevenger, C.D. Cutshall, V.H. Dale, L. Fahrig, R. France, C.R. Goldman, K. Heanue, J.A. Jones, F.J. Swanson, T. Turrentine, and T.C. Winter. 2003. Road ecology: Science and solutions. Island Press, Washington, D.C., USA.

43. Fowle, S.C. 1990. The painted turtle in the Mission Valley of western Montana. Master's Thesis, University of Montana, Missoula.

44. Gibbons, J.W. 1986. Movement patterns among turtle populations: applicability to management of the desert tortoise. Herpetologica 42(1): 104-13.

45. Gibbs, J.P. 1998. Amphibian movements in response to forest edges, roads, streambeds in southern New England. Journal of Wildlife Management 62: 584-589.

46. Gibbs, J.P., and W.G. Shriver. 2002. Estimating the effects of road mortality on turtle populations. Conservation Biology 16(6): 1647-1652.

47. Gibbs, J.P., and D.A. Steen. 2005. Trends in sex ratios of turtles in the United States: Implications of road mortality. Conservation Biology 19(2): 552-556.

48. Glista, D.J., DeVault, T.J., and DeWoody, A.J. 2009. A review of mitigation measures for reducing wildlife mortality on roadways. Landscape and Urban Planning 91: 1–7.

49. Goosem, M., Y. Izumi., and S. Turton. 2001. Efforts to restore habitat connectivity for an upland tropical rainforest fauna: A trial of underpasses below roads. Ecological Management and Restoration 2: 196-202.

50. Grgurovic, M., and P.R. Sievert. 2005. Movement patterns of Blanding's turtles (Emydoidea blandingii) in the suburban landscape of eastern Massachusetts. Urban Ecosystems 8: 203-213.

51. Griffin, K. 2005. Use of low fencing with aluminum as a barrier for turtles. Proceedings of the International Conference on Ecology and Transportation 9: 426-432. San Diego, California. USA.

52. Hagood, S., and M.J. Bartles. 2008. Use of existing culverts by eastern box turtles (*Terrapene c. carolina*) to safely navigate roads. Herpetological Conservation 3: 169-170.

53. Iglay, R.B., J.L. Bowman, and N.H. Hazdrowicz. 2007. Eastern box turtle (*Terrapene carolina carolina*) movements in a fragmented landscape. Journal of Herpetology 41(1): 102-106.

54. Jackson, S.D., and M.N. Marchand. 1998. Use of a prototype tunnel by painted turtles, Chrysemys picta. Unpublished note. University of Massachusetts, Amherst, MA, USA.

55. Jackson, S.D. 2000. Overview of transportation impacts on wildlife movement and populations. Pages: 7-20, in Messmer, T.A. and B. West, (eds.). Wildlife and highways: seeking solutions to an ecological and socio-economic dilemma. The Wildlife Society.

56. Jackson, S.D., and Griffin, C.R. 2000. A strategy for mitigating highway impacts on wildlife. Pages: 143-159, in Messmer, T.A. and B. West, (eds.). Wildlife and highways: seeking solutions to an ecological and socio-economic dilemma. The Wildlife Society.

57. Jackson, S.D., and T.F. Tyning. 1989. Effectiveness of drift fences and tunnels for moving spotted salamanders *Ambystoma maculatum* under roads. In T.E.S. Langton

(ed) Amphibians and Roads. Proceedings of the toad tunnel conference: pp. 93-99. ACO Polymer Products, Shefford, England.

58. Jackson, S.D. 2003. Proposed design and considerations for use of amphibian and reptile tunnels in New England. Unpublished report. University of Massachusetts, Amherst, MA, USA.

59. Jacobson, S.L., and T. Brennan. 2005. Integrated training course for engineers and wildlife biologists. John Muir Institute of the Environment, University of California, Davis. <http://repositories.cdlib.org/jmie/roadeco/Jacobson2005b>. Road Ecology Center eScholarship Repository. Accessed 17 Nov 2009.

60. Jaeger, J.A., Bowman, J., Brennan, J., Fahrig, L., Bert, D., Bouchard, J., Charbonneau, N., Frank, K., Gruber, B., and K. Tluk von Toschanowitz. 2005. Predicting when animal populations are at risk from roads: an interactive model of road avoidance behavior. Ecological Modeling 185: 329-348.

61. Jaeger, J.A.G., and L. Fahrig. 2004. Effects of road fencing on population persistence. Conservation Biology 18(6): 1651-1657.

62. Jaeger, J.A., Fahrig, L. and Ewald, K.C. 2006. Does the configuration of road networks influence the degree to which roads affect wildlife populations? In: Proceedings of the 2005 International Conference on Ecology and Transportation, Center for Transportation and the Environment, North Carolina State University: pp. 151-163.

63. Jochimsen, D.M., C.R. Peterson, K.M. Andrews, and J.W. Gibbons. 2004. A literature review of the effects of roads on amphibians and reptiles and the measures used to minimize those effects: final draft. Idaho Fish and Game Department and USDA Forest Service.

64. Joyal, L.A., M. McCollough, and M.L. Hunter Jr. 2001. Landscape ecology approaches to wetland species conservation: a case study of two turtle species in southern Maine. Conservation Biology 15(6): 1755-1762.

65. Kaye, D.R.J., K.M. Walsh, E.L. Rulison, and C.C. Ross. 2005. Spotted turtle use of a culvert under route 44 in Carver, Massachusetts. Proceedings of International Conference on Ecology & Transportation 9: 426-432. 29 August-2 September 2005, San Diego, California.

66. Kline N.C. and Pokorny M. 2004. A mathematical model for estimating wildlife mortality on roads, and its implications for mitigation and management. IN: Proceedings of the 2003 International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University, p. 367 (abstract only).

67. Langen, T.A., A. Machniak, E.K. Crowe, C. Mangan, D.F. Marker, N. Liddle, and B. Roden. 2007. Methodologies for Surveying Herpetofauna Mortality on Rural Highways. Journal of Wildlife Management 71: 1361–1368.

68. Langen, T.A., Ogden, K.A., and Schwarting, L.L. 2009. Predicting Hot Spots of Herpetofauna Road Mortality along Highway Networks. The Journal of Wildlife Management 73: 104-114.

69. Maine Department of Transportation, Environmental Office Waterway and Wildlife Passage Policy and Design Guide 3rd edition, July 2008.

70. Marchand, M.N., and J.A. Litvaitis. 2004. Effects of habitat features and landscape composition on the population structure of a common aquatic turtle in a region undergoing rapid development. Conservation Biology 18(3): 758-767.

71. Massachusetts General Law, Massachusetts Endangered Species Act, M.G.L c.131A. <Link to Legislation>. Accessed 10 March 2009.

72. Massachusetts River & Stream Crossing Standards. Developed by the River and Stream Continuity Partnership. Accessed 1 March 2006.

73. Mata, C., Hervás, I., Herranz, J., Malo, J.E., and Suárez, F. 2009. Seasonal changes in wildlife use of motorway crossing structures and their implication for monitoring programmes. Transportation Research Part D 14: 447–452.

74. Mata, C., Hervas, I., Herranz, J., Suarez, F., Malo, J.E. 2008. Are motorway wildlife passages worth building? Vertebrate use of road crossing structures on a Spanish motorway. Journal of Environmental Management 88: 407-415.

75. Mumme, R.L., Schoech, S.J., Woolfenden, G.W., and Fitzpatrick, J.W. 2000. Life and death in the fast lane: demographic consequences of road mortality in the Florida scrub jay. Conservation Biology 14: 501-512.

76. Natural Heritage and Endangered Species Program [NHESP]. 2009. NHESP regulatory review. <Link to Document>. Accessed 10 Mar 2009.

77. Nelson D.A., Papin M.E. and Baker T. 2006. Quick fixes: working together to address herptile road mortality in New York State. In: Proceedings of the 2005 International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University: pp. 90-93.

78. Obbard, M.E., and R.J. Brooks. 1980. Nesting migrations of the snapping turtle (*Chelydra serpentina*). Herpetologica 36(2): 158-162.

79. Pelletier, S.K., L. Carlson, D. Nein, and R.D. Roy. 2006. Railroad crossing structures for spotted turtles: Massachusetts Bay Transportation Authority–

Greenbush rail line wildlife crossing demonstration project. In: Proceedings of the 2005 International Conference on Ecology and Transportation: 414-425. Center for Transportation and the Environment, North Carolina State University. <Link to document>. Accessed 16 November 2009.

80. Puky, M. 2003. Amphibian mitigation measures in central-Europe. Proceedings of International Conference on Ecology & Transportation. Center for Transportation, and the Environment, North Carolina State University: 413-471.

81. Puky, M. 2005. Amphibian road kills: a global perspective. Proceedings of International Conference on Ecology & Transportation, Center for Transportation, and the Environment, North Carolina State University.

82. Rodriguez, A., G. Crema. and M. Delibes. 1996. Use of non-wildlife passages across a high speed railway by terrestrial vertebrates. Journal of Applied Ecology 33: 1527-1540.

83. Roof, J., and J. Wooding. 1996. Evaluation of the S.R 46 Wildlife Crossing in Lake County, Florida. Florida Game and Fresh Water Fish Commission Wildlife Research Laboratory. Gainesville, FL.

84. Ruby, D.E., J.R. Spotila, S.K. Martin, and S.J. Kemp. 1994. Behavioral responses to barriers by desert tortoises: implications for wildlife management. Herpetological Monographs 8: 144-160.

85. Smith, D.J. 2003. Monitoring wildlife use and determining standards for culvert design: final report. Florida Department of Transportation.

86. Smith, L.L., K.G. Smith, W. J. Barichivich, C.K. Dodd Jr., and K. Sorensen. 2005. Roads and Florida's herpetofauna: a review and mitigation case study. Amphibians and Reptiles: status and conservation in Florida. Krieger Publishing Company, Malabar, Florida, USA.

87. Steen, D.A., M.J. Aresco, S.G. Beilke, B.W. Compton, E.P. Condon, C.K. Dodd Jr., H. Forrester, J.W. Gibbons, J.L. Greene, G. Johnson, T.A. Langen, M.J. Oldham, D.N. Oxier, R.A. Saumure, F.W. Schueler, J.M. Sleeman, L.L. Smith, J.K. Tucker, and J.P. Gibbs. 2006. Relative vulnerability of female turtles to road mortality. Animal Conservation 9: 269-273.

88. Steen, D.A., and J.P. Gibbs. 2004. Effects of roads on the structure of freshwater turtle populations. Conservation Biology 18(4): 1143-1148.

89. Trakimas, G., and Sidaravičius, J. 2009. Road mortality threatens small northern populations of the European pond turtle, *Emys orbicularis*. Acta Herpetologica 3: 161-166.

90. Transportation Research Board. 2002. Interaction between Roadways and Wildlife Ecology. National Cooperative Highway Research Program (NCHRP) Synthesis 305. Project 20-5 FY 2000 (Topic 32-11), ISSN 0547-5570, ISBN 0-309-06923-8, Library of Congress Control No. 2002111522.

91. Trombulak, S.C., and C.A. Frissell. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. Conservation Biology 14(1): 18-30.

92. United States Department of Transportation, Federal Highway Administration. Critter Crossings; Salamander Tunnels. 2000. <http://www.fhwa.dot.gov/environment/wildlifecrossings/salamand.htm>. Accessed 17 Nov 2009.

93. U.S. Department of Transportation. Federal Highway Administration, Office of International Programs [FHWA-HPIP] 2002. Wildlife Habitat Connectivity across European Highways. Washington D.C. USA.

94. United States Federal Highway Administration [USFHA]. 2008. United States of America high statistics 2007. Office of Highway Information Management, Federal Highway Administration, U.S. Department of Transportation, Washington D.C. USA. <Link to FHWA>. Accessed 3 March 2009.

95. Woltz, H.W., J.P. Gibbs, and P.K. Ducey. 2008. Road crossing structures for amphibians and reptiles: informing design through behavioral analysis. Biological Conservation 141: 2745-2750.

96. Woodlot Alternatives, Inc. Environmental Consultants. 2003. Wildlife Studies and Mitigation Design for Greenbush Rail Corridor, Massachusetts. Unpublished report prepared for: Massachusetts Bay Transit Authority, Jacobs Civil, and CBB/STV.

97. Wray, S., P. Reason, D. Wells, W. Cresswell and H. Walker. 2005. Design, Installation, and Monitoring of Safe Crossing Points for Bats on a New Highway Scheme in Wales. Proceedings of International Conference on Ecology & Transportation 9:426-432. 2005, San Diego, California. USA.

98. Yanes, M., J.M. Velasco, and F. Suarez. 1995. Permeability of roads and railways to vertebrates: the importance of culverts. Biological Conservation 71: 217-222.