Experimental Tests of Road Passage Systems for Reducing Road Mortalities of Freshwater Turtles

EXPERIMENTAL TESTS OF ROAD PASSAGE SYSTEMS FOR REDUCING ROAD MORTALITIES OF FRESHWATER TURTLES

A Thesis Presented

by

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ABSTRACT

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Roadways are a pervasive feature of northeastern landscapes and can be a significant source of mortality for turtles. Until recently, little has been known about the design requirements for successful under-road passages for turtles and other wildlife to move safely between bisected habitat patches. At outdoor laboratories, using a factorial experimental design, we examined movements in response to varying light levels, and barrier opacity for painted turtles (*Chrysemys picta*, n=833), Blanding's turtles (*Emydoidea blandingii*, n=49), and spotted turtles (*Clemmys guttata*, n=49). Additionally, we examined tunnel size, tunnel entrance design, and artificial lighting for painted turtles only. All three species responded poorly to a 0% available light treatment. As the amount of natural light transmitted through the tops of tunnels increased, successful completion of the trials increased. Furthermore, turtles generally 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 specific entrance designs that meet the goals of the project.

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CHAPTER 1

EXPERIMENTAL TESTS OF BELOW-ROAD PASSAGE SYSTEM USE BY PAINTED TURTLES (*CHRYSEMYS PICTA*)

Introduction

Roads are major features on the landscape with many ecological effects (Jochimsen et al. 2004). There are three primary ways that roads adversely affect animal populations: (1) Barriers to movement; (2) increased mortality due to collisions with vehicles; and (3) reduced amount and quality of habitat (Jaeger and Fahrig 2004).

Many aquatic turtle species undertake terrestrial movements, which may include migrations to nesting sites, dispersal of juveniles, movement to escape unfavorable conditions, and movement of males to find mates (Gibbons 1986, Joyal et al. 2001). Turtles often encounter roads during these movements (Beaudry et al. 2008, Mumme et al. 2000) and considerable mortality occurs on roadways (Andrews et al. 2008, Aresco 2005-B, Ashley and Robinson 1996, Haxton 2000, Langen et al. 2009, Patrick et al. 2010). Additional direct effects of roads on populations include injury, alteration/restriction of movement/behavior, and loss of habitat (Forman et al. 2003, Jackson 2000, Trombulak & Frissell 2000) are well documented. Indirect effects include habitat fragmentation and degradation, isolation of turtle populations, disruption of gene flow and metapopulation dynamics (Jackson 2000, Trombulak & Frissell 2000; Spellerberg, 1998; Forman et al., 2003; Eigenbrod et al., 2008). Turtle populations are extremely vulnerable to road mortality because their life history includes low annual recruitment, high adult survival, and delayed sexual maturity (Congdon et al. 1993, 1994, Mumme et al. 2000, Beaudry et al. 2008, Fahrig et al., 1995; Gibbs and Shriver, 2002, Patrick et al. 2010). The reduction in habitat quality and size coupled with additive road mortality can alter the demographic and genetic structure of populations (Steen & Gibbs 2004, Beaudry et al 2008, Marchand and Litvaitis 2004, and Aresco 2005-B, Laporte et al 2012). Females are most vulnerable to the adverse effects of roads because of their repeated nesting migrations and because open shoulders of roads attract females seeking favorable nesting conditions (Steen et al. 2006, Ashley and Robinson 1996, Aresco 2005-B, Baldwin et al. 2004, Gibbs and Steen 2005).

Reducing collisions between vehicles and wildlife has become an area of focus in conservation (Aresco, 2005-A; Clevenger and Waltho 2005, Patrick et al. 2010). Under-road passages are being employed to allow a wide range of wildlife species to move safely between habitat patches that are bisected by roadways (Forman et al. 2003, Puky 2003, Mata et al. 2008). Increasing the permeability of road networks for turtles using under-road passages might help to alleviate direct and indirect impacts where populations are negatively affected by roads (Yanes et al. 1995; Guyot and Clobert 1997; Aresco 2005-A).

Accurately identifying the location and scale of road crossing hotspots to target for mitigation is a complex task and recent research has focused on doing just this for turtles and other reptiles and amphibians. A combination of road surveys and modeling techniques have been used to date (Cureton and Deaton 2012, Langen et al. 2012). Patrick et al (2012) determined that hotspots may be modeled reliably on the landscape for habitat specialists but not necessarily for habitat generalists.

The work of Gunson and Shueler (2012) indicates the importance of empirical data in determining where to locate mitigation structures. However, Eberhardt et al (2013) cautioned that empirical evidence of road kill hotspots may not effectively indicate mitigation locations when past road kill has depressed populations.

Road passage systems comprised of tunnels and guidance barriers have been used to mitigate the negative effects of roadways on wildlife populations. Examples of successful passage systems for reptiles and amphibian include fencing and tunnels (Dodd et al. 2004; Aresco 2005-A). Non-functioning structures are apparently still prevalent and failures of these structures may stem from inadequate design, placement and considerations of species behavior (Meinig 1989, Podloucky 1989, Puky 2003).

There are few studies that have evaluated the factors affecting passage use by turtles and other reptiles (Jochimsen et al. 2004). Several brief studies conducted basic examinations of the willingness of turtles to use tunnels and their response to barrier fencing (Ruby et al. 1994, Jackson and Marchand 1998, Griffin 2005). These studies were limited in the small number of variables they examined but results were generally suggestive that passage systems could be effective. Woltz et al. (2008) is the only published study that examined how aperture diameter, substrate type, length, and light permeability influence the preference of freshwater turtles for crossing structures. Woltz recommended that a tunnel with a diameter of ≥ 0.5 m, lined with soil or gravel, and accompanied by a 0.6-0.9 m high guide fence would best facilitate road crossings for turtles. Experimental approaches like that used by Woltz permit large samples using animals captured in the field and placed within enclosures, but there is concern that the pattern of choice exhibited by animals under experimental conditions may not be the same as that under natural conditions. Conversely, research based on monitoring arrays of already installed crossing structures affords a view of animals under ‘‘natural’’ conditions but does not permit control of site-specific variables (Patrick et al. 2010).

Installing and maintaining structures is costly and it is unclear at this time whether this approach will be implementable at the scale required for meaningful impacts and function to protect populations over the long term (Beebee 2012, Mata et al. 2008). It is important to determine what the most effective design is considering both cost and functionality in order to use resources wisely. We used a series of behavioral tests conducted in outdoor field laboratories to examine the relative influence of passage system design on the movement behavior of painted turtles. We studied painted turtles because they are a relatively common aquatic species and therefore we could achieve a sufficiently large sample size to test a variety of design characteristics, yet mortality due to roads has been documented to impact their population sizes and structures (Baldwin et al. 2004, Carr and Fahrig 2001, Fahrig et al. 1995, Fowle 1990, Marchand and Litvaitis 2004, Steen and Gibbs 2004, Steen et al. 2006).

We tested the behavioral response of painted turtles to passage system variables in four experiments; 1) we examined the influence of tunnel size, length, and lighting on the movement behavior of painted turtles; 2) we examined the effect of artificial lighting on the movement behavior of painted turtles; 3) we examined the effects of variations of tunnel entrance angle and septum use on the movement behavior of painted turtles; and 4) we tested a one-way turtle exclusion gate designed to allow turtles to pass out of an area, but not return.

We predicted the following outcomes;

- Painted turtles would prefer tunnels of the largest aperture and greatest amount of available ambient light permitted to enter the tunnel tops.
- Rates of successful passage in artificially lit tunnels would be higher than that observed in tunnels with little or no light allowed to enter through their tops but not as high as in tunnels with the 100% available ambient light treatment.
- A wider tunnel entrance and the use of guidance septa at the entrance would increase the rate at which turtles entered the tunnel.

Methods

General

In all experiments, painted turtles (n = 801) were captured using large collapsible minnow traps baited with sardines packed in soybean oil at wetlands known to contain large populations within a 15-mile radius of the field laboratories in Hampshire and Franklin counties. For identification purposes, a piece of tape marked with a unique number was attached to the carapace of each turtle for the duration of the trial. While not involved in trials, test animals were kept in holding pens that were shaded and contained water, leaf litter, and plywood structures for shelter.

We conducted Experiments 1, 2, and 4 at the Tillson Farm facility of the University of Massachusetts Amherst in Amherst, Massachusetts. The site was selected because it was easily accessible, was located close to local populations of painted turtles and provided the required electrical and water utilities. Experiment 3 was conducted at a privately-owned wood lot in Leverett, Massachusetts. We selected this site because it is immediately adjacent to a wetland containing a painted turtle population, and the field- testing setup could be oriented in a way to take advantage of the turtles' desire to escape and return to the wetland. The field laboratory was constructed in an upland forested area close to the shore of the wetland.

We recorded the behavior of turtles in the arenas for Experiments 1, 2, and 3 using Pclix LT 100 time-lapse triggers and Canon Powershot G2 and G3 digital cameras. Cameras were elevated and took photos every 5 seconds for the duration of the trial so that a detailed record of behavior could be gathered from the images and analyzed at a later date. Closed-circuit video cameras were placed at a minimum of 2 locations around the arena perimeters and were used in conjunction with direct visual observation to document the locations of turtles at intervals of 1-3 minutes throughout the trial. Closed-circuit and direct monitoring were used to keep track of trial progress and afforded a wider field of view than that of the time-lapse cameras which were trained on the tunnel entrance and its immediate vicinity. All 3 of these monitoring techniques were needed to ensure that a consistent and detailed record of behavior was documented while simultaneously minimizing human disturbance. In Experiment 4, we did not need to capture behavioral response in great detail so we used direct visual observation to monitor turtles in the arena and record their locations at regular intervals throughout the trial. For all experiments, we quantified behaviors while trials were being conducted, and later by reviewing time-lapse images where applicable.

After a turtle's use in an experiment was completed, and prior to releasing it in the wild, we gave it a permanent mark and recorded its age, sex, gravidity, maximum carapace length, maximum carapace width, and weight. All turtles were given a unique identification number by filing notches into the carapace marginal scutes (Ernst et al. 1974). We released all turtles at their point of capture (usually < 24 hours from time of capture).

Experiment 1 – We used a factorial design to experimentally test tunnel length, aperture, lighting and their interactions on the passage of painted turtles (n = 595) from 22 May to 22 July 2009. There were three aperture or opening size treatments that were crossed with 2 length treatments, and 4 lighting treatments (Table 1.1). Tunnel lengths, apertures, and lighting levels were selected based on the design recommendations found in the scientific literature and unpublished data from a previous study (Jackson 2003, Woltz et al. 2008, and Paulson unpublished data).

We used either a 40 ft or 80 ft long tunnel, with either a 2 ft x 2 ft, 4 ft x 4 ft, or 4 ft x 8 ft opening and a completely open top, except for 2 in x 4 in cross beams placed at 4 ft intervals for structural support. Overhead light transmission was manipulated using opaque cloth and shade cloth, producing four options; 0% 75%, 100% transmission and simulated median lighting. We used a Sekonic L-358 handheld digital light meter to measure transmitted light levels. The simulated median lighting treatment allowed light to enter the tunnel top only through a 2 ft x 4 ft area of shade cloth and was intended to be analogous to a tunnel with two storm drains located at its center in a highway median strip. The tunnel was oriented north-south, and the sides consisted of plywood panels reinforced with 2 inch x 4 inch wooden cross beams. The bottom of the tunnel and pens was the natural soil/sand at the site (Figure 1.1).

Experiment 1 was divided into 2 groups. Group 1 trials compared the 2 tunnel lengths, the 3 apertures, and 3 of the lighting levels (0% 75%, 100% transmission). Group 2 was comprised of a separate and later set of trials intended to compare 0% transmission to simulated median lighting across all 3 apertures for a single length tunnel (80 feet).

Enclosures attached on either end of the tunnel served as standardized start or exit pens for the trials. The enclosures were open-topped ellipses with a 15 ft minimum diameter and 20 ft maximum diameter. Pen fencing was made of 3 ft high rabbit fencing that was covered with landscaping fabric to block most visual stressors and distractions from the surrounding environment. The fence was graduated and mesh size ranged from 1 in wide x 4 in high at ground level to 4 in x 4 in near the top of the fence. Each turtle was randomly assigned a start direction, aperture, length, and lighting treatment, and given 60 minutes to complete the trial. Response variables for experiment 1 are described on page 12.

Experiment 2 - We experimentally examined the effect of artificial lighting on movement behavior of painted turtles (n = 70) from 21 June to 6 August 2010. We tested artificially illuminated tunnels in order to determine if it might be a viable alternative to an open-top design in providing high light levels. 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" from the increased light levels might be observed.

We used an 80 ft long tunnel, with a 2 ft x 2 ft opening 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 the ceiling of a closed-top tunnel. We used 15-watt "soft white" compact fluorescent bulbs with a color temperature of approximately 2700 Degrees Kelvin, which were not intended to match the color temperature of natural daylight.

We chose to use compact fluorescent bulbs rather than the more typically used 4-foot fluorescent bulbs because compact bulbs were inexpensive and easy to install in the tunnel using all-weather fixtures. We intentionally used the equivalent wattage per foot to a commonly installed fixture featuring two parallel 4 ft long bulbs.

Figures 1.1 and 1.2 show a schematic diagram of the field laboratory and a photograph of an artificially illuminated tunnel, respectively. Length of experimental trials and turtle handling protocols were the same as in Experiment 1 and are described on page 12.

Experiment 3 - We used a factorial design to experimentally test tunnel entrance angle (two angles comprised of fencing leading into the tunnel entrance), septum presence, and the interaction of these factors on movement behavior of painted turtles (n = 108) from 21 June to 5 August 2010. We tested tunnel entrance angle and septa because we felt they could play an important role in directing turtles into tunnels. We hypothesized that more turtles would successfully complete trials if the entrance angle was 45° rather than 90˚, since this modification made the tunnel entrance area much wider. We also thought that septa would be effective in helping guide turtles into the tunnel that might otherwise have bypassed the entrance.

Our experimental setup included a single tunnel connected to a large rectangular pen as shown in Figure 1.3. The tunnel was constructed in a fashion similar to those used in Experiments 1 and 2 except for the dimensions and the design of the entrance/exit. The top of the tunnel was covered with an opaque cloth and the tunnel was 36 ft long, and had cross-sectional dimensions of 4 ft x 4 ft.

The pen measured 180 ft x 16 ft and had the longest side parallel to the wetland (oriented north-south). The fence that defined the sides of the pen was supported by wooden stakes and made of chicken wire fence with a 1 in x 1 in mesh size. A silt fence, commonly used to control sediment runoff at construction sites, was added to the side of the pen that contained the entrance to the tunnel in order to block the turtles' view. The chicken wire fence had a visual barrier installed that was constructed of tarpaper and was mounted against the fence on the outside of the pen to avoid physical contact with the turtles. 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 might serve as obstacles to turtle movement or obstruct visibility for experiment observers were removed. The surface substrate that remained was comprised primarily of pine needles found at the site.

The angle of entrance relative to a turtle's path along the barrier could be manipulated to produce two options: 1) two 45˚ turns, or 2) a single 90˚ turn (Figure 1.4). The angle of wall panels in the entrance area could be manipulated by inserting the wooden stakes attached to the panels into different vertical pipes buried in the ground. The total length of the tunnel was 40 ft rather than 36 ft when the variable area of entrance angle manipulated is included. Removable septa were anchored in the ground using the same technique for anchoring the panels. We used 1 ft high panels for the 45° entrance angles to minimize the effect on incoming light from the wetland that we believed served as a motivation for the turtles to exit the pen. Figure 1.5 shows the tunnel entrance area of the field laboratory setup with an opaque visual barrier, 90˚ entrance, and a septum.

The septa were constructed of 3 ft high chicken wire attached to 4 ft long 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 in apart at the tunnel entrance and 8 ft apart at their farthest point from the tunnel entrance (Figure 1.5). The septa extended 1 ft beyond the barrier fence into the mouth of the tunnel and another 6.5 ft 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 an additional 4 ft beyond the tunnel, with rabbit fence along the perimeter to prevent turtles from escaping into the wetland after exiting the tunnel. The natural substrate found in the tunnel was used to cover the platform.

At the beginning of a trial, we randomly placed a turtle in either the northeast or southeast corner of the experimental fencing arena. Once placed in the arena, the trial continued until the turtle exited into the finish pen or the trial time reached 60 minutes, whichever came first.

Experiment 4 – We used a subset of turtles (n = 28) from Experiment 2 to test a one-way turtle exclusion gate on 16 June and 5-6 August 2010. The gate was designed to allow turtles to easily pass in a single direction. Figure 1.6 shows a turtle exclusion gate of approximately the same design, except the fence material is chain-link rather that chicken wire, and the gate base is granite rather than wood.

To test the exclusion gate we used a 10 ft x 10 ft silt fence pen divided into two sections with a chicken wire fence, and having a "gate" in the middle. The gate was a 2 ft break in the fence, with a 1 ft drop-off, made possible by situating the gate on a gently sloping hill with the lower portion excavated to produce the drop-off. Water and shade were provided for the turtles on both sides of the chicken wire fence.

A group of 10-12 turtles were placed together in the upper level of the experimental arena at the beginning of each trial. Once placed in the arena, the turtles remained there for a 60-minute duration even if all the individuals had already passed through the gate. This protocol was used to ensure that the gate was indeed a one-way passage, and hence turtles were unable to return to the upper level.

Collection and Interpretation of Behavioral Data

Experiments 1, 2, & 3 - Using seven response variables (5 categorical and 2 continuous), we quantified the reactions of turtles to the experimental trials. Categorical responses, defined below, were: (1) non-reactive (turtle was non-reactive y/n); (2) hesitated (turtle exhibited one or more hesitation behaviors (hesitated y/n); (3) entered tunnel (turtle navigated into the tunnel); (4) successful completion (turtle successfully navigated through the tunnel into the exit pen y/n); (5) successful completion with no hesitations (turtle successfully navigated through the tunnel into the exit pen and did not exhibit any hesitation behaviors y/n). Continuous variables were: (1) total number of hesitations, and (2) rate of travel in tunnel among successfully completed trials.

Non-reaction was defined as a failure to be exposed to any experimental stimulus presented during the trial period in a manner that was visible to experiment observers. Turtles that were non-reactive typically remained still for the duration of the trial or made limited movements and did not approach the tunnel entrance. Hesitating was defined as a turtle exhibiting one or more times during a trial any one of three distinct behaviors indicating hesitancy in the experimental arena: (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; (3) False start – a turtle entered the tunnel, and then returned back through the tunnel entrance without completing the trial. Entering was defined simply as a turtle moving into the tunnel, after which point it may or may not have gone on to successfully complete the trial. Successful completion was defined as navigating through the tunnel and into the exit pen in 60 minutes or less. Successful completion with zero hesitations occurred when turtles successfully completed trials and did so with zero hesitations. Once a turtle reached the exit pen or exceeded the 60-minute time limit it was removed from the trial. Turtles that did not emerge from the tunnel after 60 minutes into the exit pen were not considered to have successfully completed the trial.

The total number of hesitations observed was the pooled total number of the three hesitation behaviors (Bypass, Approach, and False start) considered collectively for each trial. Rate of travel in tunnel was the speed at which turtles moved through tunnels measured in feet per minute (FPM) and was analyzed for successfully completed trials only.

Evaluating the experimental factors of tunnel aperture, length, lighting level, and tunnel entrance design characteristics using multiple response variables allowed us to better understand the behavioral response of painted turtles so that results could be used to inform design recommendations for the construction of passage systems. We considered 3 of these response variables as primary measures by which to gauge the willingness of turtles to use a passage; (1) entered tunnel; (2) successfully completed trial; (3) successfully completed trial with zero hesitations. The least conservative measure was whether turtles entered the tunnel or not because it measured at a minimum, the willingness of a turtle to move out of the start pen and into the passage and included trials that were ultimately either unsuccessful or successful. The most conservative measure of success was that based on zero hesitations and completion of the trial in 60 minutes or less. This response variable assumed that a single hesitation would result in a turtle not using a real passage structure. Our rationale was to account for real life situations in which a turtle might hesitate and then travel in either direction and choose to pass or turn away from the tunnel. Hesitation behaviors were analyzed in order to provide a continuous scale for assessing the willingness of turtles to utilize various experimental tunnel designs.

Experiment 4 – We used two binomial responses to assess turtle behavior in the exclusion gate laboratory. These were; yes/no the turtle crossed the gate in the intended direction and yes/no the turtle subsequently returned and crossed the gate in the unintended direction. We measured the success of the exclusion gate by counting the number of turtles that went through the gate and were not able to return through it, in the allotted time.

Statistical Analysis

Preference of turtles for tunnels varying in aperture, length, entrance design, and available light in Experiments 1, 2, and 3 were assessed using Analysis of Variance (ANOVA) for continuous responses, and logistic regression for binary responses. Model selection was based on Akaike Information Criteria (AIC). Time to complete the trial was converted to a rate of travel (feet per minute) in order to standardize data for analysis, especially where two different tunnel lengths were tested in the same experiment. We used odds ratios and Tukey's HSD tests to further examine factors deemed significant. The two groups in Experiment 1 were tested separately. A t-test was used to compare means in Experiment 4. Data sets were manipulated using Microsoft Excel software, and statistical analyses were conducted in the R statistical environment (www.r-project.org). Alpha was set at 0.05 for all statistical tests. A summary of our study design and approach to statistical analyses is shown in Table 1.1.

Reducing potential biases

In the tunnel experiments, we provided turtles with a single tunnel, rather than a choice, because we wished to: (1) randomize tunnel start direction, (2) maintain a large and equal sample size per tunnel type, and (3) maintain consistency in methodology to that used in earlier studies conducted on painted turtles so that results may be compared.

In both the tunnel and fencing experiments, no food, water, or shelter was provided inside the pens so that turtles were motivated to leave the pen. Substrate of the pens was raked before each trial in order to remove vegetation and reduce any potential chemical trails left by turtles. Unless otherwise noted, each individual turtle was used in only a single experimental trial and individual turtles were only exposed to a test tunnel once in order to eliminate the effect of learning on movement behavior.

In addition to the variables described above, we also recorded potential covariates that might have affected the performance of turtles in the experimental trials. The covariates were weather, temperature inside and outside the experimental arena, experiment date, trial start time, turtle location at the trial start, each turtle's sex, age, carapace length, weight, and for females, whether or not they were gravid.

Results

Experiment 1 - Group 1 – Group 1 trials compared behavioral response of painted turtles for the 2 tunnel lengths, the 3 apertures, and 3 of the lighting levels (0% 75%, 100% transmission). Of the 452 trials, 347 turtles successfully passed through the tunnels, 98 did not, and 7 were non-reactive bringing the total number of trials for full analysis to 445 (Table 1.2). Tunnel length was a significant predictor of whether or not turtles were non-reactive during a trial ($P < 0.05$). Holding light levels and aperture constant, the odds of turtles being non-reactive were much greater in the 80 ft tunnel compared to 40 ft tunnel (Table 1.3). After analysis of non-reactive behavior, the 7 trials in which turtles were non-reactive were removed from the data set for the remainder of the analyses and were not included in Table 1.2.

When the response was frequency of trials where turtles entered the tunnel, light level, tunnel length, and the interaction between light level and tunnel aperture were all significant predictors ($P < 0.001$, $P < 0.05$, and $P < 0.01$ respectively) (Table 1.4). A greater percentage of turtles entered tunnels with treatments permitting 100% or 75% of overhead light and odds of entering were about 47 and 23 times greater than the odds of entering tunnels with the 0% light permitted treatment. A greater percentage of turtles entered tunnels that were shorter in length and the odds of turtles entering 80 ft long tunnels were about half those of entering 40 ft long tunnels (Table 1.3).

In the 0% light transmittance treatment there is a clear pattern of preference for the largest tunnel aperture. When overhead light was allowed to be transmitted, turtles showed a preference for smaller apertures and this pattern was stronger for 100%, compared to 75%, transmittance (Table 1.5 and Figure 1.7).

When the response was the frequency of successfully completed trials, there was a significant effect of light level, tunnel length, the interaction of light level and tunnel aperture, and the interaction of length and aperture ($P < 0.001$, $P < 0.001$, $P < 0.01$, $P < 0.05$ respectively) (Table 1.4). More turtles completed trials if 75% or 100% of the natural overhead light was transmitted, and the odds of success with these treatments was dramatically greater than when no light was transmitted. Turtles were much more likely to complete the trial in a 40 ft tunnel compared to an 80 ft tunnel (Table 1.3). With regard to the interaction between light treatment and tunnel aperture; turtles in trials subject to the 0% light permitted treatment had odds of success that increased with increasing tunnel aperture (Table 1.5 and Figure 1.8). Conversely, when 100% of the light was transmitted, the odds of success increased as aperture decreased.

For completed trials, tunnel length was a significant predictor of rate of travel through the tunnel ($P < 0.05$) (Table 1.6). Turtles had faster rates of travel through 80 ft tunnels compared to 40 ft long tunnels (Table 1.7).

Light level had a significant effect on the frequency of completing trials without hesitations (P < 0.001) (Table 1.4). The rate of success with zero hesitations increased as available light was increased (Figure 1.9) and the odds of success with zero hesitations for turtles subjected to the 100% and 75% light permitted treatments were 6.8 and 10.3 times (respectively) the odds for those subjected to the 0% light permitted treatment (Table 1.3).

Light level was a significant predictor (P < 0.001) of a hesitation behavior occurring one or more times during a trial (Table 1.4). Turtles in the 0% light permitted treatment group were more likely to exhibit hesitation behaviors versus those exposed to the 100% or 75% treatments (Table 1.3).

Light level, tunnel length, the 2-way interaction of light level and tunnel aperture, and the 3-way interaction of light level, tunnel length, and tunnel aperture were significant predictors of the mean total number of hesitations per trial (P < 0.001, P < 0.05, P < 0.01, P < 0.05 respectively) (Table 1.8). Significantly more hesitations were observed for trials with 0% light transmitted in comparison to 100% and 75% light transmitted (Table 1.9 and Figure 1.10). Turtles exhibited more hesitations in trials with 80 ft tunnels in comparison to trials in 40 ft tunnels (Table 1.2). With regard to the interaction between light and aperture, turtles exhibited more hesitations with the smaller aperture tunnels and there was a significant difference in the mean number of total hesitations between the 2 ft x 2 ft and 4 ft x 8 ft treatments when 0% of available overhead light was transmitted (Table 1.9). The number of hesitations increased significantly as aperture decreased but only for 0% light transmitted trials. The 3-way interaction between light, aperture, and length is a result of this same pattern differing where hesitations increase as aperture decreases in the 0% light treatment. The pattern grows more pronounced as tunnel length is increased until mean number of hesitations reaches its maximum (5.4) for trials in the 0% light treatment, 2 ft x 2 ft aperture, 80 ft long tunnel combination (Table 1.2 and Figure 1.10).

There was a significant effect of light level on the total number of hesitations among successfully completed trials ($P < 0.001$) (Table 1.6). More hesitations were observed for the 0% light permitted treatment in comparison to the either the 100% or 75% light permitted treatments (Table 1.10).

Group 2 – Group 2 trials compared behavioral response of painted turtles for 0% transmission to simulated median lighting across all 3 apertures for a single length tunnel (80 feet). Of the 143 turtles in Group 2, 66 successfully completed trials (Table 1.11). Rates of successful passage did not differ significantly between the 0% light transmitted and simulated median lighting treatments.

When the response was the frequency of successfully completed trials, there was a significant effect of tunnel aperture ($P < 0.05$) (Table 1.12). Rates of successful passage increased regardless of lighting treatment as tunnel aperture was enlarged. Odds of success were greater for the two larger aperture tunnel treatments (4 ft x 8 ft and 4 ft x 4 ft) in comparison to the 2 ft x 2 ft tunnel (Table 1.13).

Tunnel aperture was a significant predictor of successful completion with zero hesitations ($P < 0.05$) (Table 1.12). The odds of successful completion with zero hesitations increased with tunnel aperture and were greatest for the 4 ft x 8 ft aperture tunnel in comparison to either the 2 ft x 2 ft or 4 ft x 4 ft tunnels (Table 1.13).

When the response was the frequency of trials in which the turtle entered the tunnel, a hesitation behavior occurred one or more times, the mean total number of hesitations per trial, or the rate of travel in the tunnel there were no significant predictors.

Experiment 2 - Of the 70 trials in Experiment 2 where we tested the response of turtles to artificial lighting, 44 turtles successfully completed the trial, 23 did not, and 3 were non-reactive (Table 1.14). After analysis of non-reactive behavior, the 3 trials in which turtles were non-reactive were removed from the data set for the remaining analyses. These trials were removed because turtles failed to move through the field laboratory and thus failed to produce a measurable response to the experimental variables being tested.

Light level was a significant predictor ($P < 0.01$) of the frequency of trials in which test turtles entered the tunnel (Table 1.15 and Figure 1.11). In comparison to the 0% light permitted treatment, turtles were 6.6 times more likely to enter the tunnel with the artificial lighting treatment (Table 1.16). Light level was also a significant predictor ($P < 0.01$) of the frequency of successful completion of trials (Table 1.15). In comparison to the 0% light permitted treatment, turtles were 4.5 times more likely to successfully complete trials in tunnels containing artificial lighting (Table 1.16 and Figure 1.12).

Tunnel light level was a significant predictor of trials that were successfully completed with zero hesitations ($P < 0.01$). The rate of successful trial completion with zero hesitations was higher among trials with artificial lighting and the odds of successful completion with zero hesitations by turtles exposed to the artificial lighting treatment were 5.79 times those of turtles exposed to the 0% light permitted treatment (Table 1.16 and Figure 1.13).

When the response was frequency of trials where hesitations were observed, light level was a significant predictor ($P < 0.01$) (Table 1.15). Hesitations among test turtles were significantly more likely to be exhibited for the 0% light permitted treatment in comparison to the artificial lighting treatment (Table 1.16 and Figure 1.14). Light level was a significant predictor of the total number of hesitation behaviors per trial for groups including successfully completed and unsuccessfully completed trials ($P < 0.001$, Table 1.14), and for successfully completed trials considered separately ($P < 0.05$, Table 1.17) (Table 1.18). In both groups, turtles exhibited more hesitations per trial for the 0% light permitted treatment compared to the artificial lighting treatment.

Light level was not a significant predictor of the frequency of trials in which non-reactive behavior was observed or for the rate of travel in the tunnel.

Experiment 3 - Of the 108 trials in Experiment 3, where we tested the effects of tunnel entrance variables on turtle behavior, 87 turtles successfully navigated the arena to complete the trial and 21 did not (Table 1.19). There was a significant effect of both septum and entrance angle on the frequency of trials that were successfully completed without hesitations ($P < 0.05$) (Table 1.20). The odds of successful trial completion with zero hesitations were greater for trials without septa installed in comparison to those with septa installed and the odds of successful trial completion with zero hesitations were also greater for trials with the 45˚ entrance angle compared to turtles in trials with the 90˚ entrance angle (Table 1.21 and Figure 1.15). With regard to successful trial completion there was no significant interaction between septum and entrance angle.

The interaction of septum and entrance angle was significantly correlated with frequency of trials where turtles entered the tunnel ($P < 0.05$) (Table 1.20). With guidance septa installed, turtles were more likely to enter the 90˚ tunnels and without septa turtles were more likely to enter 45˚ tunnels (Table 1.21 and Figure 1.16).

Turtles were more likely to exhibit one or more hesitation behaviors when guidance septa were installed ($P<0.01$), and hesitation behaviors were observed more frequently in trials subject to the 90˚ entrance angle in comparison to the 45˚ entrance angle ($P<0.01$) (Table 1.21) (Table 1.20). When considering hesitations as the response variable there was not a significant interaction of septa use and entrance angle.

Septum use and entrance angle were significant predictors of the total number of hesitations observed per trial pooling successfully and unsuccessfully completed trials ($P< 0.01$ and $P< 0.05$ respectively) (Table 1.22). The odds of hesitating were greater in trials where the guidance septa were installed and turtles were more likely to hesitate in trials with the 90˚ entrance angle compared to trials with the 45˚ entrance angle (Table 1.21). We observed the highest mean number of hesitations when guidance septa were installed and there was a 90˚ entrance angle (Table 1.19 and Figure 1.17). For only successfully completed trials, the use of guidance septa resulted in significantly more hesitations (P<0.01) (Table 1.22) (Table 1.23). Neither septum use or angle of entrance were significant predictors for the frequency of trial success or rate of travel in the tunnel (Figure 1.18).

Experiment 4 - All turtles tested in the exclusion gate experiment successfully passed through the gate to the lower level and did not return to the upper level indicating the one-way gate functioned as intended.

Discussion

Our experiments were primarily designed to evaluate how tunnel aperture, length, available overhead light level, and entrance configuration might affect the relative effectiveness of road passage structures for freshwater turtles.

Our results of tests on the effects of tunnel length, aperture, and light transmittance indicate that transmittance of adequate ambient light through the tops of tunnels was the single most important determinant of willingness by turtles to enter and utilize a tunnel among trials. Conversely, turtles were reluctant to enter tunnels that failed to provide adequate ambient light levels by means of overhead light transmittance, aperture, length or a combination of these variables. Researchers have suggested that light level might be an important predictor of passage use by turtles but the importance of light availability remains largely unresolved (Jackson 2000, Woltz et al. 2008, Andrews et al. 2008). Light has been demonstrated to be of some importance for amphibians. Woltz (2008) found that leopard frogs and green frogs preferred an experimental tunnel with many small holes drilled in the top over a tunnel with no holes drilled in the top and Jackson and

Tyning (1989) found that spotted salamanders moved at faster rates through tunnels with an increased amount of light shined into the tunnel at the entrance or exit with a flashlight.

Available ambient light was a significant predictor of all 3 primary measures we used to assess tunnel utilization; (1) entering the tunnel; (2) successful completion of the trial, and (3) and successful completion of the trial, without hesitations. Light level was also an important predictor of hesitation measures considered separately for; (1) the mean total number of hesitations; and (2) whether or not one or more hesitations occurred during a trial. The highest rates of tunnel utilization and lowest frequencies of hesitation behaviors were observed in treatments that allowed the highest amount of overhead ambient light. Pooling tunnel length and aperture, turtles presented with tunnels where 100% of ambient light was transmitted from above were about 60 times (95% CI = 14.1-383.5) more likely to successfully complete a trial than turtles presented with 0% ambient light, and turtles presented with 75% ambient light were about 93 times (95% CI = 19.1-789.3) more likely to successfully complete a trial than turtles presented with the 0% light treatment (Table 1.3). The mean rate of successful passage examined across all dimensions in this experiment indicates major contrasts in response to light treatments (Table 1.9). Successful passage ranged from 91% for the 100% available ambient light permitted treatment to only 54 % for the 0% available ambient light permitted treatment. Our most conservative measure of willingness to utilize tunnels, success with zero hesitations, was heavily influenced by light level as well. Light level was the only significant predictor of this measure and in comparison to the 0% light treatment, turtles were 7 times and 10 times more likely to successfully navigate through tunnels without exhibiting any hesitations for the 100% and 75% light permitted treatments respectively (Table 1.3). It is unknown why turtles responded more favorably to 75% light than 100% light. These two treatments differed from each other only slightly in comparison to the difference between either of these treatments and the 0% light treatment. For practical purposes it is perhaps best to consider the two “bright” treatments (75% and 100%) in comparison to the single “dark” treatment (0%).

While the effects of lighting on various wildlife species willingness to use tunnels has not been well studied, Openness Ratio (OR = a culvert’s cross-sectional area divided by it’s length) and aperture have been important indicators. Clevenger (2005) measured the influence of a number of variables on the performance of passages by a suite of large mammals. High OR structures (shorter, larger aperture passages) strongly influenced the willingness of grizzly bears, wolves, elk, and deer while low OR structures (longer, smaller aperture passages) best explained passage use by black bears and cougars. Woltz et al. (2008) found that tunnel aperture was a significant predictor for 3 out of 4 species tested (leopard frogs, snapping turtles, and painted turtles) in a choice experiment. These species avoided the smallest aperture (0.3 m) culvert. While light was not examined explicitly in either of these examples, it is clearly tied to OR and aperture and likely to be influential.

In our experiments where light was a limiting factor, tunnel aperture and length were important predictors of turtle behavior and trial outcome. Tunnel length and the interaction of tunnel light level and aperture were significant predictors of both willingness to enter a tunnel and successful trial completion (Table 1.4). The influence of tunnel length and aperture can be attributed to their effect on the relative tunnel brightness and this is demonstrated by their lack of influence when lighting was not limited.

Pooling light treatment and aperture, turtles were half as willing to enter an 80 ft tunnel in comparison to the 40 ft tunnel (Table 1.3). This is because tunnels of widely varying actual light level are included in this sample and some of the 80 ft tunnels were considerably darker. For example, the 80 ft tunnels subject to the 0% light treatment were much darker inside than their 40 ft 0% light treatment counterparts and this affected the behavioral response of turtles. At the 80 ft tunnel length and 0% light permitted treatment there was a dramatic increase in the percentage of turtles that were willing to enter as well as successfully complete trials coinciding with an increase in tunnel aperture from 2 ft x 2 ft to 4 ft x 4 ft (Table 1.2). With this increase in aperture the percentage of turtles that were willing to enter the tunnel rose from 38% to 68% and the percentage of successful trial completion to rose from 14% to 54% (Table 1.9).

In comparing the odds of successful trial completion for different apertures within each light level, the importance of aperture as a predictor is demonstrated in a slightly different way. Among 0% light permitted trials, the odds of successful completion increased as aperture grew larger (Table 1.5). However, amongst the 75% and 100% light permitted trials where light levels were not as dependent on tunnel dimensions, the odds of successful passage were higher for the smallest aperture tunnel compared to their larger counterparts (Table 1.5). The reasons for this result are not known but suggest there may be some optimum combination of tunnel size and light treatment rather than a simpler "bigger and brighter is better" rule at work.

A complex pattern of successful trial completion in relation to experimental variables was responsible for the significant interaction between length and aperture. Among trials subject to the 100% and 75% light treatments in 80 ft tunnels, trials subject to the smallest aperture, 2 ft x 2 ft, had significantly higher rates of successful passage than trials subject to the large aperture 4 ft x 8 ft tunnels (Table 1.2). While this seemingly counterintuitive response remains unexplained, it suggests there may be some critical tradeoff between tunnel aperture size and light levels that warrants further exploration.

It was hypothesized that the rate of travel in tunnels might be influenced by light level, aperture, or length. Although this was technically the case and length was a significant predictor of rate of travel among successfully completed trials, FPM (Feet Per Minute) was only slightly higher for 80 ft tunnels compared to 40 ft long tunnels (Table 1.10 and Table 1.7) so this may have limited practical application.

Analysis of the effects of experimental variables on the mean total number of hesitations per trial provides an additional relative measure of just how strongly test turtles react to particular experimental treatments. Significant predictors of the mean total number of hesitations per trial were light level, tunnel length, the 2-way interaction of light level and tunnel aperture, and the 3-way interaction of light level, tunnel length, and tunnel aperture. Turtles exhibited about 3 times more hesitations for 0% light permitted trials in comparison to 75% and 100% light permitted trials (Table 1.9). The mean number of hesitations per trial increased steadily as aperture was reduced among 0% light permitted trials for both lengths (Table 1.9). The maximum number of 5.4 mean total hesitations per trial was observed amongst trials in the 80 ft long, 0% available overhead ambient light permitted, 2 ft x 2 ft aperture tunnel (Table 1.2). This particular combination of light, aperture and length treatments resulted in a tunnel that was darker than all others tested in this experiment and was consistently the poorest performing in all measures examined. Even among successfully completed trials, the mean number of hesitations was roughly twice as high for 0% light permitted trials in comparison to either 75% or 100% light permitted trials (Table 1.10).

Our results of tests on the effects of simulated median lighting indicate that transmittance of light with the simulated median lighting treatment had no significant effect on the effectiveness of tunnels with the 0% available ambient light treatment. It was hypothesized that allowing light to enter at the center of the tunnel, in a manner analogous to light entering through storm grates in a roadway median strip, might result in higher rates of utilization in comparison to the 0% available ambient light treatment. However, results for the simulated median lighting treatment and the 0% available ambient light treatment were essentially the same as was observed in Group 1 among trials of the 0% available ambient light treatment.

Tunnel aperture was a significant predictor of successful trial completion and successful trial completion with zero hesitations in Group 2 (Table 1.12). There were no other significant predictors for this group. We observed a clear pattern of success increasing as tunnel opening size increased. As was discussed for the previous experimental group, this may be attributed to the increase in light levels inside tunnels that occurs as tunnel aperture is increased.

In tests on the effects of artificial lighting turtles responded favorably to the artificial lighting treatment. When exposed to this treatment, test turtles performed markedly better than their 0% light counterparts and successfully navigated passages at rates comparable to those observed in Experiment 1 for trials subjected to the 100% available ambient light treatment (Table 1.10). This result suggests that artificial lighting may be a viable means of: (a) retrofitting existing tunnels and culverts that are prohibitively dark, and (b) bringing ample light levels to small aperture closed-top tunnels. However, reservations remain concerning the use of artificial lighting since it is unknown how other wildlife species might react. It is also not known if lighting should be left on day and night or only during daylight hours and how a lighting system should be controlled in terms of brightness level and timing. Additionally we have concerns that the maintenance of lighting may be logistically difficult.

Light level was a significant predictor of all 3 primary measures that we used to gauge utilization (Table 1.15); (1) entering the tunnel; (2) successful trial completion; and (3) and successful trial completion with zero hesitations.

In comparison to trials where turtles were exposed to the 0% light treatment, turtles exposed to the artificial light treatment were 6.5 times more likely to enter the tunnel; 4.5 times more likely to successfully complete a trial; and 5.8

times more likely to successfully complete a trial in the artificially illuminated tunnel treatment group (Table 1.16).

The mean total number of hesitations was inversely related to light level and turtles exhibited about three times as many hesitations in trials subject to the 0% light treatment in comparison to turtles in trials subject to the artificial light treatment (Table 1.14). Turtles were more than 5 times as likely to exhibit at least one hesitation per trial for the 0% light permitted treatment versus the artificial lighting treatment (Table 1.16).

While we did not conduct a formal comparison, it is useful to put the results of the artificial lighting trials in this experiment in the context of results for trials of the 100% light permitted treatment from Experiment 1 using tunnels of the same dimensions (2 ft x 2 ft x 80 ft) (Table 1.14). The mean number of hesitations and the percent of trials where turtles were willing to enter the tunnel were nearly identical for these groups. In comparing successful trial completion and successful trial completion with zero hesitations, test turtles exposed to the artificial light treatment exhibited rates of success that were only about 10% less than those for test turtles subjected to the 100% light treatment.

In tests on the effects of tunnel entrance angle and septa use neither varying the tunnel entrance angle or using guidance septa affected the willingness of turtles to use passages in our experimental laboratory (Table 1.20). We had hypothesized that the wider tunnel entrance design (45˚) and the guidance septa would function to guide turtles into the mouth of the tunnel and aid in achieving a higher rate of successful passage. Guidance septa did in fact guide turtles to the mouth of the tunnel but failed with regard to directing them to enter or significantly alter rates of successful trial completion.

When the response was success with zero hesitations, both septum treatment and angle of entrance were significant predictors. This result considered alone could be misleading because neither the septum treatment or angle of entrance were significant predictors of successful trial completion. Holding entrance angle constant, the percent of successfully completed trials was 83% with septa and 78 % without septa and holding septum use constant percent success differed little between trials subject to the 45˚ entrance angle compared to those subject to the 90˚ entrance angle (84% and 77% respectively) (Table 1.19).

We tested septa in the field laboratory to determine if they could be used as a means of briefly interrupting turtles at the mouth of the tunnel and causing them to hesitate in hope that they would be directed into the tunnel rather than bypass it. It is worth noting that there were two different configurations of septum and angle of entrance that increased the odds that turtles would enter tunnels but did not significantly affect rates of successful trial completion. The odds of success with zero hesitations were about half as great in trials with septa in place versus trials without septa (Table 1.21) but the significance of this result can be attributed to the hesitations that resulted from turtles reacting to the septum and entrance angle treatments. As described above, the septa were successful in directing turtles into tunnel entrances but this was not enough of a "push" to alter the frequency of successfully completed trials.

Overall, turtles exhibited a higher frequency of mean hesitations per trial among trials with septa in comparison to trials without septa (Table 1.24). When septa were coupled with a 90˚ entrance turtles most often entered the tunnel briefly to move around the septa. This combination led to turtles being more than 4 times more likely to hesitate in trials with a 90˚ angle of entrance versus a 45˚ angle of entrance (Table 1.21). In trials without the septa installed, fewer turtles were willing to enter the tunnel when the 90˚ entrance angle was used in comparison to the 45˚ entrance angle (Table 1.19). This was a result of the tendency by turtles to follow the more gradual contour of the 45˚ turns and move into the tunnel momentarily.

Ultimately, neither septum nor entrance angle success were significant predictors of success and it was probably the turtles perception of the tunnel itself that mattered. That being said, there are other reasons such as structural and load bearing concerns that the angle of entrance may need to 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.

Our results of tests on the effects of the turtle exclusion gate indicate the gate functioned as it was designed to. 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 inch 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 inch height we tested, with a "no-grip" polished surface that prevents climbing, may be the best option.

Conclusions/Management Implications

Our findings have a number of implications for informing the design of under-road passage systems for freshwater turtles as well as highlighting elements of design that may benefit from additional research. One, our findings indicate the importance of designing road passage structures that provide adequate lighting for freshwater turtles. Of the tunnel design variables examined, transmitted light was more important than tunnel opening size and length in promoting movement of painted turtles.

Two, our results indicate that artificial lighting may be nearly as effective as 100% natural light in encouraging turtles to pass through tunnels. 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 likely be serving many species in addition to turtles, research that explicitly investigates these concerns is well warranted. There are significant cost constraints at work driving the selection of smaller than optimum structures and the use of artificial lighting has the potential to provide the benefits of more traditionally recommended large and expensive structures within a budget that is generally more palatable.

Three, in our tests neither varying the angle of entrance or installing guidance septa had any significant effect on successful completion of trials by turtles. Employing wider tunnel entrances and using septa did in fact guide turtles into the mouth of the tunnel but failed to significantly improve the frequency of tunnel entry or trial success and make tunnels more effective.

Four, the exclusion gate, as tested, appears to work well and is a simple and straightforward means of allowing one-way passage into and out of areas. The exclusion gate or "jump out" gate is frequently employed in passage systems for large mammals as a means of allowing them to safely escape a fenced road corridor should they access it by entering at the end of a fence. Such a device may too be useful in allowing turtles that have trespassed onto the fenced-in roadway to safely escape to the surrounding environment.

In an actual road passage system, a turtle that hesitates to use a tunnel even once and moves on without passing through could potentially lose its life on the road. For this reason, managers looking for some guidance on the design of effective passage systems may want to consider rates of success with zero hesitations as a conservative base measure of performance to provide indication of levels of minimal potential use. At the same time, it is important to recognize that the physiological state of turtles used in these experiments and they way in which turtles were placed in experimental arenas against their will could affect results as well.

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. Large aperture closed top structures will still provide adequate passage for most of a population upon first interaction with the crossing structure. Over time, the rate of successful passage may increase due to acclimation. Further testing of this hypothesis is needed.

Table 1.1 Study design and analysis approach for experiments examining tunnel lighting level, entrance angle, and septum, and effectiveness of exclusion gate on the movement behavior of 3 turtle species.

Experiment purpose (number of trials)	Experimental variables	Response variables	Data treatment
Experiment 1 Test relative importance of varying natural lighting level and tunnel size with regard to movement behavior (n=595)	Aperture -2ft x 2ft -4ft x 4ft -4ft x 8ft Length 40ft 80ft Light level (Group 1) -100% - 75% - 0% Light level (Group 2) -simulated median - 0%	Categorical data: - non-reactive y/n - hesitated y/n - successful completion y/n -successful completion with zero hesitations y/n	GLM with logit link
		Continuous: - total trial time - total number of hesitations - rate of travel in tunnel	ANOVA
Experiment 2 Test effectiveness of artificial lighting (n=70)	Aperture -2ft x 2ft Length -40ft Light level -artificial - 0%	Categorical: - non-reactive y/n - hesitated y/n - successful completion y/n - successful completion with zero hesitations y/n	GLM with logit link
		Continuous: - total trial time - total number of hesitations - rate of travel in tunnel	ANOVA
Experiment 3 Test relative importance of varying entrance angle, and septum (n=108)	Entrance angle -45 deg -90 deg Guidance septa used -yes -no	Categorical: - non-reactive y/n - hesitated y/n - successful completion y/n - successful completion with zero hesitations y/n	GLM with logit link
		Continuous: - total trial time - total number of hesitations - rate of travel in tunnel	ANOVA
Experiment 4 Test effectiveness of exclusion gate (n=28)	N/A	Categorical only: - turtle crossed gate in the intended direction y/n -turtle subsequently returned crossing gate in unintended direction y/n	T-test

Table 1.2 Mean values in Experiment 1, Group 1 for total number of hesitation behaviors observed (Mnhes), percent of trials where turtles entered the tunnel (%enter), percentage of trials that were successfully completed (%success), and % of trials that were successfully completed with 0 hesitation behaviors observed

Tunnel Length	Light Treatment	Aperture	n	Mnhes (sd)	%enter	%success	%nohes
40 ft (n = 223) (Mnhes (sd) = 1.5 (2.3)) (%enter = 88 %) (%success = 84 %) (%nohes = 49 %)	0% light permitted	2x2 ft	26	3.2 (3.2)	65%	58%	15%
		4x4 ft	23	2.4 (3.0)	70%	61%	44%
		4x8 ft	27	2.3 (3.0)	85%	70%	41%
		All apertures	76	2.6 (3.1)	74 %	63%	33 %
	75% light permitted	2x2 ft	25	0.6 (0.8)	100%	100%	60%
		4x4 ft	26	1.7 (2.6)	85%	81%	42%
		4x8 ft	24	0.5 (0.8)	100%	100%	71%
		All apertures	76	0.9 (1.7)	95 %	93 %	57 %
	100% light permitted	2x2 ft	25	1.0 (1.1)	100%	100%	48%
		4x4 ft	22	0.6 (0.7)	95%	95%	55%
		4x8 ft	25	0.8 (1.9)	96%	96%	72%
		All apertures	72	0.8 (1.3)	97%	97 %	58 %
80 ft (n = 222) (Mnhes (sd) = 1.9 (2.6)) (%enter = 82 %) (%success = 72 %) (%nohes = 41 %)	0% light permitted	2x2 ft	21	5.4 (4.2)	38%	14%	10%
		4x4 ft	28	3.1 (3.0)	68%	54%	25%
		4x8 ft	23	2.0 (2.0)	91%	61%	26%
		All apertures	72	3.4 (3.4)	67 %	44 %	21 %
	75% light permitted	2x2 ft	25	0.9 (1.4)	92%	92%	60%
		4x4 ft	26	0.9 (1.3)	92%	85%	50%
		4x8 ft	25	1.3 (1.8)	80%	76%	48%
		All apertures	76	1.0 (1.5)	82 %	72 %	41 %
	100% light permitted	2x2 ft	25	1.0 (1.5)	96%	88%	52%
		4x4 ft	25	1.4 (0.5)	88%	88%	56%
		4x8 ft	24	1.4 (1.4)	83%	79%	37%
		All apertures	74	1.2 (1.7)	89 %	85 %	49 %

Table 1.3 Logistic regression, odds ratio for 5 response variables in Experiment 1, Group 1 (probability modeled is non-reactive = 0, hesitated = 0, entered = 0, success = 0, success with no hesitations = 0).

Response	Effect	Point estimate	Wald 95% confidence limits	
Non-reactive	Intercept	7.68e-03	0.0004	3.87e-02
	100% AL vs. 0% AL	1.67e-09	NA	2.06e+296
	75% AL vs. 0% AL	7.25e-01	0.14	3.37e+00
	80 ft vs. 40 ft	6.14e+00	1.03	1.17e+02
Hesitated	Intercept	2.81	1.62	4.97
	100% AL vs. 0% AL	0.37	0.23	0.60
	75% AL vs. 0% AL	0.33	0.21	0.54
	80 ft vs. 40 ft	0.92	0.47	1.81
	4x4 ft vs. 2x2 ft	0.85	0.43	1.66
	4x8 ft vs 2x2 ft	0.45	0.23	0.87
	80 ft and 4x4 ft vs. 40 ft and 2x2 ft	1.15	0.45	2.95
	80 ft and 4x8 ft vs. 40 ft and 2x2 ft	2.69	1.04	7.03
Entered	Intercept	1.54	0.82	2.95
	100% AL vs. 0% AL	47.01	8.96	869.71
	75% AL vs. 0% AL	22.98	6.04	151.89
	80 ft vs. 40 ft	0.51	0.28	0.90
	4x4 ft vs. 2x2 ft	2.10	0.91	4.93
	4x8 ft vs 2x2 ft	6.78	2.52	20.69
	100% AL and 4x4 ft vs. 0% AL and 2x2 ft	0.11	<0.00	0.90
	75% AL and 4x4 ft vs. 0% AL and 2x2 ft	0.15	0.02	0.87
	100% AL and 4x8 ft vs. 0% AL and 2x2 ft	0.03	<0.00	0.22
	75% AL and 4x8 ft vs. 0% AL and 2x2 ft	0.05	0.01	0.36

Table 1.3 Continued

Success	Intercept	1.47	0.69	3.24
	100% AL vs. 0% AL	59.70	14.12	383.47
	75% AL vs. 0% AL	92.85	19.08	789.25
	80 ft vs. 40 ft	0.09	0.02	0.32
	4x4 ft vs. 2x2 ft	1.01	0.35	2.89
	4x8 ft vs 2x2 ft	2.43	0.82	7.46
	100% AL and 4x4 ft vs. 0% AL and 2x2 ft	0.14	0.02	0.96
	75% AL and 4x4 ft vs. 0% AL and 2x2 ft	0.04	<0.00	0.25
	100% AL and 4x8 ft vs. 0% AL and 2x2 ft	0.07	0.01	0.44
	75% AL and 4x8 ft vs. 0% AL and 2x2 ft	0.05	<0.00	0.33
	80 ft and 4x4 ft vs. 40 ft and 2x2 ft	8.57	1.91	48.81
	80 ft and 4x8 ft vs. 40 ft and 2x2 ft	3.22	0.67	19.07
Success w/no hesitations	Intercept	0.148	0.536	0.343
	100% AL vs. 0% AL	6.84	2.59	20.57
	75% AL vs. 0% AL	10.26	3.88	31.10
	80 ft vs. 40 ft	0.98	0.47	2.01
	4x4 ft vs. 2x2 ft	3.63	1.21	12.08
	4x8 ft vs 2x2 ft	5.37	1.82	17.67
	100% AL and 4x4 ft vs. 0% AL and 2x2 ft	0.36	0.09	1.30
	75% AL and 4x4 ft vs. 0% AL and 2x2 ft	0.17	0.04	0.59
	100% AL and 4x8 ft vs. 0% AL and 2x2 ft	0.38	0.10	1.40
	75% AL and 4x8 ft vs. 0% AL and 2x2 ft	0.31	0.08	1.15
	80 ft and 4x4 ft vs. 40 ft and 2x2 ft	0.90	0.34	2.39
	80 ft and 4x8 ft vs. 40 ft and 2x2 ft	0.36	0.13	0.98

Table 1.4 Generalized linear model results indicating the importance of predictor variables in relation to 5 categorical response variables in Trial Group 1 of Experiment 1.

Response	Effect	Df	Deviance	Resid. Df	Resid. Dev	P
Non-reactive	Null	-	-	17	15.67	-
	Light	2	5.67	15	9.99	0.06
	Length	1	3.99	14	6.00	0.05*
Hesitated	Null	-	-	17	42.17	-
	Light	2	23.80	15	18.38	<0.001***
	Length	1	2.27	14	16.11	0.13
	Aperture	2	1.88	12	14.23	0.39
	Length x Aperture	2	4.90	10	9.33	0.09
Entered	Null	-	-	17	75.66	-
	Light	2	35.42	15	40.25	<0.001***
	Length	1	4.93	14	35.31	0.03*
	Aperture	2	3.61	12	31.70	0.16
	Light x Aperture	4	17.60	8	14.10	0.001**
Success	Null	-	-	17	116.42	-
	Light	2	71.04	15	45.38	<0.001***
	Length	1	13.96	14	31.42	<0.001***
	Aperture	2	1.03	12	30.39	0.60
	Light x Aperture	4	14.28	8	16.11	0.006*
	Length x Aperture	2	8.36	6	7.75	0.02*
Success w/no hesitations	Null	-	-	17	53.71	-
	Light	2	30.46	15	23.25	<0.001***
	Length	1	3.72	14	19.53	0.054
	Aperture	2	2.24	12	17.28	0.33
	Light x Aperture	4	8.83	8	8.46	0.07
	Length x Aperture	2	5.06	6	3.40	0.08

Table 1.5 Logistic regression, odds ratio for 2 response variables in Experiment 1, Group 1 and are presented by aperture within each light treatment (probability modeled is entered = 0, success = 0).

Response	Light treatment	Effect	Point estimate	Wald 95% confidence limits	
Entered	0% light permitted	Intercept	1.14	0.64	2.03
		4x4 vs. 2x2	1.92	0.85	4.44
		4x8 vs. 2x2	6.45	2.43	19.49
	75% light permitted	Intercept	24.00	7.45	146.88
		4x4 vs. 2x2	0.32	0.05	1.47
		4x8 vs. 2x2	0.37	0.05	1.80
	100% light permitted	Intercept	49.00	10.75	867.51
		4x4 vs. 2x2	0.22	0.01	1.55
		4x8 vs. 2x2	0.18	0.01	1.17
Success	0% light permitted	Intercept	0.62	0.34	1.11
		4x4 vs. 2x2	2.12	0.95	4.83
		4x8 vs. 2x2	3.13	1.38	7.31
	75% light permitted	Intercept	24.00	7.45	146.88
		4x4 vs. 2x2	0.20	0.03	0.83
		4x8 vs. 2x2	0.30	0.04	1.37
	100% light permitted	Intercept	15.67	5.75	64.50
		4x4 vs. 2x2	0.69	0.13	3.28
		4x8 vs. 2x2	0.46	0.09	1.85

Table 1.6 Analysis of variance model results indicating the importance of predictor variables in relation to the mean total number of hesitation behaviors and rate of travel is measured in feet per minute (FPM) observed in Trial Group 1 of Experiment 1.

Response	Effect	Df	Sum Sq	Mean Sq	F value	P
FPM	Light	2	111	55.71	3.01	0.05
	Length	1	86	86.13	4.65	<0.05*
	Residuals	343	6348	18.51	-	-
Total hesitations	Light	2	29.3	14.67	8.15	<0.001***
	Residuals	344	619.6	1.80	-	-

Table 1.7 Mean values in Experiment 1, Group 1 for 2 length treatments amongst successfully completed trials only. Mean values are given for total number of hesitations behaviors observed (Mnhes), total trial times, and the rate of travel in feet per minute (FPM).

Tunnel Length	n	Mnhes (sd)	Mean total trial time (SD)	Mean FPM (SD)
40 ft	188	0.80 (1.30)	33.07 (20.71)	12.52 (4.07)
80 ft	159	0.88 (1.45)	27.13 (21.24)	13.46 (4.62)

Table 1.8 Analysis of variance model results indicating the importance of predictor variables in relation to the mean total number of hesitation behaviors observed in Trial Group 1 of Experiment 1

Response	Effect	Df	Sum Sq	Mean Sq	F value	P
Total Hesitations	Light	2	401.8	200.9	41.69	<0.001***
	Length	1	21.6	21.6	4.48	<0.05*
	Aperture	2	25.2	12.6	2.62	0.07
	Light x Length	2	8.7	4.34	0.90	0.41
	Light x Aperture	4	91.5	22.9	4.75	<0.001***
	Length x Aperture	2	7.0	3.5	0.72	0.49
	Light x Length x Aperture	4	49.9	12.5	2.59	<0.05
	Residuals	427	2058.0	4.8	-	-

Table 1.9 Mean values in Experiment 1, Group 1 for total number of hesitations behaviors observed (Mnhes), percent of trials where turtles entered the tunnel (%enter), percent of trials that were successfully completed (%success), and percent of trials that were successfully completed with zero hesitation behaviors observed (%nohes).

Light Treatment	Aperture	n	Mnhes (sd)	%enter	%success	%nohes
0% light permitted	2x2 ft	47	4.2 (3.8)	53%	38 %	13 %
	4x4 ft	51	2.8 (3.0)	69%	57 %	33 %
	4x8 ft	50	2.1 (2.6)	88%	66 %	34 %
	All apertures	148	3.0 (3.2)	70 %	54 %	27 %
75% light permitted	2x2 ft	50	0.8 (1.1)	96 %	96 %	60 %
	4x4 ft	52	1.3 (2.0)	88 %	83 %	46 %
	4x8 ft	49	0.9 (1.4)	90 %	88 %	59 %
	All apertures	151	1.0 (1.6)	91 %	89 %	55 %
100% light permitted	2x2 ft	50	1.0 (1.3)	98 %	94 %	50 %
	4x4 ft	47	0.9 (1.6)	91 %	92 %	55 %
	4x8 ft	49	1.1 (1.7)	90 %	88 %	55 %
	All apertures	146	1.0 (1.5)	93 %	91 %	53 %

Table 1.10 Mean values in Experiment 1, Group 1 for 3 light treatments amongst successfully completed trials only. Mean values are given for total number of hesitations behaviors observed (Mnhes), total trial times, and the rate of travel in feet per minute (FPM).

Light Treatment	n	Mnhes (sd)	Mean total trial time (sd)	Mean FPM (sd)
0% light permitted	80	1.36 (2.00)	26.24 (19.20)	13.69 (4.74)
75% light permitted	134	0.63 (0.97)	33.77 (21.54)	12.28 (4.16)
100% light permitted	133	0.74 (1.17)	29.37 (21.44)	13.18 (4.22)

Table 1.11 Mean values in Experiment 1, Group 2 for total number of hesitations behaviors observed (Mnhes), percent of trials where turtles entered the tunnel (%enter), percent of trials that were successfully completed (%success), and percent of trials that were successfully completed with zero hesitation behaviors observed (%nohes).

Light Treatment	Aperture	n	Mnhes (sd)	%enter	%success	%nohes
0% light permitted	2x2 ft	27	3.7 (3.3)	70 %	30 %	4 %
	4x4 ft	18	2.9 (2.0)	61 %	50 %	11 %
	4x8 ft	24	3.9 (3.6)	67 %	54 %	17 %
	All apertures	69	3.6 (3.1)	67 %	43 %	10%
Simulated median lighting	2x2 ft	24	4.6 (4.4)	63 %	33 %	8 %
	4x4 ft	26	4.4 (3.9)	65 %	54 %	23 %
	4x8 ft	24	2.1 (2.7)	83 %	58 %	29 %
	All apertures	74	3.7 (3.8)	70 %	49 %	20 %

Table 1.12 Generalized linear model results indicating the importance of predictor variables in relation to 4 categorical response variables in Trial Group 2 of Experiment 1. Stepwise AIC was used to select the reduced models depicted.

Response	Effect	Df	Deviance	Resid. Df	Resid. Dev	P
Hesitated	Null	-	-	5	4.65	-
	Light	1	1.9	4	2.72	0.17
Entered	Null	-	-	5	3.78	-
	Light	1	0.21	4	3.56	0.64
Success	Null	-	-	5	7.48	-
	Aperture	2	7.25	3	0.23	<0.05*
Success w/no hesitations	Null	-	-	5	9.21	-
	Light	1	2.88	4	6.34	0.09
	Aperture	2	6.32	2	0.02	<0.05*

Table 1.13 Logistic regression, odds ratio for 2 response variables in Experiment 1, Group 2 (probability modeled is success = 0, success with no hesitations = 0).

Response	Effect	Point estimate	Wald 95% confidence limits	
Success	Intercept	0.46	0.25	0.81
	4x4 vs. 2x2	2.40	1.05	5.62
	4x8 vs. 2x2	2.81	1.25	6.51
Success w/no hesitations	Intercept	0.04	0.008	0.129
	4x4 vs. 2x2	3.30	0.875	6.294
	4x8 vs. 2x2	4.77	1.360	22.384

Table 1.14 Mean values in Experiment 2 for total number of hesitations behaviors observed (Mnhes), percent of trials where turtles entered the tunnel (%enter), percent of trials that were successfully completed (%success), and percent of trials that were successfully completed with zero hesitation behaviors observed (%nohes).

Light Treatment	n	Mnhes (sd)	%enter	%success	%nohes
0% light permitted	28	3.2 (2.7)	57 %	46 %	11 %
Artificial lighting	39	1.0 (1.3)	90 %	79 %	41%
100% light permitted (Exp. 1)	25	1.0 (1.5)	96%	88%	52%

Table 1.15 Generalized linear model results indicating the importance of predictor variables in relation to 5 categorical response variables in Experiment 2.

Response	Effect	Df	Deviance	Resid. Df	Resid. Dev	P
Non-reactive	Null	-	-	1	0.72	-
	Light	1	0.72	0	0	0.4
Hesitated	Null	-	-	1	8.02	-
	Light	1	8.02	0	0	<0.01**
Entered	Null	-	-	1	9.62	-
	Light	1	9.62	0	0	<0.01**
Success	Null	-	-	1	7.93	-
	Light	1	7.93	0	0	<0.01**
Success w/no hesitations	Null	-	-	1	8.03	-
	Light	1	8.03	0	0	<0.01**

Table 1.16 Logistic regression, odds ratio for 4 response variables in Experiment 2 (probability modeled is hesitated = 0, entered = 0, success = 0, success with no hesitations = 0).

Response	Effect	Point estimate	Wald 95% confidence limits	
Hesitated	Intercept	6.00	2.32	20.42
	Artificial vs. 0% AL	0.19	0.05	0.62
Entered	Intercept	1.33	0.63	2.88
	Artificial vs. 0% AL	6.56	1.96	26.48
Success	Intercept	0.87	0.41	1.82
	Artificial vs. 0% AL	4.47	1.57	13.68
Success w/no hesitations	Intercept	0.12	0.29	0.34
	Artificial vs. 0% AL	5.79	1.67	27.29

Table 1.17 Mean values Experiment 2 amongst successfully completed trials only. Mean values are given for total number of hesitations behaviors observed (Mnhes), total trial times, and the rate of travel in feet per minute (FPM).

Light Treatment	n	Mnhes (sd)	Mean total trial time (SD)	Mean FPM (SD)
Artificial lighting	13	0.81 (1.22)	18.32 (9.98)	11.38 (16.06)
0% light permitted	31	1.85 (1.34)	30.00 (11.49)	6.96 (9.96)

Table 1.18 Analysis of variance model results indicating the importance of predictor variables in relation to the mean total number of hesitation behaviors observed in Experiment 2.

Response	Effect	Df	Sum Sq	Mean Sq	F value	P
Total Hesitations (all trials)	Light	1	86.8	86.8	21.3	<0.001 ***
	Residuals	68	276.7	4.1	-	-
Total Hesitations (successful trials only)	Light	1	9.9	9.9	6.3	<0.05
	Residuals	42	66.5	1.58	-	-

Table 1.19 Mean values in Experiment 3 for total number of hesitations behaviors observed (Mnhes), percent of trials where turtles entered the tunnel (%enter), percent of trials that were successfully completed (%success), and percent of trials that were successfully completed with zero hesitation behaviors observed (%nohes).

Septum Treatment	Entrance Angle	n	Mnhes (sd)	%enter	%success	%nohes
With septa	90 degrees	25	0.9 (1.1)	88 %	88 %	44 %
	45 degrees	23	0.4 (0.8)	78 %	78 %	65 %
	All Angles	48	0.7 (1.0)	83 %	83 %	54 %
Without septa	90 degrees	32	0.3 (0.6)	69 %	69 %	62 %
	45 degrees	28	0.1 (0.4)	89 %	89 %	85 %
	All Angles	60	0.2 (0.5)	78 %	78 %	73 %

Table 1.20 Generalized linear model results indicating the importance of predictor variables in relation to 4 categorical response variables in Experiment 3. Stepwise AIC was used to select the reduced models depicted.

Response	Effect	Df	Deviance	Resid. Df	Resid. Dev	P
Hesitated	Null	-	-	3	18.3	-
	Septum	1	9.76	2	8.54	<0.01**
	Entrance angle	1	8.38	1	0.16	<0.01**
Entered	Null	-	-	3	4.45	-
	Septum	1	0.35	2	4.10	0.55
	Entrance angle	1	0.25	1	3.85	0.62
	Septum x Entrance angle	1	3.85	0	0	<0.05*
Success	Null	-	-	3	4.16	-
	Septum	1	0.75	2	3.41	0.38
	Entrance angle	1	0.57	1	2.83	0.45
	Septum x Entrance angle	1	2.83	0	0	0.09
Success w/no hesitations	Null	-	-	3	10.77	-
	Septum	1	4.30	2	6.48	<0.05*
	Entrance angle	1	6.26	1	0.22	<0.05*

Table 1.21 Logistic regression, odds ratio for 4 response variables in Experiment 3 (probability modeled is hesitated = 0, entered = 0, success = 0, success with no hesitations = 0).

Response	Effect	Point estimate	Wald 95% confidence limits	
Hesitated	Intercept	0.06	0.02	0.16
	Y septa vs. N septa	4.83	1.86	13.69
	90 vs. 45 deg	4.16	1.56	12.34
Entered	Intercept	6.25	2.43	21.22
	Y septa vs. N septa	0.48	0.11	1.92
	90 vs. 45 deg	0.35	0.09	1.22
	Y septa w/90 vs. N septa w/45 deg	6.94	1.00	57.37
Success	Intercept	6.25	2.43	21.22
	Y septa vs. N septa	0.61	0.13	2.60
	90 vs. 45 deg	0.35	0.09	1.22
	Y septa w/90 vs. N septa w/45 deg	5.48	0.76	46.24
Success w/no hesitations	Intercept	5.18	2.40	12.37
	Y septa vs. N septa	0.40	0.17	0.92
	90 vs. 45 deg	0.35	0.14	0.80

Table 1.22 Analysis of variance model results indicating the importance of predictor variables in relation to the mean total number of hesitation behaviors observed in Experiment 3. Stepwise AIC was used to select the reduced models depicted.

Response	Effect	Df	Sum Sq	Mean Sq	F value	P
Total Hesitations (all trials)	Septum	1	6.12	6.12	11.60	<0.001***
	Entrance angle	1	3.37	3.37	6.38	<0.05*
	Septum x Entrance angle	1	1.03	1.03	1.95	0.17
	Residuals	104	54.91	0.53	-	-
Total Hesitations (successful trials only)	Septum	1	4.75	4.75	9.25	<0.01**
	Entrance angle	1	1.16	1.16	2.27	0.14
	Residuals	84	43.08	0.513	-	-

Table 1.23 Mean values for Experiment 3 amongst successfully completed trials only. Mean values are given for total number of hesitations behaviors observed (Mnhes), total trial times, and the rate of travel in feet per minute (FPM).

Treatment	n	Mnhes (sd)	Mean total trial time (SD)	Mean time in tunnel (SD)
With guidance septa	40	0.57 (0.96)	18.35 (12.74)	85.50 (32.97)
Without guidance septa	47	0.11 (0.43)	18.36 (12.55)	94.68 (54.71)

Table 1.24 Mean values for Experiment 3 given for total number of hesitations behaviors observed (Mnhes), total trial times, and the rate of travel in feet per minute (FPM).

Treatment	n	Mnhes (sd)	Mean total trial time (SD)	Mean time in tunnel (SD)
With guidance septa	48	0.65 (0.97)	25.29 (19.51)	71.25 (44.03)
Without guidance septa	60	0.17 (0.49)	28.28 (23.03)	74.17 (62.29)
90 degree entrance angle	57	0.54 (0.89)	26.58 (21.28)	74.21 (65.09)
45 degree entrance angle	51	0.20 (0.60)	27.37 (21.93)	71.37 (40.69)

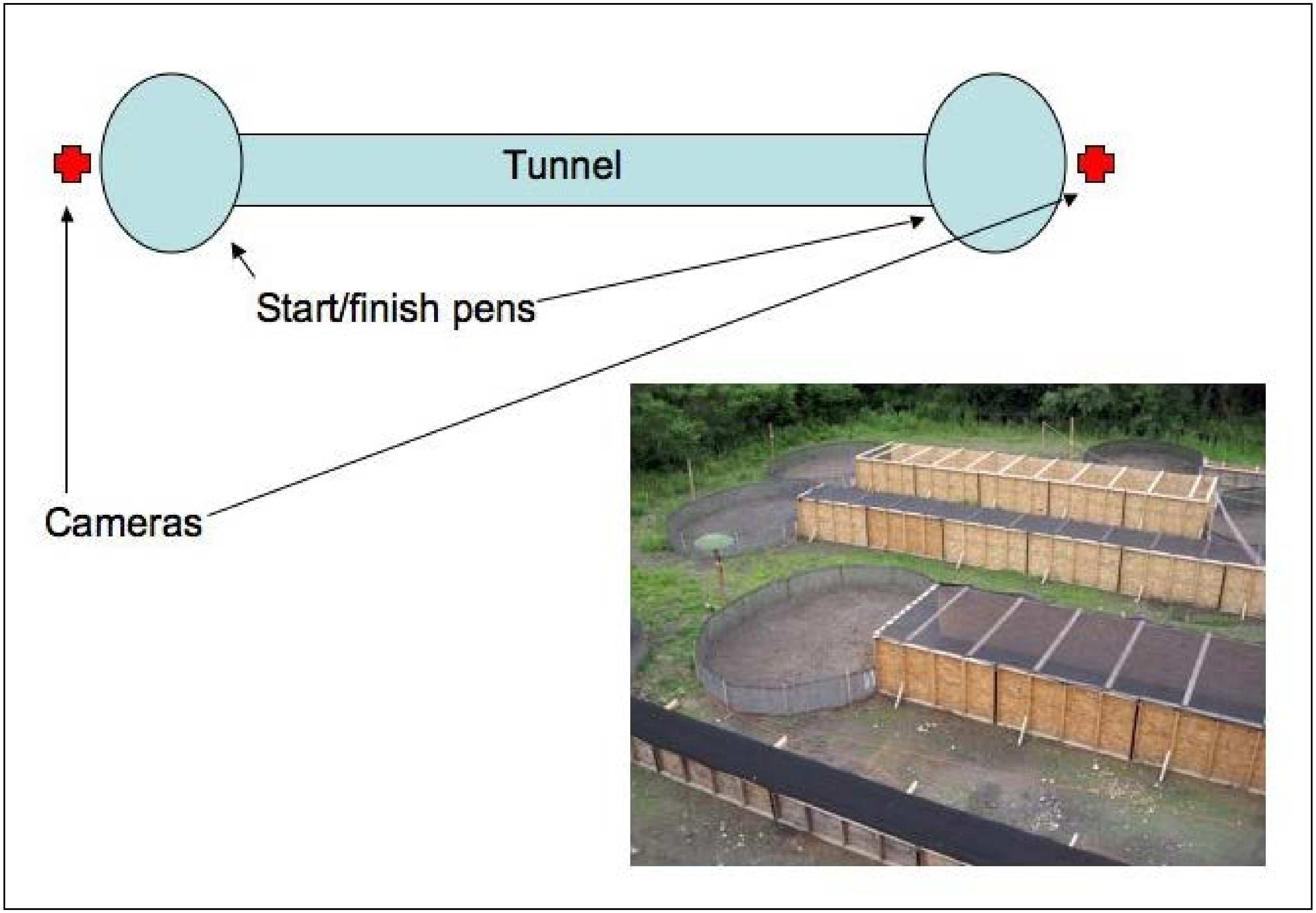

Figure 1.1 Schematic diagram of the experimental setup, and photograph depicting several of the test tunnels at the Tillson Tunnel Field Laboratory.

Figure 1.2 Photograph picturing the interior of artificially illuminated tunnel used in Experiment 2 with the fluorescent lighting turned on.

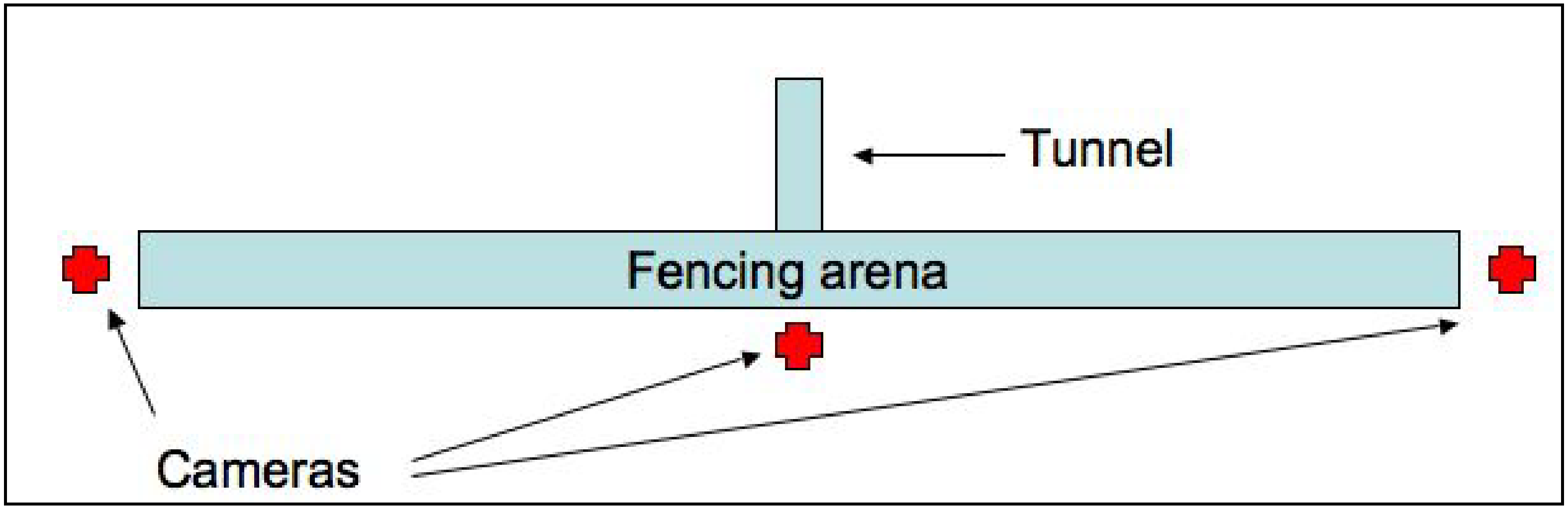

Figure 1.3 Schematic diagram depicting the layout of field laboratory used in Experiment 3.

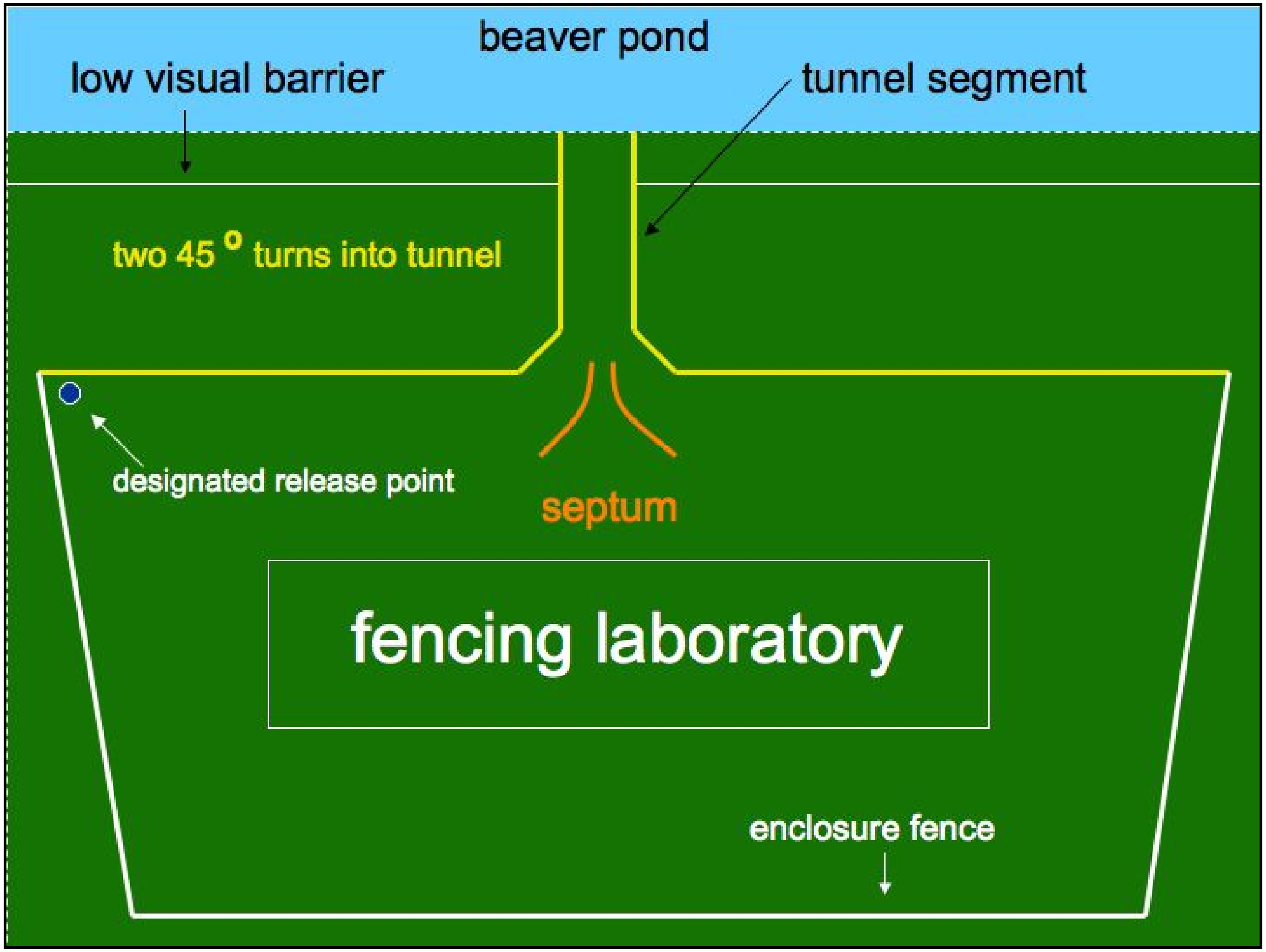

Figure 1.4 Schematic diagram of fencing field laboratory in the context of its location immediately adjacent to a wetland. Laboratory is shown here with 45 degree entrance angles and guidance septa in place.

Figure 1.5 Photograph of the tunnel entrance area of the field laboratory used in Experiment 3. The laboratory is depicted here with the guidance septa installed and the entrance angle at 90 degrees.

Figure 1.6 Photograph depicting a turtle exclusion gate, Groton MA. The design of this gate is similar to that of the gate tested in Experiment 4 at the Tillson exclusion gate field laboratory.

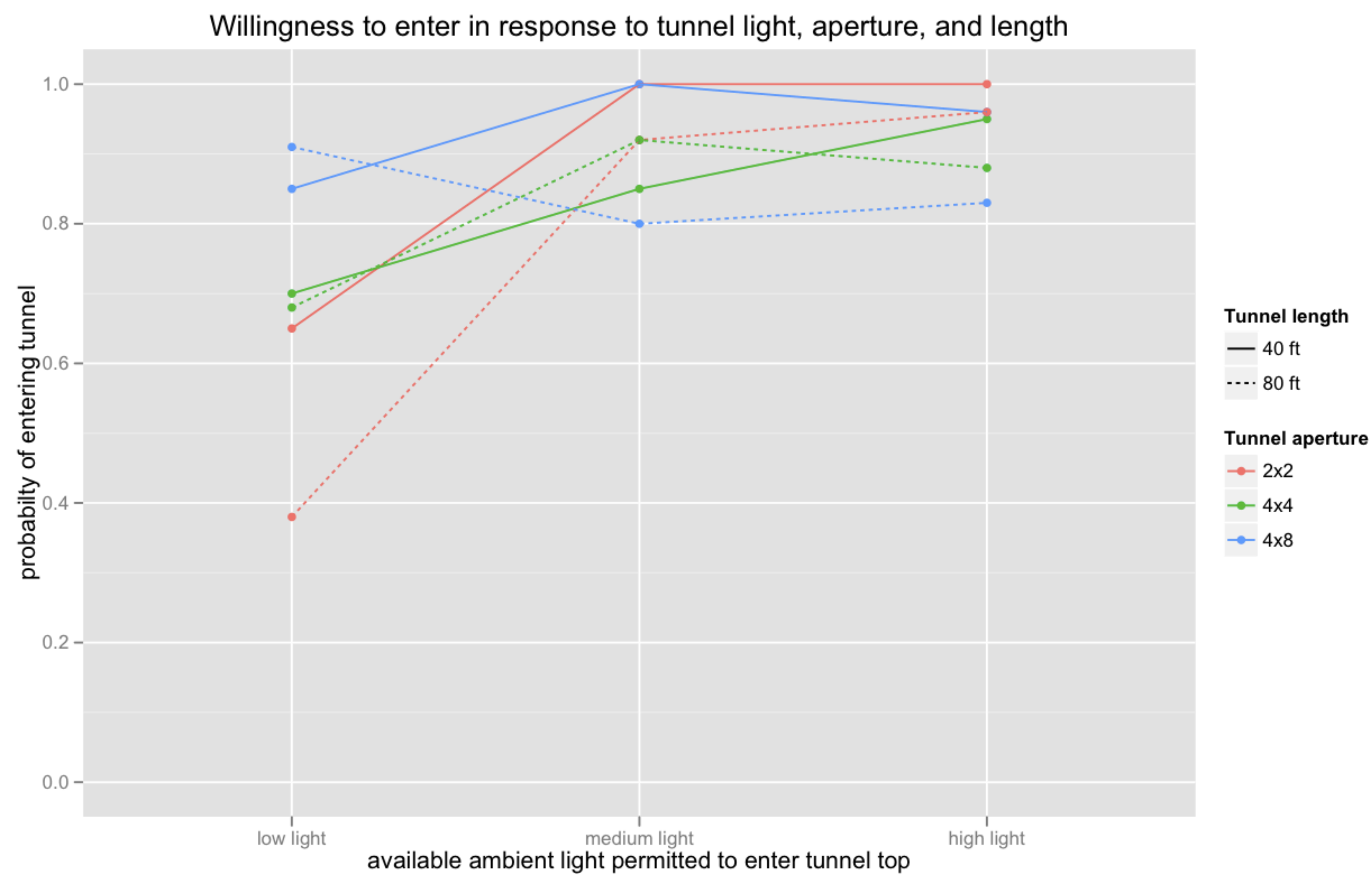

Figure 1.7 Willingness to enter in response to tunnel light, aperture, and length. Medium light = 75% overhead transmittance, and High light = 100% overhead transmittance.

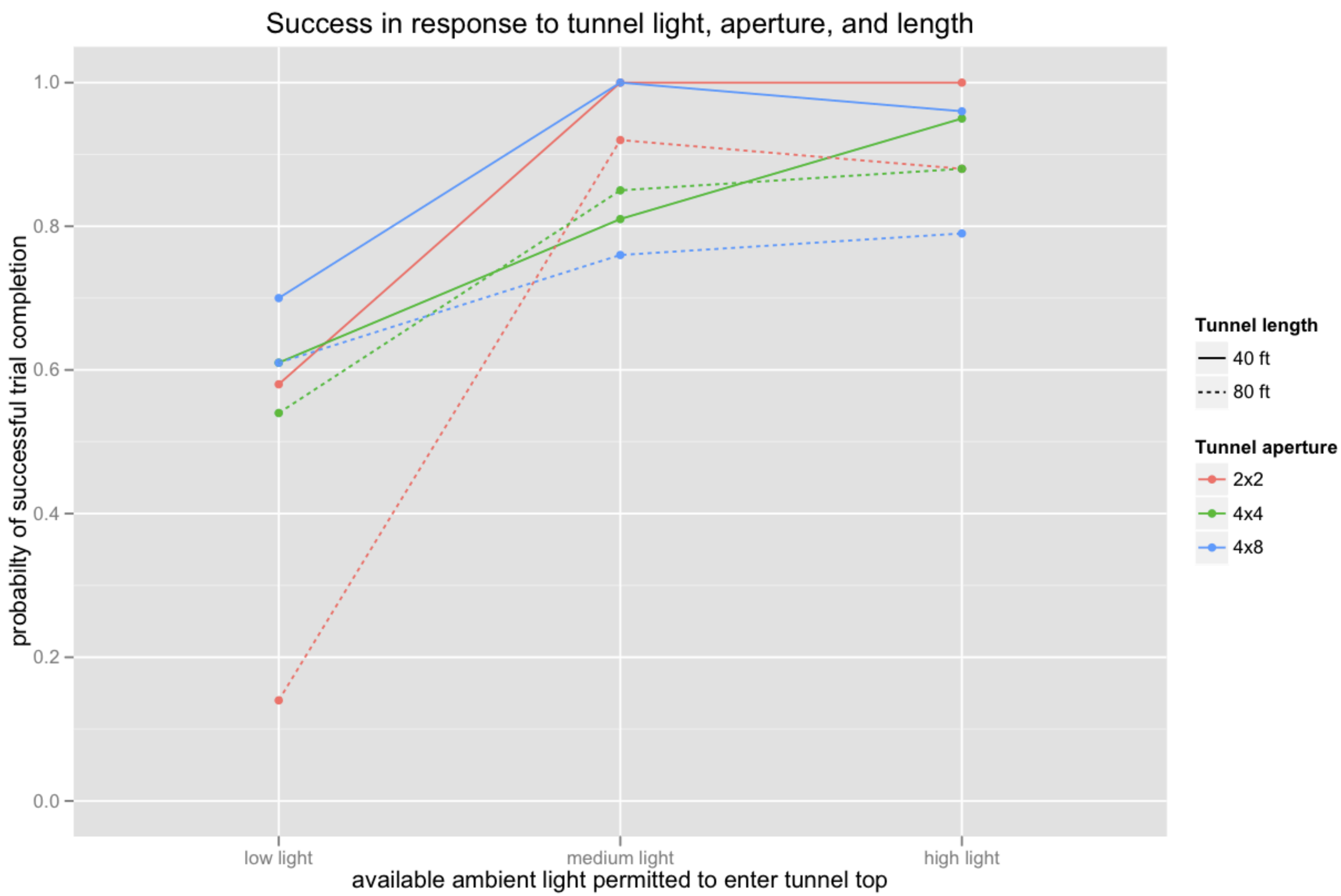

Figure 1.8 Success in response to tunnel light, aperture, and length. Medium light = 75% overhead transmittance, and High light = 100% overhead transmittance.

Figure 1.9 Success with zero hesitations in response to tunnel light, aperture, and length. Medium light = 75% overhead transmittance, and High light = 100% overhead transmittance.

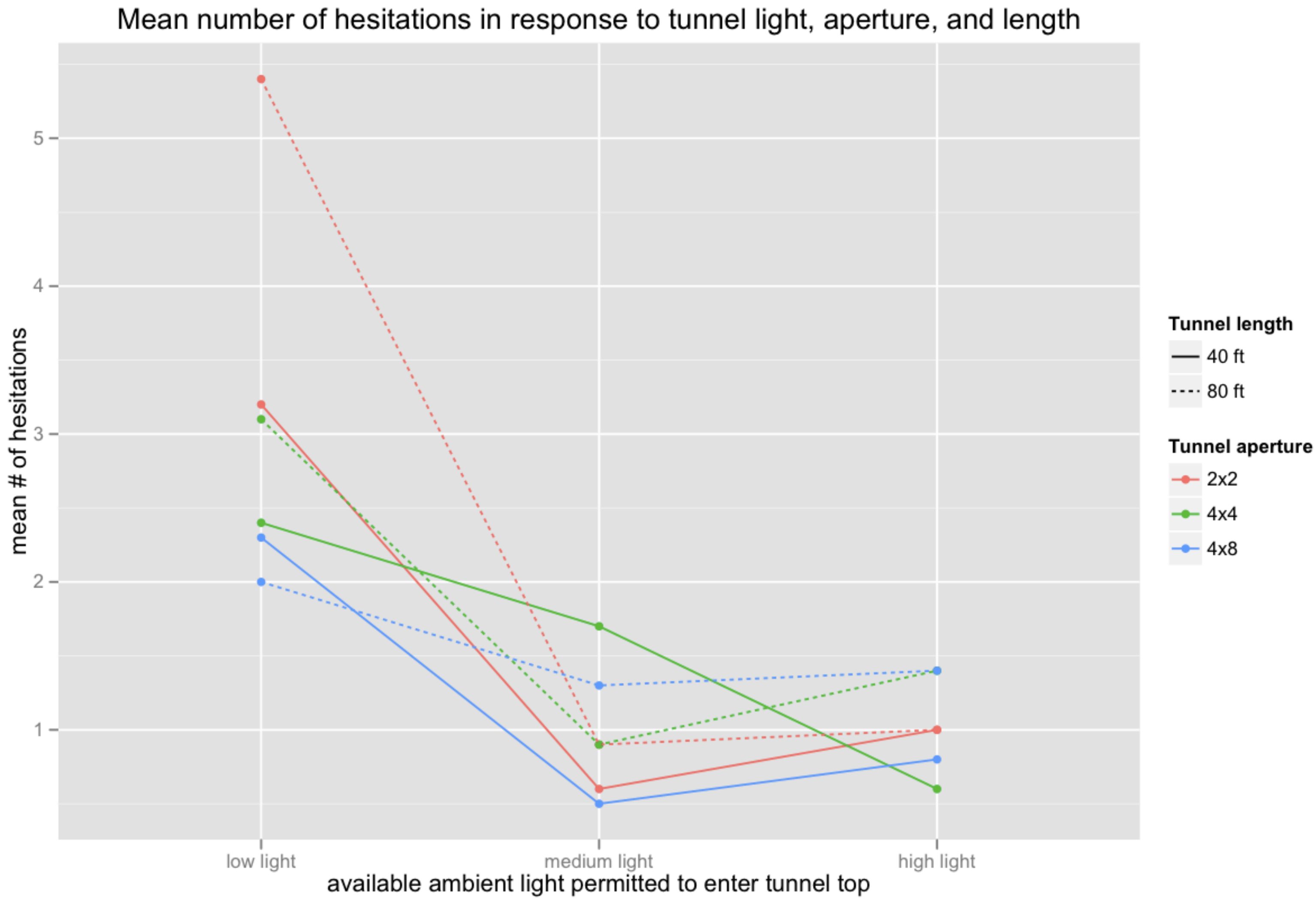

Figure 1.10 Mean number of hesitations in response to tunnel light, aperture, and length. Low light = 0% overhead transmittance, Medium light = 75% overhead transmittance, and High light = 100% overhead transmittance.

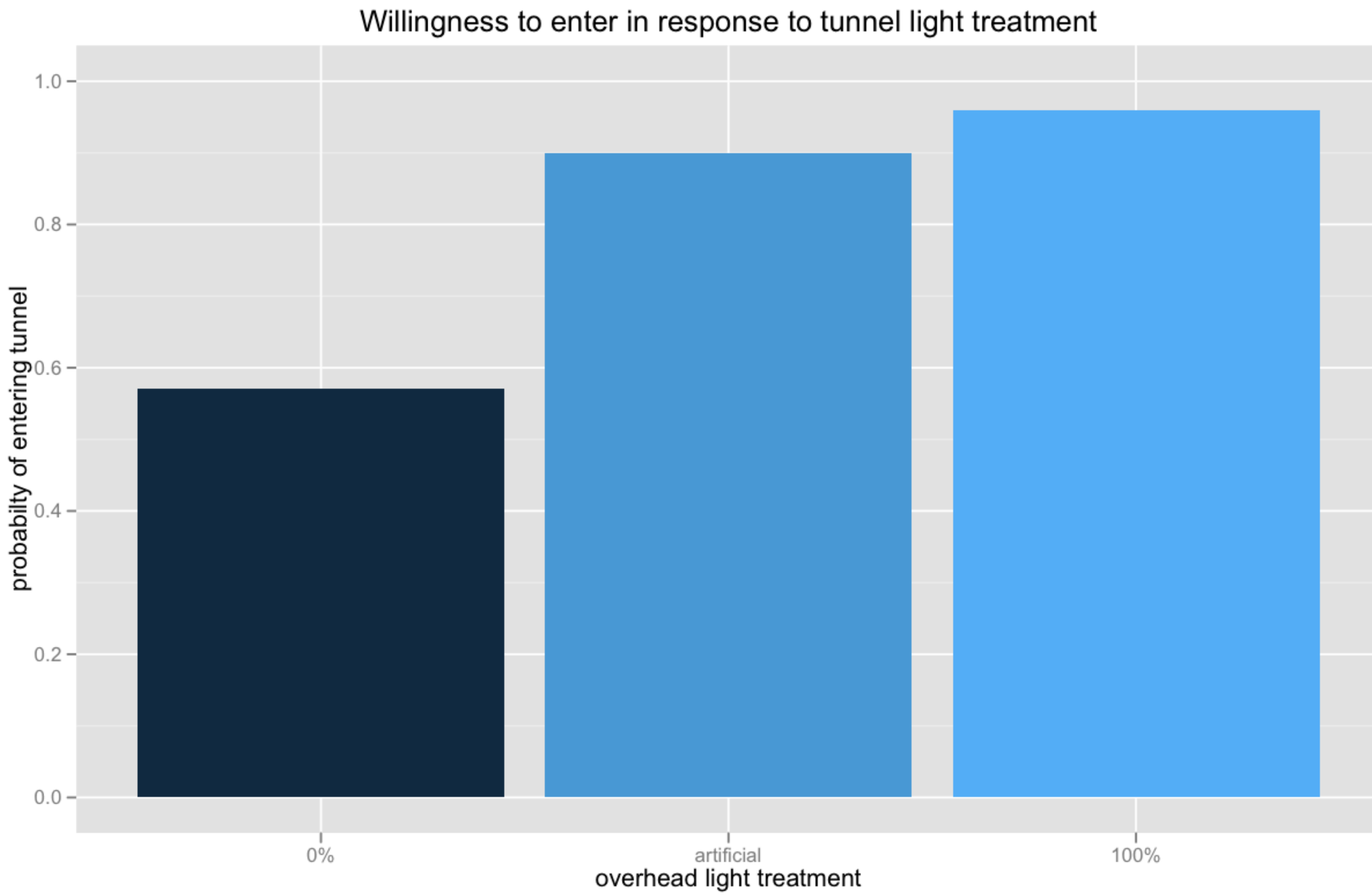

Figure 1.11 Willingness to enter tunnel in response to light treatment. Trials subject to 100% light permitted treatment were tested separately in Experiment 1 and are included for purposes of comparison.

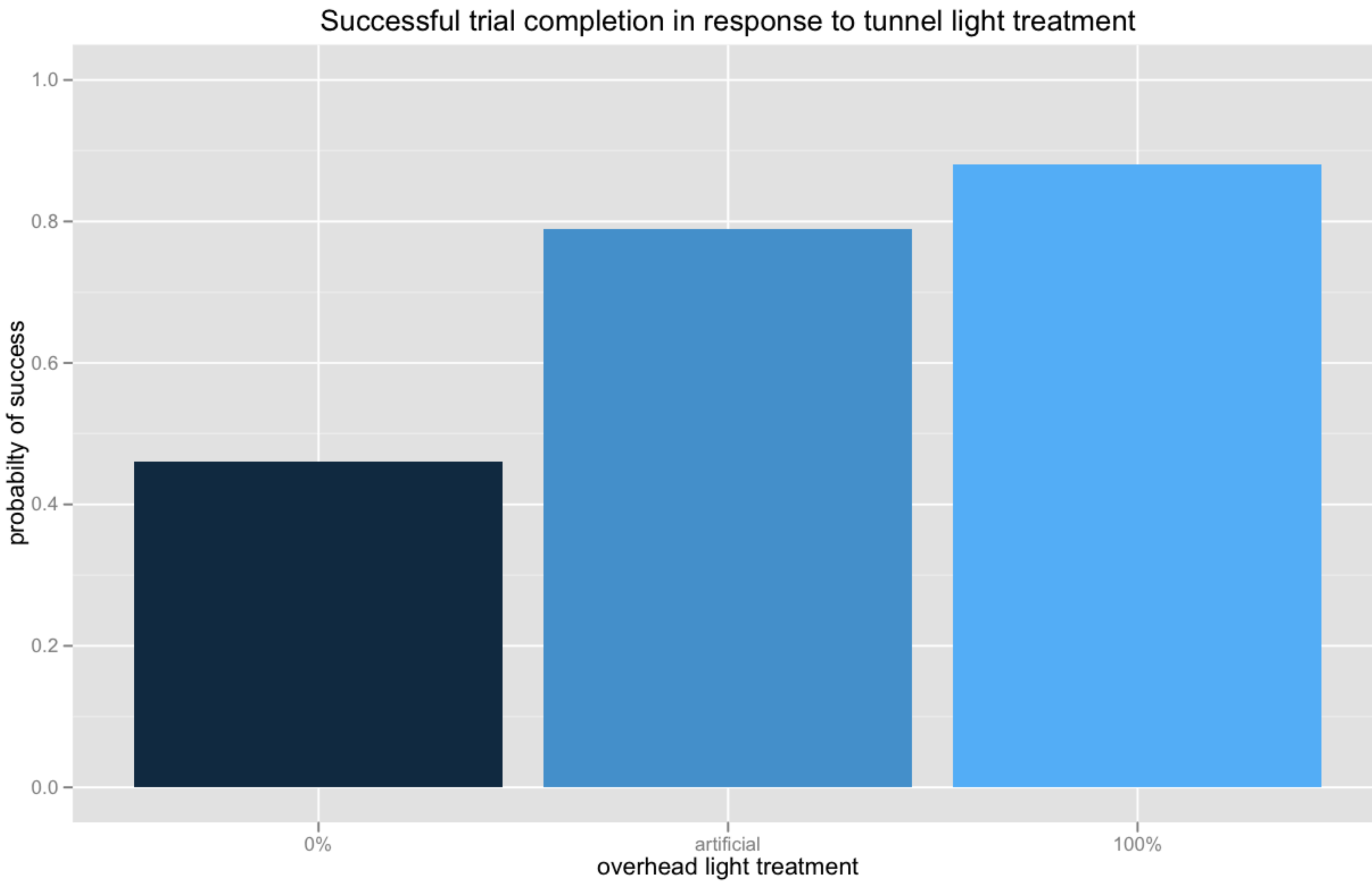

Figure 1.12 Success in response to light treatment. Trials subject to 100% light permitted treatment were tested separately in Experiment 1 and are included for purposes of comparison.

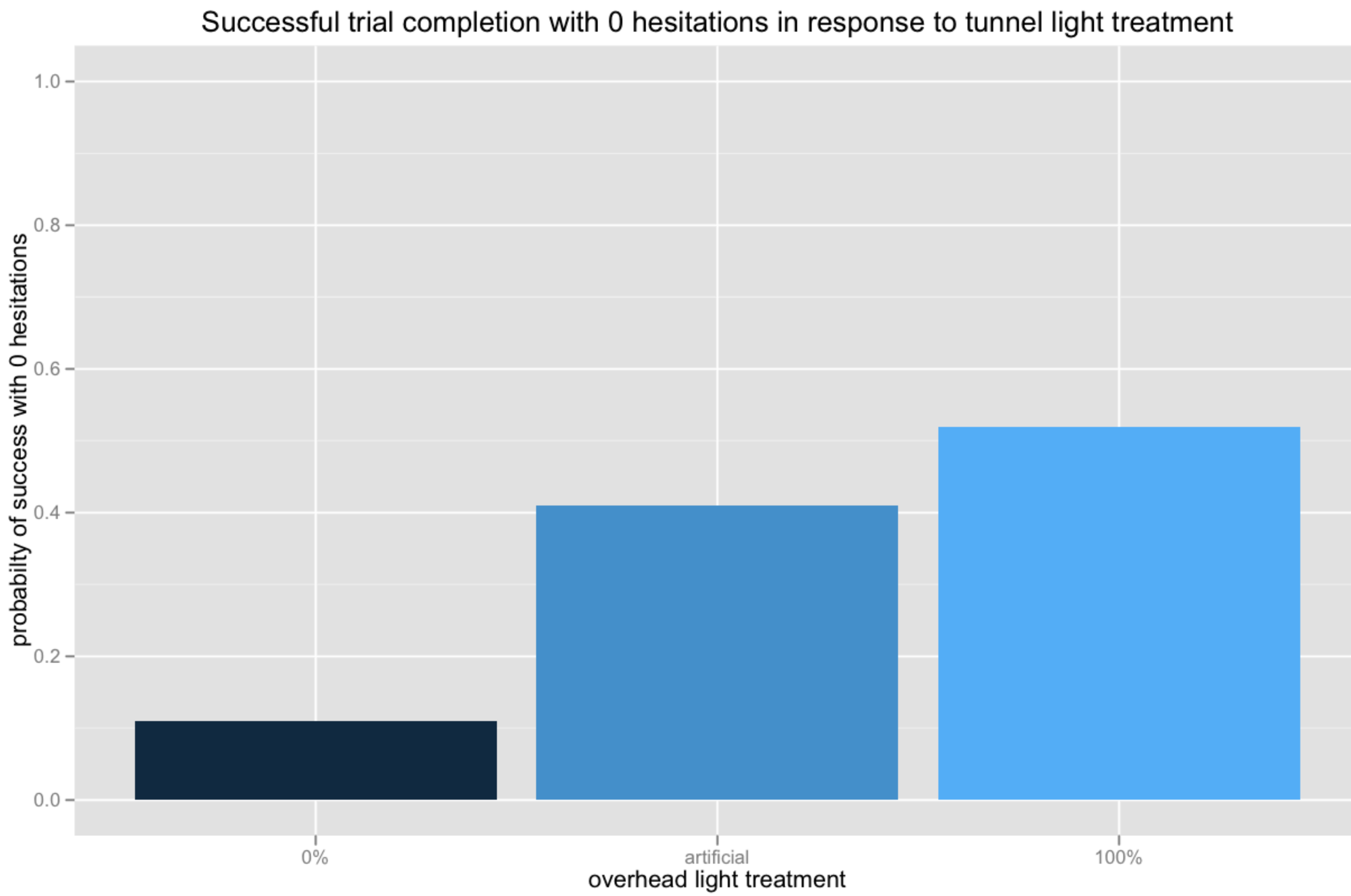

Figure 1.13 Success with zero hesitations in response to light treatment. Trials subject to 100% light permitted treatment were tested separately in Experiment 1 and are included for purposes of comparison.

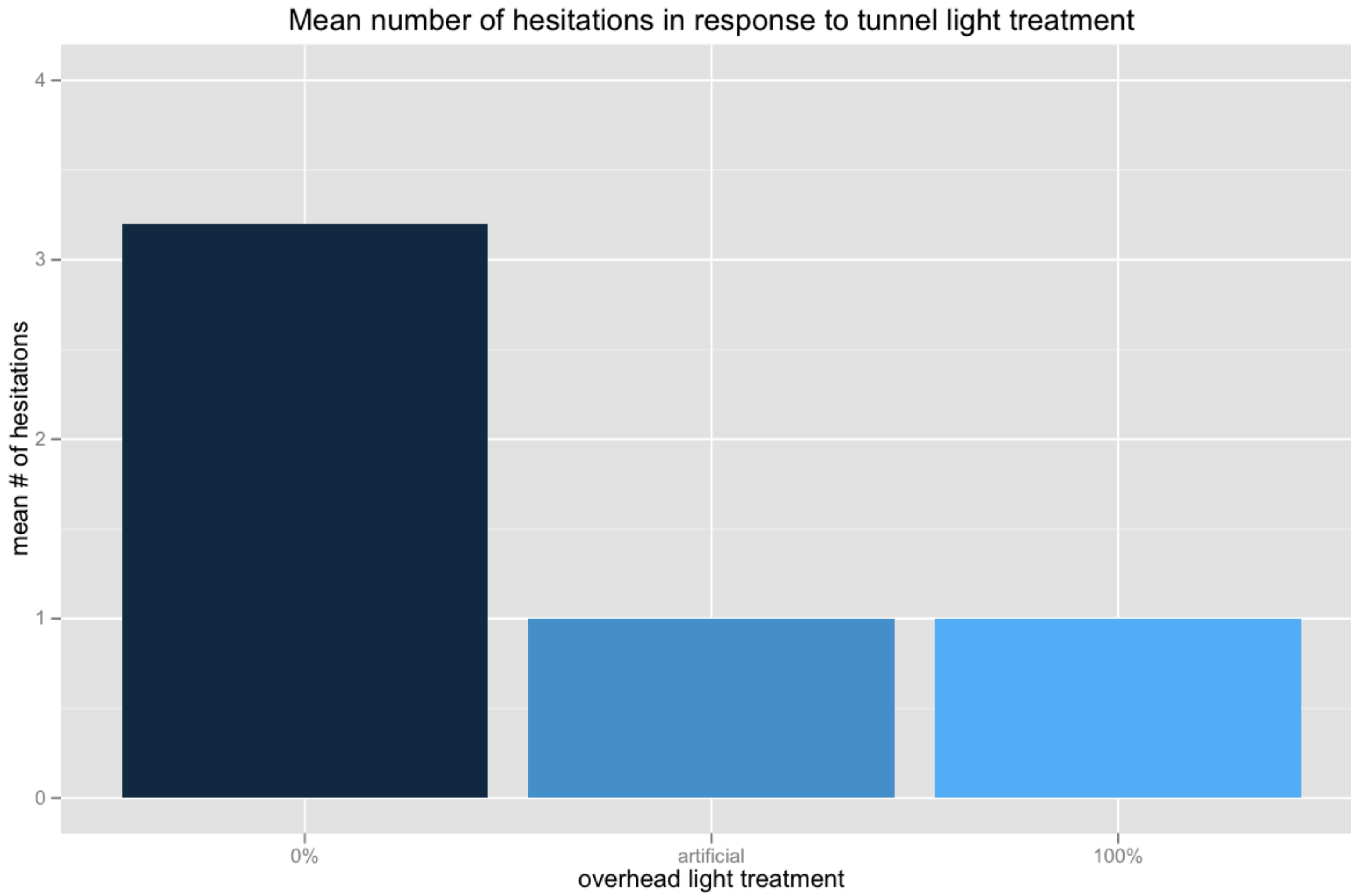

Figure 1.14 Mean number of hesitations in response to light treatment. Trials subject to 100% light permitted treatment were tested separately in Experiment 1 and are included for purposes of comparison.

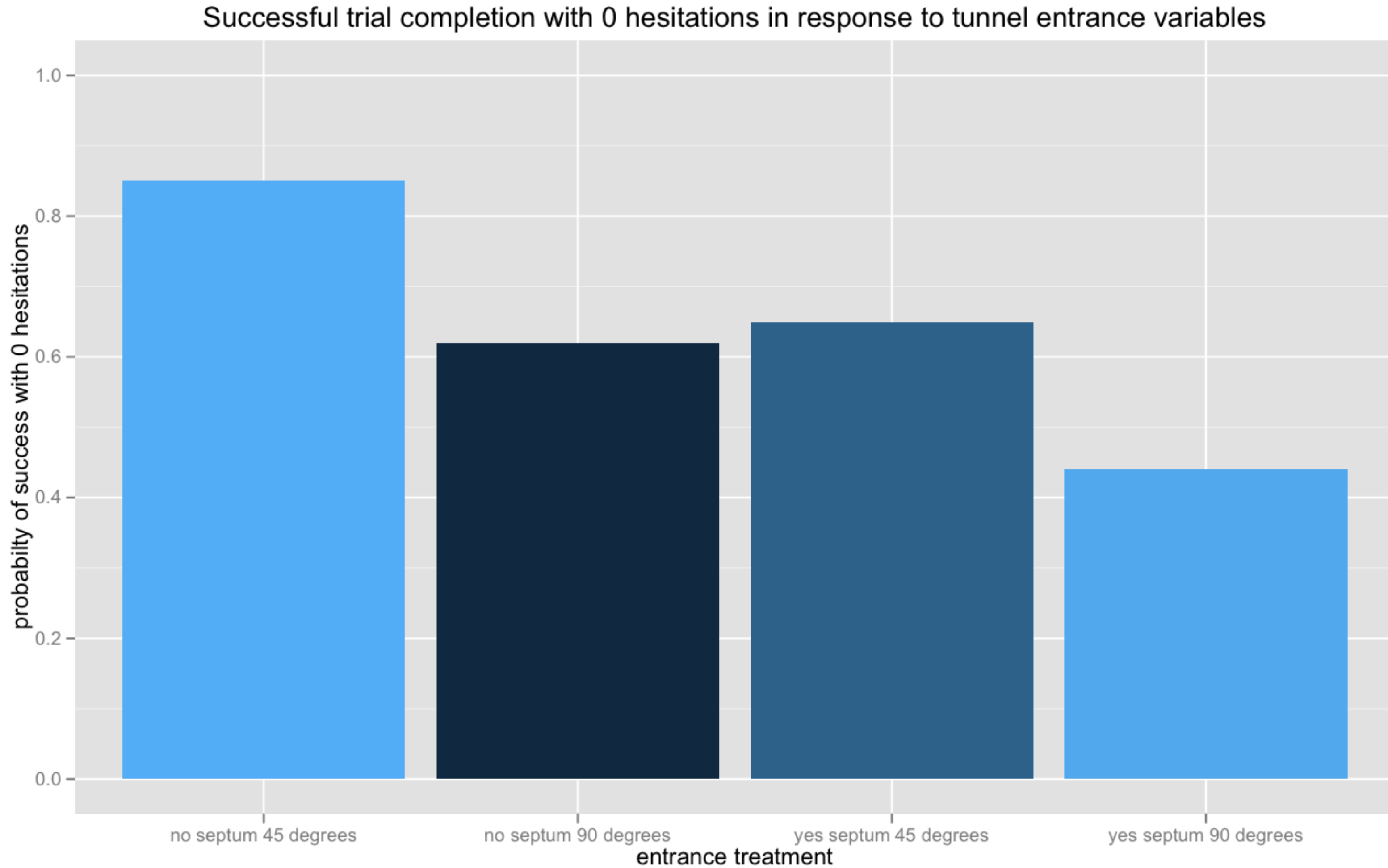

Figure 1.15 Success with zero hesitations in response to entrance angle and septum.

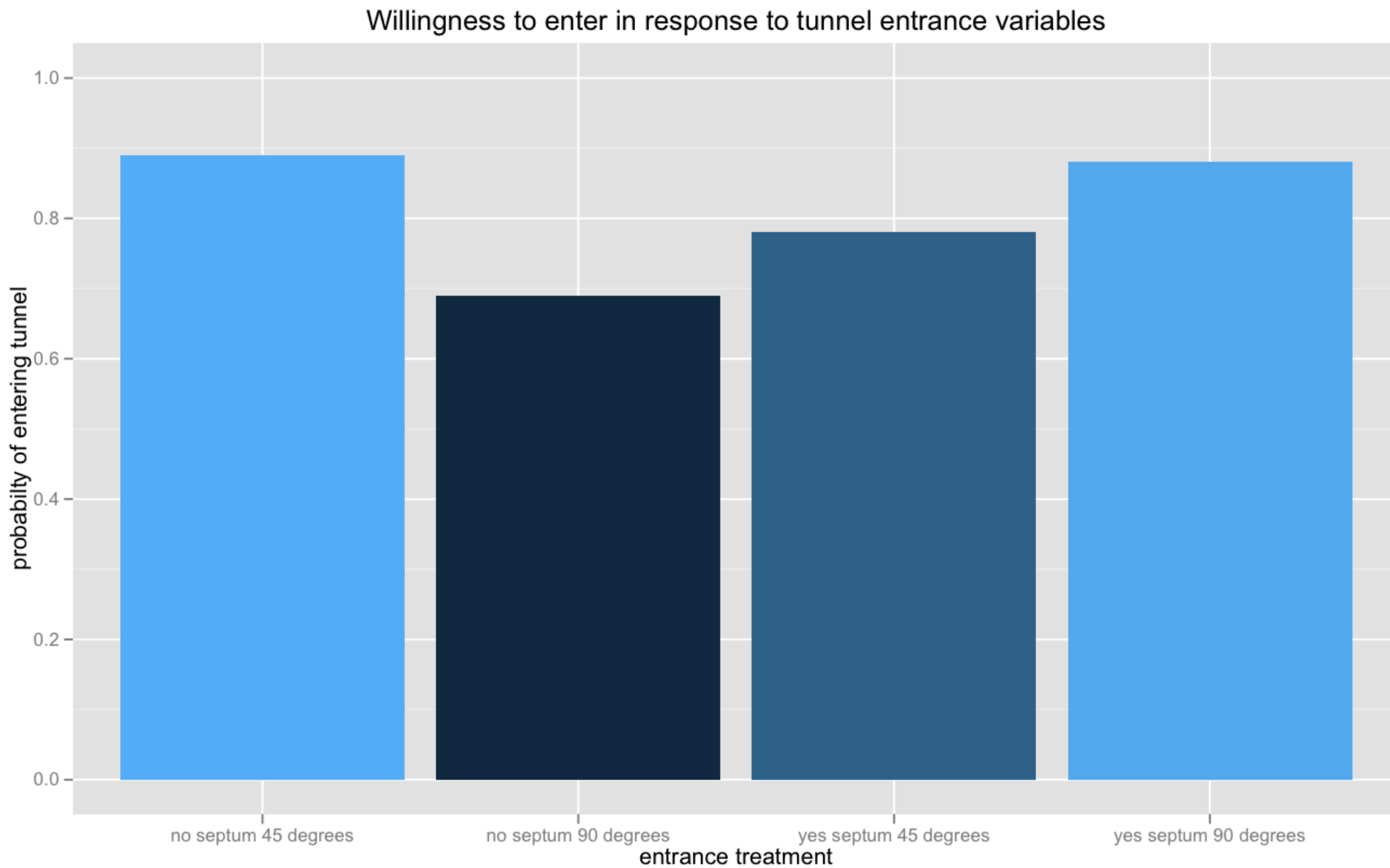

Figure 1.16 Willingness to enter tunnel in response to entrance angle and septum.

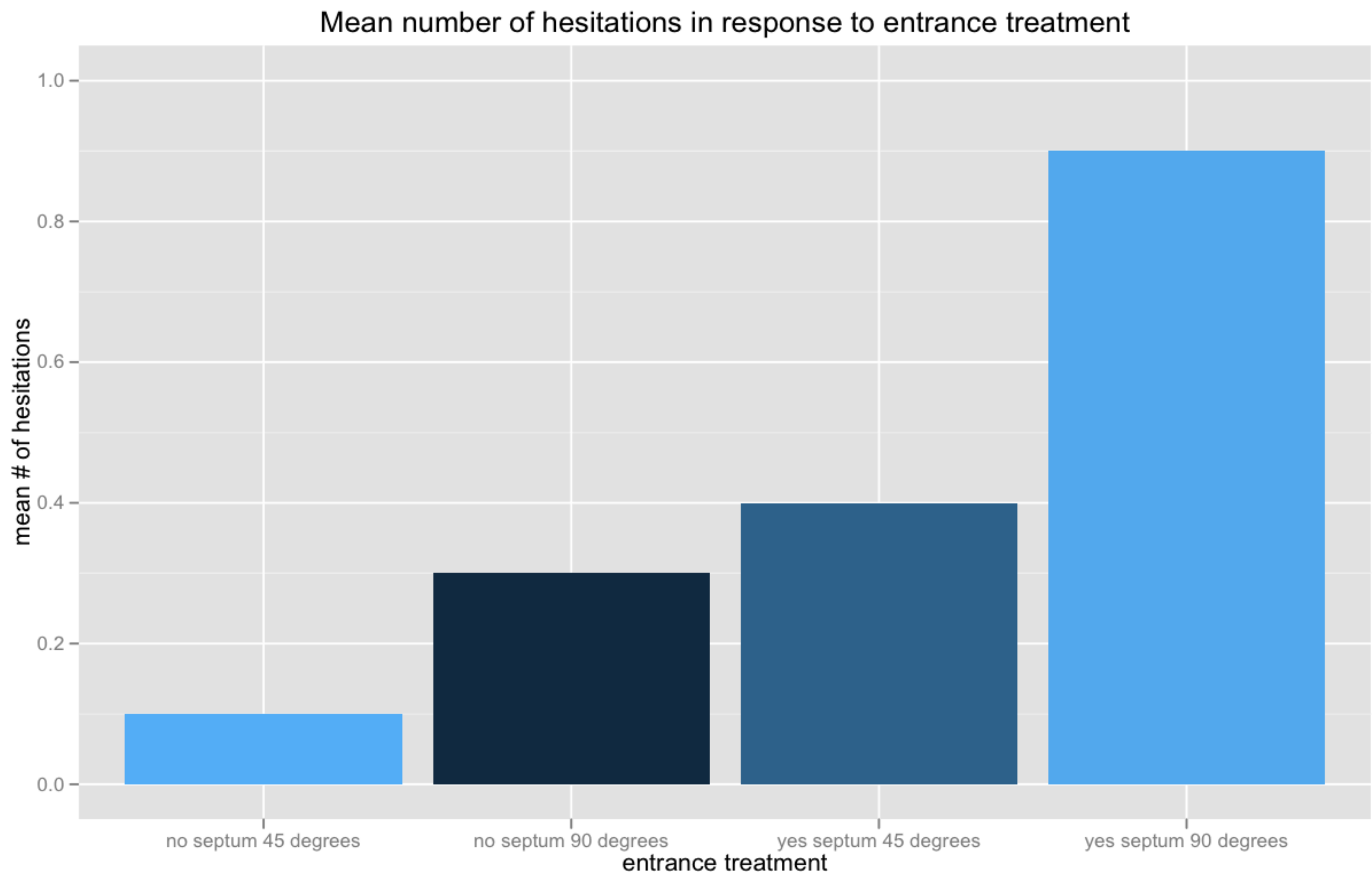

Figure 1.17 Mean number of hesitations in response to entrance angle and septum.

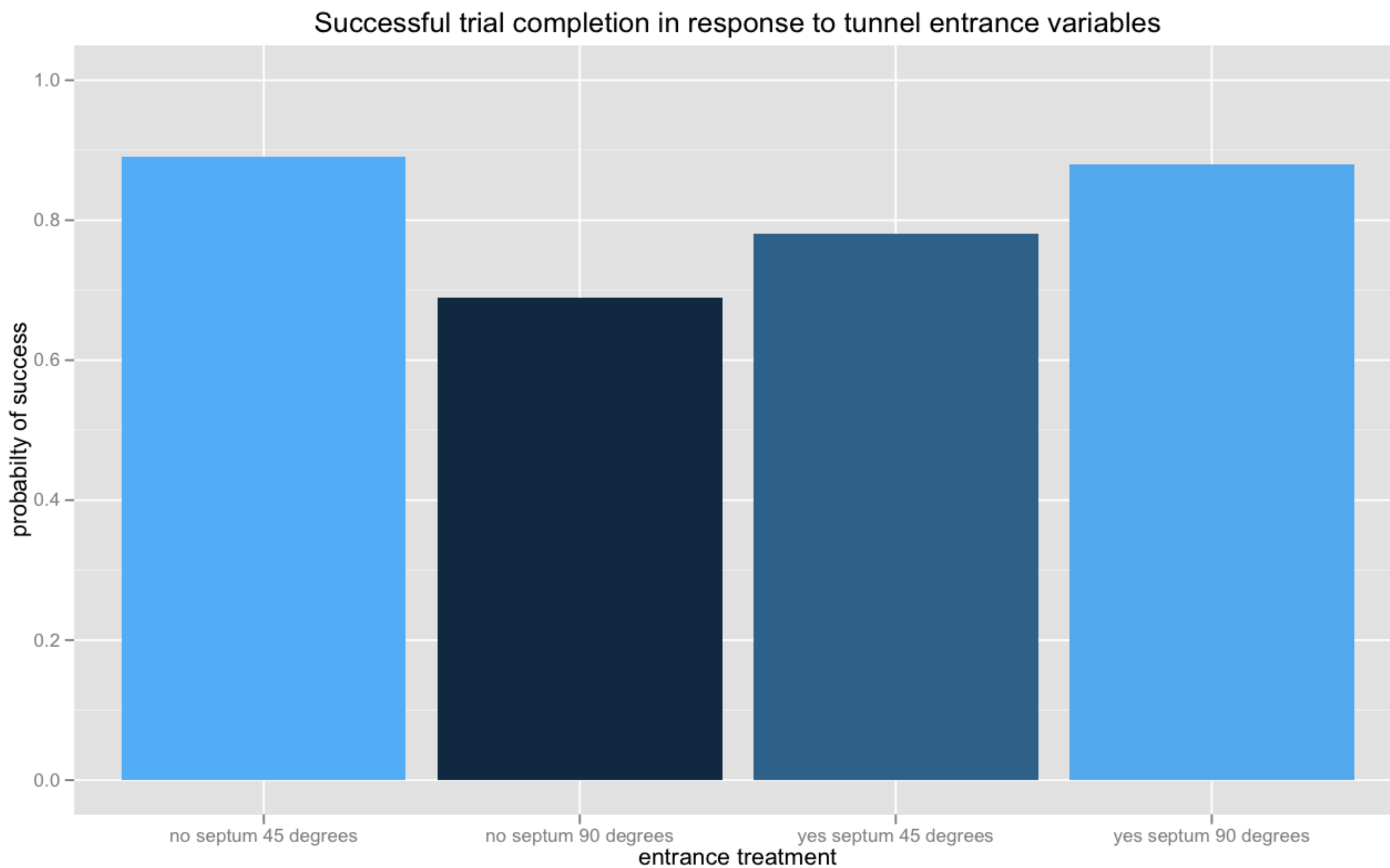

Figure 1.18 Success in response to entrance angle and septum.

CHAPTER 2

EXPERIMENTAL TESTS OF BARRIER TYPE AND TUNNEL LIGHTING EFFECTS ON ROAD PASSAGE USE BY THREE MASSACHUSETTS TURTLE SPECIES

Introduction

Roads can cause harmful impacts to a wide range of wildlife species (Fahrig et al 2009, Forman and Alexander 1998) and the negative effects they have on freshwater turtles on roadways are well documented (Ashley and Robinson 1996, Haxton 2000, Steen et al. 2006). Aquatic turtles often encounter roads when they undertake terrestrial movements including seasonal migrations, migrations to nesting sites, dispersal of juveniles, movement to escape unfavorable conditions, and movement of males to find mates—and considerable mortality can occur (Aresco 2005, Beaudry et al. 2008, Gibbons 1983, Steen & Gibbs 2004, Steen et al. 2006). Other direct effects of roads on turtle populations include nonfatal injuries, alteration and restriction of movement and behavior, and loss of habitat (Andrews et al. 2007). Indirect effects include habitat fragmentation and degradation, isolation of turtle populations, and disruption of metapopulation dynamics and gene flow (Andrews et al. 2007, Gibbs & Shriver 2002, Laporte et al 2013, Marsack & Swanson 2009, Steen & Gibbs 2004).

Mitigating road threats to turtle populations requires an understanding of where it might be possible to mitigate with barrier and passage systems (Beaudry et al. 2008, Langen et al. 2009, Patrick et al. 2010), and how effective these methods will be (Clevenger et al. 2005). The important role of testing and monitoring wildlife passages has been clearly articulated (Mata et al. 2009), but we still know little about which passage systems are useful for turtles and other small animals (Woltz et al. 2008). Research on passage systems for turtles has been limited, but is needed in order to provide technical guidance on passage design (Dodd et al. 2004, Jackson and Marchand 1998, Ruby et al. 1994). Woltz et al. (2008) conducted the only experimental study examining passage of freshwater turtles (painted turtles, *Chrsyemys picta*, and snapping turtles, *Chelydra serpentina*) through crossing structures that varied in aperture diameter, length, substrate type, and light permeability. Woltz et al. found that both turtle species preferred tunnels of mid-size aperture (0.5 and 0.6 m) and that painted turtles showed some degree of avoidance toward the longest tunnels. Ruby et al. (1994) conducted the only tests on the behavioral responses of any turtle species to different types of solid and non-solid barriers using captive desert tortoises (*Gopherus agassizii*). Ruby et al. (1994) found that a 1 cm hardware cloth barrier performed best when the goal was to guide tortoises to a passageway or beyond an area because it was perceived by tortoise as solid and impenetrable yet still allowed them to see through it. Likewise they found that if the goal was to repel tortoises from an area then a solid barrier was the best choice. These studies provide important insight into which specific design elements are key to constructing effective passage systems for turtles and this type of data is critical to practitioners who wish to implement these systems.

In Massachusetts, the Blanding's turtle (*Emydoidea blandingii*), listed as threatened, and the spotted turtle (*Clemmys gutatta*), not state-listed, but uncommon and prohibited to possess, are two species whose populations may be suffering from high rates of road mortality. Though widespread, both species are of conservation concern over much of their respective ranges. Blanding's turtles and spotted turtles are identified as Species of Greatest Conservation Need (SGCN) for the northeast region (NEPARC 2010). The ranges of these species overlap with some of the most densely developed parts of eastern North America where habitat fragmentation and associated problems are well documented (Grgurovic and Sievert, 2005, Joyal et al. 2001, Milam and Melvin 2001). Even though road mortality is one of the greatest threats to semi-terrestrial freshwater turtles, as a result of their life-history traits (Beaudry et al. 2008), there are currently no peer-reviewed publications providing guidance on design of passage systems for Blanding's and spotted turtles.

Installing and maintaining road passage structures is expensive (Mata et al. 2009, Paulson 2009) so it is important to determine the best design in order to use resources wisely. We conducted behavioral tests in outdoor field laboratories to examine the relative influence of passage design characteristics on the movement behavior of spotted turtles, Blanding's turtles, and painted turtles. This work was done with the goal of informing the design of effective passage systems. We studied spotted turtles and Blanding's turtles because they are species of conservation concern that have been documented to be impacted negatively by roads. Painted turtles were included in the study because they are a relatively common aquatic species we have studied in previous passage system experiments (Yorks, unpublished data) and yet mortality due to roads has been documented to impact the size and demographic structure of their populations (Baldwin et al. 2004, Fowle 1996, Marchand & Litvaitis 2004, Steen and Gibbs, 2004, Steen et al. 2006).

In our first experiment, we examined the influence of tunnel lighting on the movement behavior of spotted turtles, Blanding's turtles, and painted turtles, and in our second experiment, we examined the effect of barrier opacity on the movement behavior of these three species. We predicted that turtles would prefer tunnels having tops that transmitted the most ambient light, and they would move greater distances, travel at faster rates, and take a less tortuous path in a pen with an opaque barrier versus a translucent one. We also predicted that spotted turtles and Blanding's turtles would be more willing to use tunnels receiving little ambient light compared to painted turtles since they commonly use forested wetlands, a habitat that is darker than those typically used by painted turtles.

Methods

General

In all experiments, spotted turtles (n = 49), Blanding's turtles (n = 49), and painted turtles (n = 32) were captured using collapsible minnow traps baited with sardines packed in soybean oil. Traps were set in wetlands known to contain large populations of these species in Middlesex and Plymouth Counties, Massachusetts.

For identification purposes, a piece of tape marked with a unique number was attached to the carapace of each experimental turtle for the duration of the trial. While not involved in trials, test animals were kept in holding pens that were shaded, and contained water, leaf litter, and plywood structures for cover.

We conducted all experiments at the Assabet River National Wildlife Refuge (NWR), Sudbury, Massachusetts. This site was selected because it was in close proximity to our study populations and provided the necessary space to set up our field laboratories in a vacant gravel pit.

We recorded the behavior of turtles in the arenas using Pclix LT 100 time-lapse triggers and Canon Powershot G2 and G3 digital cameras. Cameras were elevated 5 ft above the ground and took photos every 5 seconds for the duration of the trial so that a detailed record of behavior could be gathered from the images and analyzed at a later date. Closed-circuit video cameras were placed at a minimum of 2 locations around the arena perimeters and were used in conjunction with direct visual observation to document and record the locations of turtles at 2-minute intervals throughout the trial. During the tunnel experiments, turtles in the arena were also directly observed to record their locations at 2-minute intervals throughout the trial. Closed-circuit and direct monitoring were used to keep track of trial progress and afforded a wider field of view than that of the time-lapse cameras which were trained on the tunnel entrance and its immediate vicinity. All 3 of these monitoring techniques were needed to ensure that a consistent and detailed a record of behavior was documented while simultaneously minimizing human disturbance.

After a turtle's use in an experiment was completed, and prior to releasing it in the wild, we gave it a permanent mark and recorded its age, sex, gravidity, maximum carapace length, maximum carapace width, and weight. All turtles were given a unique identification number by filing notches into the carapace marginal scutes (Ernst et al. 1974). We released all turtles at their point of capture (usually < 24 hours from time of capture).

Experiment 1 - We experimentally examined the effect of lighting level on movement behavior of spotted turtles, Blanding's turtles, and painted turtles from 6 June to 29 July 2011. We used an 80 ft long tunnel, with a 2 ft x 2 ft opening and a completely open top, except for 2 in x 4 in cross beams placed at 4 ft intervals for structural support. Overhead light transmission was manipulated using opaque cloth, producing two options: 0% and 100% transmission. The tunnel was oriented east-west, and the sides consisted of plywood panels reinforced with 2 in x 4 in wooden cross beams. The bottom of the tunnel and pens was the natural soil/sand at the site. Figure 2.1 shows a schematic diagram of the experimental tunnel laboratory.

Enclosures attached to either end of the tunnel served as standardized start or exit pens for the trials. The enclosures were open-topped ellipses with a 15 ft minimum diameter and 20 ft maximum diameter. Pen fencing was made of 3 ft high rabbit fencing that was covered with landscaping fabric to block most visual stressors and distractions from the surrounding environment. The fence had a graduated mesh size that was 1inch wide x 4 inches high at ground level and increased to 4 inch x 4 inch at the top.

Each experimental turtle was randomly assigned a start direction and tunnel lighting treatment, and given 60 minutes to complete the trial. We used the most extreme light treatments to maximize the likelihood of detecting an effect of light on passage rates of the three turtle species.

Experiment 2 - We experimentally examined the effect of 2 barrier opacities (0% and 100%) on movement behavior of spotted turtles, Blanding's turtles, and painted turtles from 5 June to 29 July 2011. We were interested in barrier opacity because we believed it could be used in directing turtles into tunnels by altering their rates and patterns of movement.

The field laboratory for barrier opacity tests consisted of a large square pen (50 ft x 50 ft) delineated with chicken wire (1 inch mesh) supported by wooden stakes. A turtle's ability to see through the fence could be reduced by attaching a 1 ft high piece of tar paper to the outside of the fence. The ground substrate of the arena was the natural soil/sand at the site (Figure 2.2). To keep track of the location of a turtle within the arena, the pen was divided into a semi-regular grid consisting of 48 cells. The distance between pairs of turtle locations was estimated as the distance between cell centers.

Turtles were randomly assigned to barrier treatments (translucent or opaque), and to start locations within the arena. Possible start locations were the four corners of the experimental barrier arena, and once a turtle was placed in the arena, the trial continued for 60 minutes.

Collection and Interpretation of Behavioral Data

Experiment 1 - Using seven response variables (5 categorical and 2 continuous), we quantified the reactions of turtles to the experimental trials. Categorical response variables included: (1) non-reactive (turtle was non-reactive y/n); (2) hesitated (turtle exhibited one or more hesitation behaviors (hesitated y/n); (3) entered tunnel (at a minimum, turtle navigated into the tunnel); (4) successful completion (turtle entered the tunnel and successfully navigated through into the exit pen y/n); (5) successful completion with no hesitations (turtle successfully navigated through the tunnel into the exit pen and did not exhibit any hesitation behaviors y/n). Continuous variables were: (1) total number of hesitations, and (2) rate of travel in tunnel for turtles successfully completing trials.

Non-reaction was defined as a failure to react to any experimental stimulus presented during the trial period in a manner that was visible to experiment observers. Turtles that were non-reactive typically remained still for the duration of the trial or made limited movements and did not approach the tunnel entrance. Hesitating was defined as a turtle exhibiting any one of the three following behaviors during the trial: (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; (3) False start – a turtle entered the tunnel, and then returned back through the tunnel entrance without completing the trial. Entering was defined simply as a turtle moving into the tunnel, after which point it may or may not have gone on to successfully complete the trial. Successful completion was defined as navigating through the tunnel and into the exit pen of the trial in 60 minutes or less. Turtles that successfully completed trials and did so with no hesitations were classified in the fifth response group listed above. Once a turtle reached the exit pen or exceeded the 60-minute time limit it was removed from the trial. Turtles that did not emerge into the exit pen after 60 minutes were classified as unsuccessful.

The total number of hesitations observed was the pooled total number of the three hesitation behaviors (Bypass, Approach, and False start) considered collectively for each trial. Rate of travel in tunnel was the speed at which turtles moved through tunnels measured in feet per minute (FPM) and was calculated for turtles that successfully passed through the tunnel.

We used multiple response variables to evaluate the effect of lighting so that we could provide reliable design recommendations for the construction of passage systems. Three of our response variables were considered primary measures of the willingness of turtles to use a passage: (1) entered tunnel; (2) successfully completed trial; (3) successfully completed trial with zero hesitations. The least conservative measure was whether turtles entered the tunnel or not because it measured at a minimum, the willingness of a turtle to move out of the start pen and into the passage and included trials that either could have been successful or unsuccessful. The most conservative measure of success was that based on zero hesitations and completion of the trial in 60 minutes or less. This response variable assumed that a single hesitation would result in a turtle not using a real passage structure. Our rationale was to account for real life situations in which a turtle might hesitate and then travel in either direction and choose to pass or turn away from the tunnel.

Experiment 2 - We quantified the reaction of turtles to fencing types using four response variables (one categorical and three continuous). The categorical response was deviation from the barrier (yes or no), and the continuous responses were; (1) total distance traveled, (2) rate of travel, and (3) tortuosity of the movement path.

Deviation from the barrier was defined as a turtle moving a distance > 1 m from the barrier at some point during the trial and entering any or all of the central grid cells (Figure 2.3: cells 45-48). Total distance traveled was the sum of distances traveled by a turtle based on locations recorded at 2-minute intervals over the 60-minute duration of the trial. Rate of travel was the speed at which turtles moved through the arena measured in feet per minute (FPM). Because some trials were < 60 minutes long rate of travel (FPM), is not simply the total distance divided by 60. Tortuosity of the path was a measure used to indicate the relative amount of change in the trajectory of a turtle, independent of distance traveled. For each observation interval, a value of 1 was given when a turtle moved in a different direction from its trajectory in the previous interval and a value of 0 was given when a turtle moved in the same direction from its trajectory in the previous interval. Non-movement observations where the turtle remained still between observations or moved a very short distance and remained in the same cell were excluded. The resulting mean tortuosity value for each trial ranged from 0-1. A value of 0 indicated no change in direction relative to movements made (e.g. moving clockwise around the pen for the entire trial), and a value of 1 indicated a change in direction for each movement made (e.g. moving back and forth along a single wall of the pen changing direction at 2 minute intervals for the entire trial).

Statistical Analysis

Preferences of turtles for tunnels varying in available light were analyzed using Analysis of Variance (ANOVA) for continuous responses, and logistic regression for binary responses. Because sample sizes were unequal in ANOVAs, we used Type III sums of squares. Model selection was conducted using Akaike Information Criteria (AIC) statistics. We used odds ratios and Tukey's HSD tests to further examine factors deemed significant. Data sets were manipulated using Microsoft Excel software, and statistically analyzed in the R statistical environment (www.r-project.org). An alpha of 0.05 was used in all statistical tests. A summary of study design, and analysis approach, is provided in Table 2.1.

Reducing potential biases

In the tunnel lighting experiment, we provided turtles with a single tunnel, rather than a choice, because we wished to: (1) randomize tunnel start direction, (2) maintain a large and equal sample size per tunnel type, and (3) maintain consistency in methodology to that used in earlier studies conducted on painted turtles so that results may be compared.

In both the tunnel and fencing experiments, no food, water, or shelter was provided inside the pens so that turtles were motivated to leave the pen. Substrate of the pens was raked before each trial in order to remove vegetation and reduce any potential chemical trails left by turtles. Unless otherwise noted, individual turtles were only exposed to a test tunnel once in order to eliminate the effect of learning on movement behavior.

In addition to the variables described above, we also recorded data on potential covariates that might have affected the performance of turtles in the experimental trials. The covariates were weather, temperature inside and outside the experimental arena, experiment date, trial start time, turtle location at the trial start, each turtle's sex, age, carapace length, weight, and if female, whether the individual was gravid.

Results

Experiment 1 - Of the 110 tunnel trials conducted, 52 turtles successfully passed through the tunnels, 54 did not, and 4 were non-reactive and therefore were not included in the analyses. Light level and species were significant predictors of the frequency of trials where turtles entered the tunnel ($P< 0.001$ and $P < 0.05$ respectively) (Table 2.2). Turtles were 8.4 times more likely to enter a tunnel when 100% of the overhead light was transmitted, compared to 0% lighting (Table 2.3). Spotted turtles and Blanding's turtles were less likely to enter a tunnel than painted turtles (Table 2.3).

When the response was the frequency of successfully completed trials, light level and species were again significant predictors ($P< 0.001$ and $P < 0.05$ respectively) (Table 2.2). The odds of success for turtles subjected to the 100% light transmitted treatment were a dramatic 84.3 times higher in comparison to those subject to the 0% treatment (Table 2.3). With regard to the species effect, spotted turtles, followed by Blanding's turtles were the least likely to successfully complete trials and percentage of successfully completed trials was highest for painted turtles

(Tables 2.3 and 2.4). For the 0% light treatment, only 25% of painted turtles successfully passed through the tunnel and both Blanding's and spotted turtles were either extremely reluctant, or unwilling, to pass through the dark tunnel, having 9% and 0% passage rates, respectively (Table 2.4). Most painted, Blanding's, and spotted turtles tested were willing to use the tunnel with the 100% available light treatment but passage rates for spotted turtles were reduced relative to the other species (Table 2.4).

There was a significant effect of both light level and species on the frequency of completing trials without hesitations ($P < 0.001$ and $P < 0.05$ respectively; Table 2.2). The odds of success with zero hesitations for turtles subjected to the 100% light treatment were 21.3 times the odds of success for those subjected to the 0% light treatment (Table 2.3). Spotted turtles were least likely to successfully complete trials with zero hesitations and painted turtles were the most likely. Spotted turtles were about one fifth as likely to successfully complete a trial with zero hesitations as painted turtles and Blanding's turtles were about half as likely (Table 2.3).

Light level and species were significant predictors of a hesitation occurring 1 or more times during a trial ($P < 0.001$ and $P < 0.05$ respectively) (Table 2.2). Turtles were far less likely to exhibit a hesitation behavior (point estimate of odds ratio = 0.13) for the 100% light treatment compared to the 0% treatment and spotted turtles were more likely to hesitate than either Blanding's turtles or painted turtles (Table 2.3). Of the three species, painted turtles had the greatest proportion of trials where no hesitation behaviors were exhibited (Table 2.4).

Species, and the interaction between light level and species, were significant predictors of the total number of hesitations observed per trial pooling successfully and unsuccessfully completed trials ($P < 0.01$ and $P < 0.05$ respectively) (Table 2.2). Significantly more hesitations were observed for trials with spotted turtles and Blanding's turtles compared to painted turtles ($P < 0.05$: Table 2.4). With regard to the interaction of species and light level, there were significantly more hesitations observed among spotted turtles and Blanding's turtles in the 0% treatment compared to all other combinations of species and light level treatments ($P < 0.05$: Table 2.4). In addition, though not statistically significant, the mean number of total hesitations was higher for tunnels with the 0% light treatment compared to the 100% light treatment (Table 2.4). Neither light level nor species were significant predictors of the rate of travel in tunnels.

Experiment 2 – There were a total of 130 behavioral trials conducted in the barrier only laboratory. Barrier opacity was a significant predictor of whether or not turtles deviated from the barrier during a trial ($P < 0.01$) and species was nearly significant ($P = 0.07$). Turtles were 3.1 times more likely to deviate from the opaque barrier than the translucent barrier. Blanding's turtles were the least likely to deviate from a barrier, followed by spotted turtles then painted turtles (Table 2.5).

Barrier opacity and species were significant predictors of the total distance traveled during trials ($P < 0.001$). All three species traveled farther when presented with an opaque barrier compared to a translucent one (Table 2.6). The mean distance traveled by spotted turtles was significantly shorter than that traveled by either painted turtles or Blanding's turtles ($P < 0.01$: Table 2.6).

Rate of travel (feet per minute, or FPM) was significantly influenced by barrier opacity and species ($P < 0.001$). Rates of travel were faster in trials with the opaque visual barrier in place compared to those with the translucent barrier (Table 2.6). Spotted turtles moved more slowly than the other two species regardless of barrier type ($P < 0.01$: Table 2.6). The interaction between barrier and species was nearly significant ($P < 0.07$). Spotted turtles and painted turtles moved significantly faster along opaque barriers, compared to translucent ones (Table 2.6).

Barrier opacity and species were both significant predictors of path tortuosity ($P < 0.05$ and $P < 0.001$ respectively). Tortuosity ranged from 0 to 1, representing few to many changes of trajectory, respectively. Considering the number of sampling intervals where movements were recorded, turtles changed their trajectory more often with a translucent barrier in place (Table 2.6), and spotted turtles changed their trajectory more than Blanding's or painted turtles (Table 2.6).

Discussion

Our results of tests on the effects of lighting level indicate that full transmittance of ambient light through the tops of tunnels is significant in facilitating the movement of all three turtle species examined. Conversely, turtles are reluctant to enter close-topped tunnels. Spotted turtles were significantly more hesitant than the other two species to enter tunnels under either lighting condition, indicating that they may have been inhibited by the width and/or length of the passage. Based on our previous work examining light level effects on use of passages by painted turtles (Chapter 1), we predicted Blanding's turtles and spotted turtles might respond similarly because they occupy structurally and ecologically similar environments. Researchers have suggested that light level might be an important predictor of passage use by turtles but the importance of light availability remains largely unresolved (Jackson 2000, Woltz et al. 2008, Andrews et al. 2007; but see Yorks chapter 1 in prep.). Light has been demonstrated to be of some importance for amphibians. Woltz (2008) found that leopard frogs and green frogs preferred an experimental tunnel with many small holes drilled in the top over a tunnel with no holes drilled in the top, and Jackson and Tyning (1989) found that spotted salamanders moved at faster rates through tunnels with an increased amount of light shined into the tunnel at the entrance or exit with a flashlight.

Available ambient lighting level was a significant predictor of 3 primary responses we used to assess tunnel utilization: (1) entering the tunnel, (2) successful completion of the trial, and (3) successful completion of the trial, without hesitations. Turtles presented with tunnels where 100% of ambient light was transmitted from above were 8.4 times more likely to enter the tunnel, 84.3 times more likely to successfully complete a trial, and 21.3 times more likely to successfully complete a trial with zero hesitations compared to turtles presented with close-topped tunnels. While the effects of lighting on various wildlife species willingness to use tunnels has not been well studied, Openness Ratio (OR = a culvert's cross-sectional area divided by it's length) and aperture are commonly used as one indicator of lighting. Clevenger et al. (2005) measured the influence of a number of variables on the performance of passages by a suite of large mammals.

High OR structures (shorter, larger aperture passages) strongly influenced the willingness of grizzly bears, wolves, elk, and deer while low OR structures (longer, smaller aperture passages) best explained passage use by black bears and cougars. Woltz et al. (2008) found that tunnel aperture was a significant predictor for 3 out of 4 species tested (leopard frogs, snapping turtles, and painted turtles) in a choice experiment. These species avoided the smallest aperture (0.3 m) culvert. While light was not examined explicitly in either of these examples, it is clearly tied to OR and aperture and may be playing a role here. There are however other possible factors to consider such as predator avoidance that may be influential in determining whether or not an animal is willing to enter a space it may consider to be confining.

Of the three species examined, painted turtles were the most willing to utilize tunnels of either light treatment, while spotted turtles were the least willing. Response to the close-topped tunnel was very poor for spotted turtles and only marginally better for Blanding's turtles. Painted turtles were about 6 times more likely to enter a tunnel than either spotted turtles or Blanding's turtles and were 8.5 times more likely to successfully complete a trial than spotted turtles. The poor performance by spotted turtles was somewhat unexpected given their documented use of a 60 ft long 6 ft x 6 ft concrete box culvert at a Massachusetts site (Kay et al. 2005). Perhaps the confined space of a 2 ft x 2 ft culvert was smaller than most spotted turtles were willing to utilize and the effect of the confining space was more important to this species than light level.

Results from analysis of hesitation behaviors further support the limitations of close-topped tunnels for encouraging passage of freshwater turtles.

For the 2 ft x 2 ft aperture 80 ft long tunnel tested here, more turtles hesitated when presented with close-topped tunnels compared to 100% transmittance tunnels, and the mean number of hesitations was greater when no overhead light was transmitted into the tunnels. In this size range, hesitations associated with close-topped tunnels were most prevalent for spotted and Blanding's turtles, suggesting that these tunnel types may be inadequate to facilitate successful passage for both species.

Our tests of barrier fencing indicate that opacity is a significant predictor of turtle deviation from barriers, total distance traveled, rate of travel and path tortuosity. Species was also a significant predictor of the total distance traveled and the rate of travel.

All three turtle species were more likely to deviate from the opaque barrier than the translucent barrier and traveled farther when presented with an opaque barrier compared to a translucent one. In general, rates of travel were faster in trials with an opaque barrier compared to those with a translucent one, but interestingly, rates did not differ for Blanding's turtles. All three turtle species exhibited a higher degree of tortuosity when the barrier was translucent, and the paths of spotted turtles were more tortuous than either Blanding's turtles or painted turtles

Distance traveled and rate of travel may be partly explained by average body sizes of the species we tested. Mean total distances traveled by each species increased in correspondence to an increase of mean carapace lengths for each species providing an indication of similar effort expended by each species. Spotted turtles, the smallest species, traveled the shortest mean distances, and at the slowest rates.

These results suggest that turtle movements can be modified by the choice of either opaque or translucent barriers. While an opaque barrier may be the best tool to facilitate swift movement, the increased frequency of deviations from the opaque barrier is worrisome in situations where it is desirable for turtles to persist in their efforts to find a way beyond the barrier and eventually move through a passageway. Therefore, an opaque barrier is probably best utilized in barrier-only situations where a passageway is not installed.

Andrews et al (2007) provide no indication of studies on the importance of barrier opacity in their synthesis of data on desert tortoises (*Gopherus agassizii*) with regard to roadway mitigation. In the most extensive test of behavioral response by a turtle species to barriers, Ruby et al. (1994) tested the effects of different types of barriers and barrier materials on desert tortoises by placing captive tortoises in pens made of selected materials. The authors found that tortoises responded differently to solid and non-solid barriers when placed in small pens constructed of various materials. In total, 12 different opaque and translucent materials were tested and Ruby concluded that if the object of a barrier is to guide tortoises to a passageway, solid barriers will tend to inhibit this behavior so a translucent barrier is preferable. Specifically, he concluded that a small mesh (1 cm hardware cloth) barrier performed best because it allowed tortoises to see beyond the barrier yet the small mesh elicited far fewer attempts by tortoises to push or move through it than was observed for chain link or chicken wire. Fusari (1982) examined the behavioral response of desert tortoises to three fence types in a study on the feasibility of using a fence and culvert system to allow tortoises to pass under roads. Fusari found tortoises pushed more against open-mesh fences than against solid ones and also spent more time walking along open-mesh fences than along solid fences.

Both Ruby and Fusari determined that the choice of barrier type depends upon the goal of the barrier. They argue that if a barrier is to repel tortoises from an area, then a solid barrier is preferred. If it is to repel and guide tortoises along and then beyond a structure such as a highway, then a non-solid barrier is best. The results of our work testing reactions to barrier types by freshwater turtles lead us to the same conclusions regarding the use of translucent barriers to guide turtles through an area and solid barriers to repel turtles from an area. It is worth considering also that motivational factors may play a large role outside of experimental situations and the motivation by turtles may outweigh any effects of barrier opacity if strong enough.

Conclusions/Management Implications

Our results from tests of ambient lighting in tunnels 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 that lacks overhead light may be of little or no use in passing turtles. Spotted turtles were more hesitant than painted and Blanding's turtles to enter tunnels under either lighting treatment, indicating that they may be inhibited by the width and/or length of the passage itself.

It appears that barriers can be an effective means of directing turtles into passages and that varying the opacity can be used to manipulate their behavior. We found that the mean total distance traveled by all 3 species tested was considerably greater and the rate of travel was considerably faster when a barrier was opaque versus translucent. At the same time, turtles were significantly more likely to deviate from an opaque barrier versus a translucent one so we conclude that translucent barrier material is a better choice for directing turtles toward a passageway and an opaque barrier is a better choice when simply deterring and restricting access from a roadway or other hazardous area.

Though we did not test scent cues, it is possible that they may lead to increased use of closed topped culverts in the field, compared to what we measured under experimental circumstances. Over time, the rate of successful passage may increase due to acclimation, but this hypothesis remains to be tested. Considering the low rates of successful passage for spotted turtles and Blanding's turtles, and accompanying high numbers of hesitation behaviors, in close-topped tunnels, it is unlikely that willingness to utilize the tunnel will improve to an acceptable level, even with acclimation.

Table 2.1 Study design and analysis approach for experiments examining tunnel lighting level and barrier opacity on the movement behavior of 3 turtle species.

Experiment purpose (number of trials)	Experimental variables	Response variables	Data treatment
<u>Experiment 1</u> Test relative importance of varying lighting level with regard to movement behavior among 3 species (n = 110)	<u>Light level:</u> - 100% - 0% <u>Species:</u> - spotted turtle - Blanding's turtle - painted turtle	<u>Categorical:</u> - non-reactive y/n - hesitated y/n - entered y/n - successful completion y/n -successful completion with zero hesitations y/n	GLM with logit link
		<u>Continuous:</u> - total number of hesitations - rate of travel in tunnel (successful trials only)	ANOVA w/Type III Sums of Squares
<u>Experiment 2</u> Test effectiveness of barrier design among 3 species (n = 130)	<u>Barrier opacity:</u> - opaque -translucent <u>Species:</u> - spotted turtle - Blanding's turtle - painted turtle	<u>Categorical:</u> - deviated from barrier y/n	GLM with logit link
		<u>Continuous:</u> - total distance traveled - rate of travel -tortuosity of path	ANOVA

Table 2.2 GLM with logit link. Reduced models were chosen with stepwise Akaike Information Criteria (AIC).

Response	Effect	Df	Deviance	Resid. Df	Resid. Dev	P
Non-reactive	Null	-	-	5	4.36	-
	Light	1	1.23	4	3.12	0.27
	Species	2	1.40	2	1.72	0.50
	Light x species	2	1.72	0	0	0.42
Hesitated	Null	-	-	5	31.58	-
	Light	1	21.63	4	9.96	3.31e-06 ***
	Species	2	7.93	2	2.02	0.01893 *
Entered	Null	-	-	5	30.92	-
	Light	1	22.56	4	8.36	2.038e-06 ***
	Species	2	6.60	2	1.77	0.03705 *
Success	Null	-	-	5	78.75	-
	Light	1	70.64	4	8.11	<2e-16 ***
	Species	2	6.35	2	1.76	0.0418 *
Success w/no hesitations	Null	-	-	5	45.91	-
	Light	1	36.94	4	8.97	1.219e-09 ***
	Species	2	6.68	2	2.29	0.03551 *

Table 2.3 Logistic regression, odds ratio (probability modeled is non-reactive = 0, hesitated = 0, entered = 0, success = 0, success w/no hesitations = 0). 100% AL (100% available light), 0% AL (0% available light), CHPI (painted turtle), CLGU (spotted turtle), EMBL (Blanding's turtle). *Non-reactive trials were excluded from odds ratio analysis because there were no significant predictors.

Response	Effect	Point estimate	Wald 95% confidence limits	
Hesitated	Intercept	1.69	0.54	5.39
	100% AL vs. 0% AL	0.13	0.05	0.31
	CLGU vs. CHPI	5.51	1.56	21.62 2
	EMBL vs. CHPI	2.14	0.64	7.71
Entered	Intercept	3.80	0.97	25.07
	100% AL vs. 0% AL	8.4	3.37	23.09
	CLGU vs. CHPI	0.15	0.02	0.71
	EMBL vs. CHPI	0.17	0.02	0.76
Success	Intercept	0.24	0.04	0.99
	100% AL vs. 0% AL	84.3	23.55	434.8 4
	CLGU vs. CHPI	0.11	0.01	0.71
	EMBL vs. CHPI	0.38	0.06	2.28
Success w/no hesitations	Intercept	0.18	0.04	0.67
	100% AL vs. 0% AL	21.3	7.09	2.32
	CLGU vs. CHPI	0.19	0.04	0.75
	EMBL vs. CHPI	0.54	0.13	2.08

Table 2.4 Mean values by species for behavioral response to tunnel light treatment. CHPI (painted turtle), CLGU (spotted turtle), EMBL (Blanding's turtle).

Species	Light Treatment (% light permitted)	n	Mnhes (sd)	%enter	%success	%nohes
Spotted turtle	0%	21	5.1 (2.9)	38 %	0 %	0 %
	100%	22	0.9 (1.2)	82 %	73 %	45 %
	0% and 100%	43	3.0 (3.0)	60 %	37 %	23 %
Blanding's turtle	0%	23	5.6 (4.2)	35 %	9%	9%
	100%	25	0.6 (1.1)	88 %	88 %	68 %
	0% and 100%	48	3.0 (3.9)	63 %	50 %	40 %
Painted turtle	0%	8	2 (2.3)	88 %	25 %	25 %
	100%	11	0.6 (1.3)	91 %	91 %	73 %
	0% and 100%	19	1.2 (1.8)	89 %	63 %	53 %
All species	0%	52	4.9 (3.6)	44 %	8 %	8 %
	100%	58	0.7 (1.2)	86 %	83 %	60 %
	0% and 100%	110	2.7 (3.3)	66%	47%	35%

Table 2.5 Logistic regression, odds ratio (probability modeled is separated from wall = 0). opaque (opaque barrier), translucent (translucent barrier), CHPI (painted turtle), CLGU (spotted turtle), EMBL (Blanding's turtle).

Response	Effect	Point estimate	Wald 95% confidence limits	
Deviated from barrier	Intercept	0.91	0.40	2.12
	opaque vs. translucent	3.11	1.51	6.60
	CLGU vs. CHPI	0.72	0.27	1.84
	EMBL vs. CHPI	0.36	0.13	0.91

Table 2.6 Mean values by (a) by barrier only & by barrier and species; and (b) by species only. Tortuosity of path - ranges from 0-1. The closer the value is to 1, the greater proportion of moves made were deviations from the barrier.

Barrier treatment	Species	n	Total distance traveled (SD)	Rate of travel FPM (SD)	Tortuosity of path (SD)	% trials with deviations
Opaque	CHPI	18	512.33 (199.41)	9.41 (3.05)	0.35 (0.10)	78 %
	CLGU	24	365.65 (179.82)	7.32 (2.74)	0.42 (0.11)	63 %
	EMBL	27	454.32 (179.09)	7.79 (2.90)	0.33 (0.08)	52 %
	All species	69	438.61 (191.20)	8.05 (2.97)	0.37 (0.10)	62 %
Translucent	CHPI	14	301.58 (143.17)	5.12 (2.34)	0.34 (0.12)	43 %
	CLGU	25	218.19 (116.07)	3.65 (1.94)	0.46 (0.13)	44 %
	EMBL	22	340.74 (90.31)	5.90 (1.42)	0.40 (0.11)	23 %
	All species	61	281.53 (125.44)	4.80 (2.10)	0.41 (0.13)	36 %
Both treatments	CHPI	32	420.12 (204.17)	7.53 (3.48)	0.35 (0.11)	63 %
	CLGU	49	290.42 (166.67)	5.44 (2.98)	0.44 (0.12)	53 %
	EMBL	49	403.32 (155.56)	6.94 (2.52)	0.36 (0.10)	39 %
	All species	130	364.90 (181.06)	6.52 (3.06	0.39 (0.12)	50 %

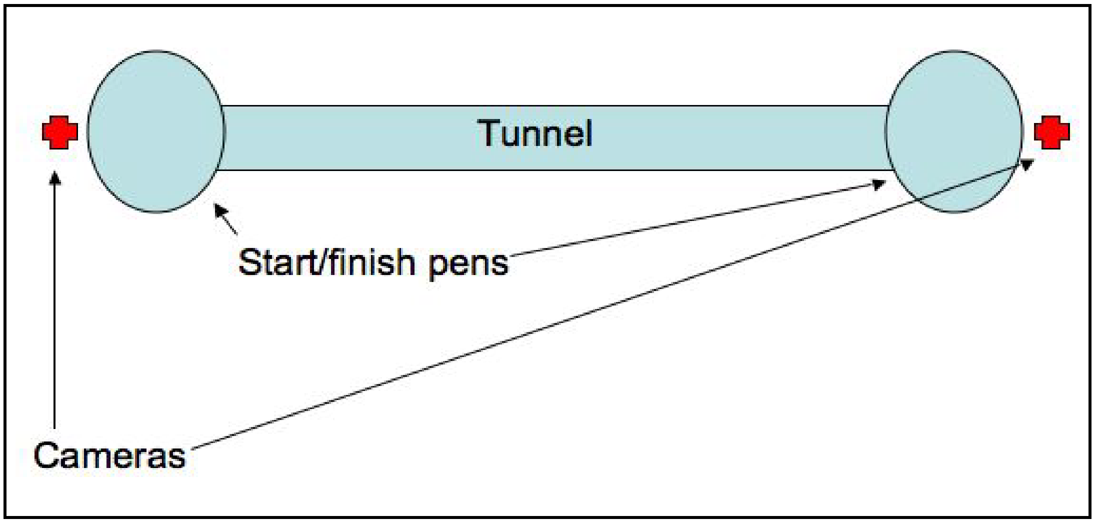

Figure 2.1 Schematic diagram depicting the tunnel field laboratory used in tests of artificial lighting.

a.

b.

Figure 2.2 Photographs of barrier laboratory illustrating the two fence treatments; (a) translucent (fence only) (b) opaque (fence with opaque visual barrier).

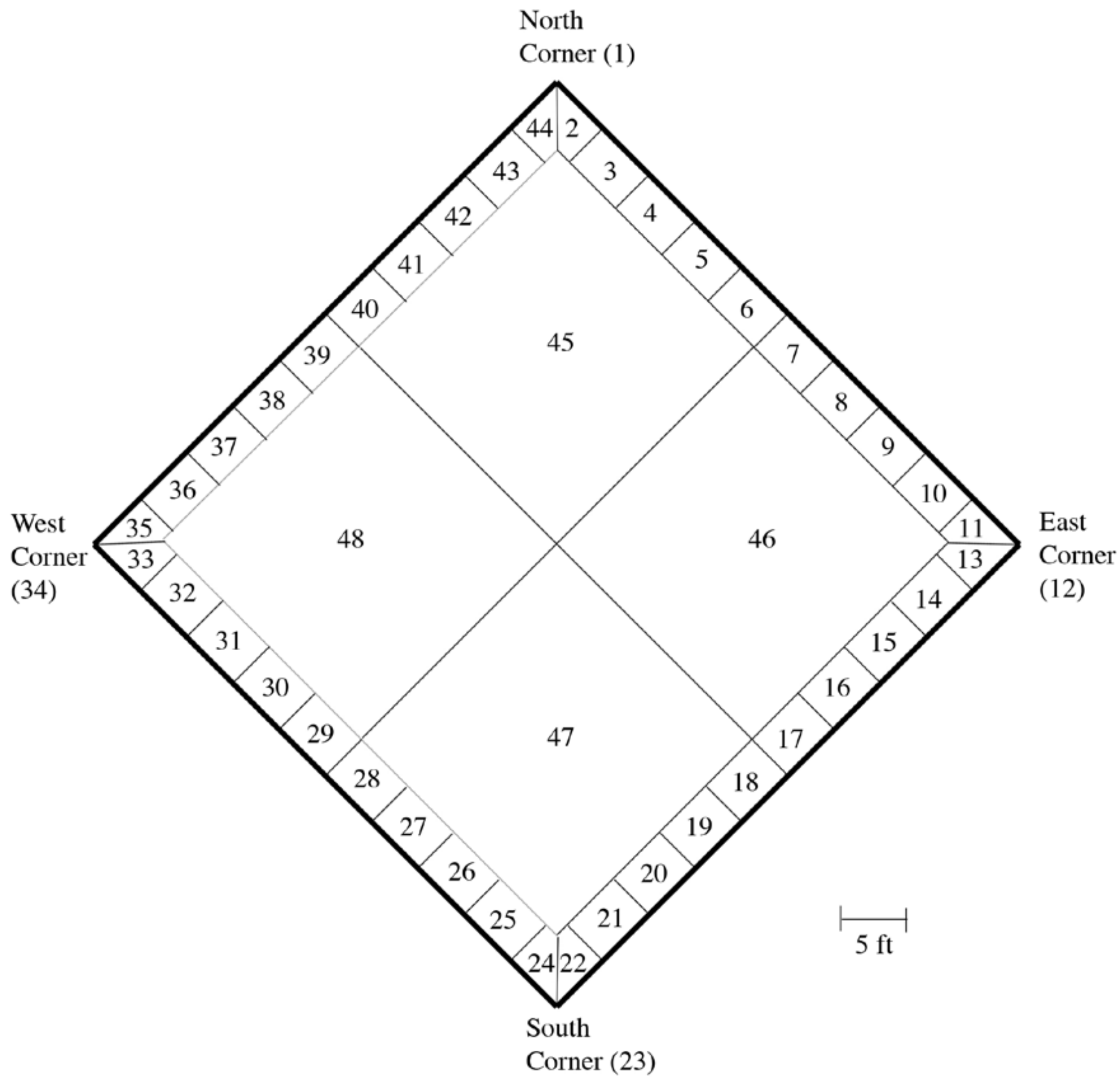

Figure 2.3 Schematic of barrier laboratory showing numbered sampling locations. During experiments, locations of turtles were recorded at any of the 44 cells or 4 corner points at 2-minute intervals. Movement patterns were analyzed to assess the reactions of turtles to the fencing types.

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