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# EVALUATING THE EFFECTS OF ABIOTIC AND BIOTIC FACTORS ON MOVEMENT THROUGH WILDLIFE CROSSING TUNNELS DURING MIGRATION OF THE CALIFORNIA TIGER SALAMANDER, *AMBYSTOMA CALIFORNIENSE*

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**Abstract.**—Road networks cut through ecosystems causing habitat fragmentation, genetic isolation, and wildlife mortality. Wildlife crossing structures are used often as a solution to overcome this growing problem, but are frequently installed without further study on the features that can influence their efficacy. We addressed this issue by investigating the effects of several abiotic and biotic factors, including precipitation, ambient temperature, humidity, sex, size, and within-tunnel moisture levels, on the movement of endangered California Tiger Salamanders (*Ambystoma californiense*) during their annual migration. We examined the effects of these factors on the rate of tunnel completion and the time it took individuals to traverse a tunnel, with an experimental focus on within-tunnel moisture. We examined 77 individuals in this study, and 39 of these individuals passed through the tunnels regardless of moisture levels. Our results showed that within-tunnel moisture levels had no significant effect on the probability of tunnel crossing completion or rejection, but did have an effect on crossing time, resulting in individuals spending about 20% less time traveling through wet tunnels as compared to dry tunnels. Among the other factors evaluated, only precipitation increased the probability of completions. Our findings suggest that internal moisture levels have little effect on the use of road tunnels and the migration of *A. californiense*. Rather, external precipitation largely drives tunnel usage and migratory behavior. Nonetheless, our study demonstrated the successful use of wildlife crossing structures in safely facilitating *A. californiense* under a hazardous roadway.

**Key Words.**—amphibians; behavior; conservation; endangered; precipitation; moisture; road ecology; road tunnels

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## INTRODUCTION

A growing human population and the desire for connectivity and accessibility have driven the expansion of roadway networks across the globe. This has had countless negative impacts on the land and ecosystems through which these roads traverse (Coffin 2007; Bissonette and Adair 2008; van der Ree et al. 2011). Examples of road impacts include habitat fragmentation, genetic isolation, edge effects, wildlife mortality, and environmental pollution (Forman and Alexander 1998; Clevenger and Waltho 2000; Forman and Deblinger 2000; Corlatti et al. 2009). Although roads can negatively affect many species, there are particular groups that are more disrupted by roads than others. Wildlife that undergo migrations, have expansive ranges, or are highly mobile tend to encounter roads more frequently than less mobile fauna (Gloyne and Clevenger 2001; Tigas et al. 2002). Additionally, small, slow-moving animals, such as amphibians, increase their chances of vehicular impact because of their inability to evade vehicles (Carr and Fahrig 2001; Hels

and Buchwald 2001; Allaback and Laabs 2003; Woltz et al. 2008). Many species of amphibians must complete annual migrations across roadways, thus compounding their chances of mortality (Gibbs and Shriver 2005; Mazerolle et al. 2005; Andrews et al. 2008). One such amphibian is the endangered California Tiger Salamander (*Ambystoma californiense*), which migrates every winter between its upland habitat and breeding vernal pools.

California Tiger Salamanders are endemic to California, USA, and typically occur in grassland and woodland habitats near breeding pools and ponds. The historical range of this species included the Central Valley and surrounding foothills, and lowlands of the Coast Range Mountains (U.S. Fish and Wildlife Service 2003). However, urbanization, agricultural expansion, and suburban development have reduced *A. californiense* habitat dramatically (Davidson et al. 2002; Cook et al. 2006). Today, *A. californiense* is limited to patchy regions of its historic range, and the species is listed as either threatened or endangered under the federal and California state endangered species acts (Shaffer et

al. 2004; California Natural Resources Agency. 2011. California Natural Diversity Database. Available from [http://www.dfg.ca.gov/biogeodata/cnddb/plants\\_and\\_animals.asp](http://www.dfg.ca.gov/biogeodata/cnddb/plants_and_animals.asp) [Accessed 23 August 2011]).

During rainy winter nights, *A. californiense* leave their burrows en masse and travel to nearby breeding pools (Barry and Shaffer 1994; Cook et al. 2006). After breeding is finished, both sexes return to their upland habitat for the remainder of the year. In Sonoma County, California, a breeding population of *A. californiense* completes its yearly migration across a high speed, heavily trafficked road to reach the only breeding pool in the vicinity. This trek increases the risk of vehicular mortality, population decline, and even local extinction compared to a migration free of road crossings (Beebee 2013). One possible option to provide safe passage for migrating salamanders is to install wildlife crossing structures under the road and directional fencing within the migration corridor.

Wildlife crossing structures are frequently used in many countries to effectively connect migration corridors, ecosystems, and to link habitats across fragmented landscapes (Ng et al. 2004; Goosem et al. 2006; Trocmé 2006; Mata et al. 2008). There are numerous designs for crossing structures that are influenced by factors including wildlife type, road topology, structural attributes, habitat variations, and available funding (Jackson and Griffin 2000; Glista et al. 2009). Most amphibian crossing structures are pipes made from concrete or metal that may or may not include a natural substrate (Lesbarrères et al. 2004; van der Ree et al. 2007). Other designs have open grated tops that allow rain to come through and increase air flow. Additionally, some type of low fencing is used to direct amphibians to the tunnel entrance and prohibit them from entering the roadway (Jackson and Tynning 1989).

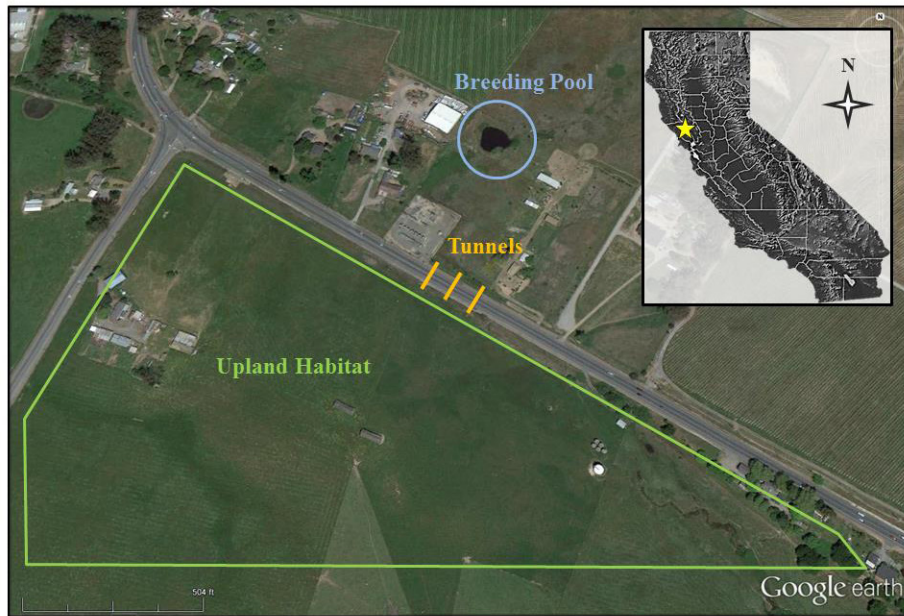
The efficacy of various tunnel designs has been studied by scientists around the globe. Most studies measured the success rate of amphibian passage or the effects of structural attributes (e.g., length, diameter, structure material; Mata et al. 2005; McCollister and Van Manen 2010; Patrick et al. 2010). Fewer studies tested internal tunnel conditions such as moisture, and their effects on amphibian behavior (van der Ree et al. 2007; Woltz et al. 2008). Because most amphibians require damp surroundings to avoid desiccation, long, dry tunnels could deter or even inhibit *A. californiense* use. Furthermore, wildlife tunnels often have been questioned as potential prey traps, and dry tunnels could slow amphibian movement, thus increasing the chance of predation (Little et al. 2002). This project addressed these concerns by manipulating the internal tunnel conditions, which is essential for understanding amphibian behavior and use of crossing structures.

Our primary goal was to study the effect of moisture in underground crossing tunnels on the migration and behavior of *A. californiense*. We conducted experiments to analyze the behavior and movement of *A. californiense* when they encountered tunnels with or without water added. We hypothesized that water would change the rate at which *A. californiense* would cross through tunnels. We predicted that with added water, *A. californiense* would cross through tunnels at a faster pace and with greater frequency than without water. We used project data to determine the effectiveness of each tunnel and provide input on future crossing structure systems.

## MATERIALS AND METHODS

**Study site.**—Our study site was located along Stony Point Road in Sonoma County, California, USA. Most of the *A. californiense* upland habitat was located south of the road in a pasture used for livestock grazing; whereas, a single breeding pool was located north of the road and bordered by vineyards and minimal upland habitat (Fig. 1). The Stony Point Road study site consisted of a two-lane asphalt road with surrounding grassland habitat. We measured the *A. californiense* migration corridor to be approximately 644 m along the road based on the locations of the furthest captured salamanders. The tunnel and fencing system covered 85 m of road, including approximately 13% of the central portion of the migration corridor. The tunnel and fencing system was limited in coverage because of roadside restrictions from the construction of the road bed and private property limitations. Within the tunnel area, the road was elevated 1–1.5 m above the surrounding terrain. The road had steep earthen shoulders and shallow drainage ditches along both sides. We installed three steel pipes, 22 m long and 25 cm in diameter, underneath the road, and we placed them 35 m apart. The unperforated pipe tunnels were installed near the vernal pool, but not directly across from it because of limiting factors in the roadbed. We connected all three tunnels with directional fencing made of plastic mesh that was 43-cm tall. We chose this fencing to allow water to flow through and for its durability to withstand multiple years of direct sunlight. *Ambystoma californiense* have difficulty climbing vertically and so this fencing was installed in a vertical fashion to prevent salamander escape (Trenham and Cook 2008). The directional fencing formed a zig-zag pattern as it angled toward each tunnel entrance to funnel salamanders under the road (Fig. 2a).

On the south side of the road, a 25-cm diameter PVC pipe spanned the ditch connecting the steel tunnel to the directional fencing, thus preventing salamanders from entering the ditch (Fig. 2b). Each PVC pipe connector



**FIGURE 1.** Aerial photograph of study area in Sonoma County, California, USA, showing California Tiger Salamander (*Ambystoma californiense*) upland habitat, breeding pool, and location of tunnels. (Photograph from Google Earth, 5 February 2014).

had slits cut into the top to allow rain to enter and increase airflow to encourage use by *A. californiense*. On the north side of the road, we used a double fencing design to allow water to flow freely through the ditch. A short, angled fence led out of the tunnel and then ended before the roadside ditch (Fig. 2c). A second, longer angled fence continued on the other side of the ditch. We used the double fencing design on the south side of the road as there was less space available for a single, connected, zig-zag fence.

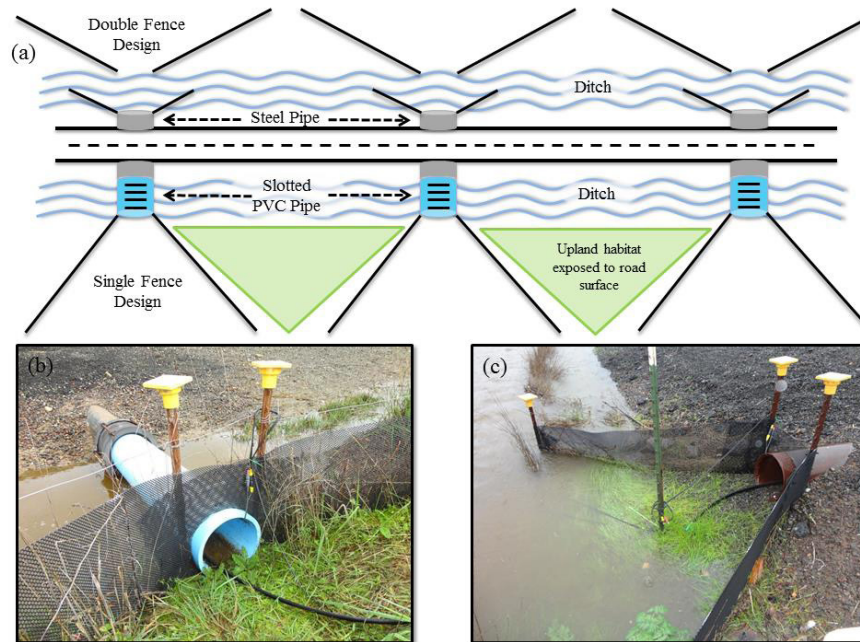
**Road surveys.**—We performed road surveys at night 1700–2300 from October through March 2011–2013 to capture living or dead salamanders on the road and in the roadside areas. We conducted surveys only on nights when there was at least a 30% chance of rain according to local weather stations. Observers with flashlights walked a continuous loop approximately 725 m long along both sides of Stony Point Road through the *A. californiense* migration corridor, searching for salamanders. We translocated salamanders 400 m or less from their capture site to the tunnel locations. We did not look for salamanders in the grassland area behind the fencing as it was too dark to locate them in the grass. We placed each salamander found in individual buckets and recorded the age, sex, snout-vent length (SVL), and condition of each individual (alive/dead). We categorized salamanders as either juvenile or adult, with juveniles being 90 cm or less SVL and usually moving toward the upland habitat away from the vernal pool. We photographed living salamanders to document the spot pattern for later identification. We

used living adult salamanders for our wildlife crossing tunnel experiments.

**Tunnel experiments.**—We conducted tunnel passage experiments during the winter migration of *A. californiense* for two seasons beginning in October 2011 (winter 2012) and ending in February 2013 (winter 2013). We compared salamander behavior and travel times between dry and wet tunnels. We extended a porous soaker hose through all three tunnels to ensure an equal distribution of water along the length of each tunnel. These hoses remained in the tunnels for the duration of the study. On rainy nights prior to sunset, we haphazardly chose one or two of the tunnels to be wet tunnels for the experiments. The selected hose(s) received 19–26 L of water over approximately 15 min to ensure the tunnel floor remained wet for the evening experiment. Any excess water drained from the ends. Tunnels with added water were visibly wet throughout the duration of the experiment while tunnels without water added were visibly dry the entire time. We used all three tunnels in the experiments.

We used each salamander captured during the road surveys only once to reduce stress in the animals and to minimize the potential for handling effects (Cash et al. 1997). Furthermore, we used only adult *A. californiense* migrating toward the breeding pool in the experiments as juveniles were moving in the opposite direction across the road and their movement was not driven by a physiological motivation to breed. We haphazardly chose and placed salamanders one at a time in front of and facing a tunnel entrance in their natural direction





**FIGURE 2.** Tunnel system design for California Tiger Salamander (*Ambystoma californiense*). (a) diagram of tunnel and fencing system; (b) slotted PVC pipe tunnel entrance crossing ditch on south side of road; (c) steel tunnel exit on north side of road with video camera inside. (Photographed by Tracy Bain).

of migration. After moving a short distance away, we stood still to record the behaviors and reactions of salamanders to the tunnel entrance. We recorded the following behaviors: hesitation to enter the tunnel, retreating, tunnel rejection, burrowing, and climbing.

We recorded tunnel completion and calculated crossing time, or the time it took a salamander to traverse a tunnel and emerge on the other side. During the first season, we synchronized watches and recorded the exact time in minutes when a salamander entered and exited a tunnel. During the second season, we installed Sony Effio infrared security cameras (Sony Effio Weatherproof IR Bullet Camera System, Sony Corporation, Tokyo, Japan) at the six tunnel openings and positioned them outward to record *A. californiense* as they exited or entered the tunnels. During post-processing analysis we reviewed the time-stamped video footage and located the crossing salamanders. Each individual was identified as either an animal used in the experiments or one that crossed through the tunnels on its own. To identify individual salamanders, we manually matched the spot pattern of a salamander in the video footage to the photographs taken of each individual prior to the experiments. Spot pattern identification has been shown to accurately identify individual salamanders in previous studies (Searcy and Shaffer 2011; Searcy et al. 2013; Wayne 2013). We recorded completed tunnel crossings after a salamander exited a tunnel on the north side of the road in the direction of migration. We

calculated total crossing time in minutes by subtracting the exit video time stamp from the entrance video time stamp. The cameras also served as a means to record *A. californiense* and other wildlife movements throughout the evening on rainy nights.

**Statistical analyses.**—To determine whether there was an effect from a wet or dry tunnel floor on tunnel completion, we analyzed data with a generalized linear mixed model (SAS 9.2). We used a linear mixed model to compare tunnel crossing time in wet or dry tunnels (JMP 11). For each experiment, we chose the best model to minimize the Akaike Information Criterion (AIC). We included average temperature, relative humidity, and total precipitation during the experimental period as covariates in both original models. We acquired these data from the National Climatic Data Center, Santa Rosa Airport weather station (National Oceanic and Atmospheric Administration. 2013. Quality Controlled Local Climatological Data. Available from <http://www.ncdc.noaa.gov/data-access/land-based-station-data/land-based-datasets/quality-controlled-local-climatological-data-qclcd> [Accessed 23 January 2014]). We included salamander sex and SVL as fixed effects in the original models, but we removed them from the models if not significant. We included tunnel identifier as a random effect in both models. We evaluated linear mixed model residuals for approximate normality after log transformation of movement times.

**TABLE 1.** Road observations of California Tiger Salamanders (*Ambystoma californiense*) at Stony Point Road migration corridor during winter 2012 and 2013. Age group is based on snout to vent length. We could not identify some dead salamanders to age class or sex.

Field Season	Adult	Juvenile	Male	Female	Alive	Dead
2012	18	6	7	6	23	5
2013	71	20	35	30	84	43
Total	89	26	42	36	107	48

## RESULTS

**Road surveys.**— We captured 155 *A. californiense*, including 89 adults, 26 juveniles, and 40 individuals we could not assign to an age class because of mutilation from vehicular collisions (Table 1). We collected all *A. californiense* found on the roadway and roadside areas regardless of which direction they were traveling. However, most adults were moving north across the road toward the breeding pool; whereas, most juveniles were moving south toward their upland habitat.

**Salamander tunnel transits.**—Over the course of two breeding seasons, winter 2012 and winter 2013, we used 77 *A. californiense* in the behavioral experiment. All were adults and in breeding condition moving north toward the breeding pool. Of these 77, 39 (51%) individuals successfully crossed through the tunnels regardless of moisture conditions. Twenty *A. californiense* completed the wet tunnels and 19 completed the dry tunnels. The remaining 38 salamanders turned around and rejected the tunnels during the project hours. Other recorded behaviors included one failed attempt to climb the walls inside a tunnel and one attempt to climb the fencing leading to the tunnels. A more common behavior we observed nine times, was for a salamander to burrow under the grass after release, if rainfall had decreased or stopped altogether. We observed most salamanders moving at a staggered pace, either through the grass or when crossing the road, stopping to check their surroundings, or alter their direction.

Although almost half of the salamanders included in the behavior experiment successfully crossed through the tunnels, tunnel completion was not significantly affected by tunnel moisture levels ( $F_{1,72} = 0.01$ ,  $P = 0.972$ ). This was further substantiated by the equal numbers of rejections to both wet and dry tunnels. However, tunnel completion was significantly affected by precipitation ( $F_{1,72} = 6.71$ ,  $P = 0.012$ ). As precipitation increased, tunnel completion through both wet and dry tunnels increased (Fig. 3). Ninety-seven percent of tunnel rejections occurred on nights with < 25 mm of total rainfall and 84% of tunnel rejections occurred on

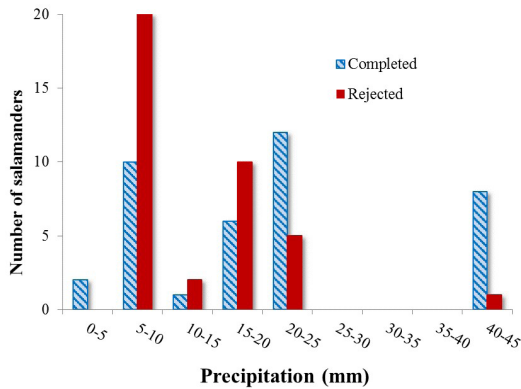
**TABLE 2.** Aikake Information Criteria (AIC) Values for GLIMMIX models tested using the following fixed effects: temp = average temperature, hum = percent humidity, precip = daily precipitation, TT = tunnel type (wet/dry), sex (male/female). Non-significant fixed effects were removed from the best model.

Model	AIC
temp, hum, precip, TT, sex, svl	108.59
temp, hum, precip, TT, sex	106.59
hum, precip, TT, sex	105.40
precip, TT	104.34
precip	102.90

nights with < 20 mm of total rainfall. All other fixed effects (average temperature, relative humidity, sex, and snout-vent length) were removed from the final model as these were not significant and did not improve model strength according to AIC values (Table 2).

Of 39 *A. californiense* that successfully traveled through the tunnels, it took an average of 22.4 min ( $\pm 17.0$  min [SD]) for salamanders to cross through a wet tunnel (crossing time ranged from 2–61 min) versus 28.4 min ( $\pm 20.75$  min) through a dry tunnel (crossing time ranged from 11–83 min). Crossing time of 27 of these salamanders (incomplete data on SVL and sex for 12 individuals evaluated by cameras) was significantly associated with tunnel moisture levels ( $F_{1,22} = 5.77$ ,  $P = 0.025$ ). Fixed effects associated with SVL and sex were not significant and were excluded from the final model. Additionally, although not significant ( $F_{1,22} = 3.91$ ,  $P = 0.060$ ), there was a trend towards a decrease in travel time through tunnels as precipitation increased (Fig. 4).

**Tunnel camera monitoring.**—Infrared video cameras at each of the tunnel openings recorded the movements of *A. californiense* and other species using the tunnels on nights when rain occurred during the breeding season. The cameras captured observations of a variety of mammals including: Virginia Opossum (*Didelphia virginiana*), Northern Raccoon (*Procyon lotor*), Gray Fox (*Urocyon cinereoargenteus*), Domestic Cat (*Felis catus*), and various rodents as well as amphibians such as the Arboreal Salamander (*Aneides lugubris*) and the Sierra Tree Frog (*Pseudacris sierra*) using or exploring the tunnels. In addition to providing data on the crossing times of salamanders used in the experiments, the cameras also captured 31 additional observations (identified by researchers) of *A. californiense* using the tunnels to cross to the breeding pool unaided after project hours during the 2013 winter season. These observations were not included in the statistical analysis as the individuals were not exposed to the same experimental conditions as the captured salamanders.

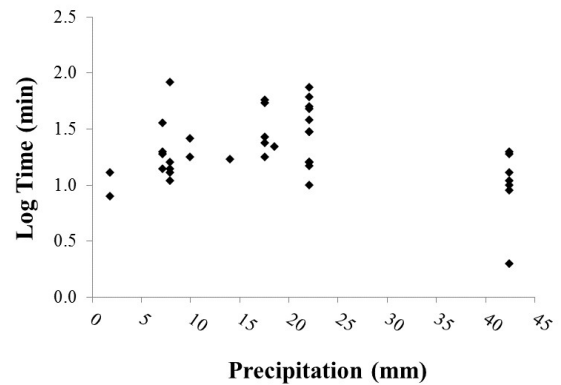


**FIGURE 3.** Number of California Tiger Salamanders (*Ambystoma californiense*) that completed or rejected tunnels with regard to precipitation levels.

## DISCUSSION

This study showed that moisture levels within road tunnels had little effect on whether a salamander would cross through a tunnel. However, the moisture levels in the tunnels did effect transit times. Precipitation was found to be the key factor in this study, increasing the propensity for both *A. californiense* behavior and movement. Finally, although the installation of the tunnel system did not prevent all salamanders from accessing the road surface, this study documented 31 salamanders after study hours moving unaided through the tunnels toward the breeding pool. The three-tunnel system allowed a portion of the population to reach the breeding pool without risk of mortality on the roadway during migration (exact percentage cannot be calculated as the population size is unknown). Future studies would be useful to determine what proportion of breeding *A. californiense* use the tunnels relative to those still accessing the road during migratory movements both to and from the breeding pool.

**Effects of tunnel moisture on movements.**—We found that 51% of *A. californiense* captured and placed at a tunnel entrance successfully and immediately crossed through the tunnels regardless of tunnel moisture levels. Our results revealed that evening precipitation, which did not provide direct moisture into the pipe tunnels, had a greater effect on tunnel completion than moisture levels. Precipitation outside the tunnels appeared to be the driving factor for *A. californiense* to pass through the tunnels with more frequency. This effect was supported by the data that revealed 97% of the tunnel rejections occurred on nights with < 25 mm of rainfall during project hours. Although it is interesting that the solid tunnels, which precluded rain, did not seem to deter their movement, it is reasonable that this behavioral



**FIGURE 4.** Relationship of time adult California Tiger Salamanders (*Ambystoma californiense*) used to complete passage through tunnels under road relative to precipitation levels.

drive is stronger during heavier rainfall (Trenham 2001; Cook et al. 2006; Orloff 2011).

Tunnel moisture levels did have an impact on crossing time. *Ambystoma californiense* moved an average of 6 min faster through a wet tunnel than a dry tunnel. However, the biological significance of this difference in time is uncertain. This time difference could increase the chance of predation in dry tunnels, but it is doubtful there would be a substantial effect as previous studies have shown few instances of intentional predation in or near crossing structures (Little et al. 2002; Ford and Clevenger 2010). Further, it was observed that most *A. californiense* did not move at a fixed rate. We observed that salamanders moved at a staggered pace, stopping frequently to take in their surroundings, and presumably to check for predators and readjust their course of direction. This staggered movement occurred during all levels of precipitation and on all substrate types, and could be the reason for the minimal difference between crossing times.

**Impacts of environmental variables on movements.**—Precipitation has been a principle and driving factor throughout this study. This is not wholly unexpected as it has been shown in previous studies of ambystomatid salamanders that migration will not occur on dry nights. When rainfall does occur there is often mass movement to breeding grounds to capitalize on the weather conditions (Loredo and Van Vuren 1996; Pagnucco et al. 2012). It is not unreasonable then to propose that with heavier rainfall, salamanders will be driven to reach their breeding grounds both at a faster pace and with less regard to obstacles in their path, such as underground tunnels. They are following reproductive instincts responding to the wet weather stimulus to more successfully breed. On the other hand, lack of rainfall will slow *A. californiense* movement

and even halt it altogether. This could explain why salamander crossing times were more varied during nights with less rainfall. Many project nights began with heavy precipitation and salamander movement, but when the rain slowed or stopped, most salamanders discontinued their trek and immediately burrowed under the wet grass, returning to their journey and tunnel crossing later in the evening. This could be a behavior to avoid desiccation, but is curious because the ground was still saturated and desiccation was unlikely. These behaviors could explain why a wet tunnel floor had little impact on both tunnel usage and salamander speed. It seems that water from above, rather than below, is the key driver of *A. californiense* movement.

Additional environmental variables measured in this study included relative humidity and average temperature. It was not surprising that humidity had little effect on *A. californiense* movement as humidity was generally constant during rainfall. Temperature varied throughout each season and we presumed colder temperatures would discourage salamander movement as suggested in previous studies (Trenham et al. 2000; Orloff 2011). However, temperature did not significantly affect movement in our study, and so was removed from the analyses.

We hypothesized that *A. californiense* body size might influence crossing time, and that larger salamanders would move through the tunnels more quickly. Larger individuals could have an increased stride and cover more ground in a shorter time. Bennett et al. (1989) showed that locomotor performance and endurance is positively correlated with body size in salamanders. However, in our study, there was no correlation between body size and crossing time as salamanders of all sizes moved at varying rates with no significant pattern.

Lastly, we evaluated whether sex influenced crossing time. Males arrive at the breeding pools first to stage at the pool and await the arrival of females (Cook et al. 2006). Males might move faster through the tunnels in their attempt to outcompete other males for the best pool positions. Further, females would likely be weighed down by their egg sac, thus making the journey slow and arduous (Finkler et al. 2003). On the contrary, we found no pattern of one sex moving more quickly through the tunnels than the other sex. In fact, the fastest time recorded (2 min) through a tunnel was from a gravid female.

**Management implications.**—This study can be used to enhance the efficacy of the tunnel and fencing system at this site and offers insights for small pipe crossing structures at other sites. Often wildlife crossing structures are added long after the design and construction of a roadway in response to high levels of wildlife vehicular mortality (Grilo et al. 2010).

Although most structures are built following blueprints derived from previous sites, many assumptions are made as to which design will be most effective for the focal species. Additionally, there are often funding or site limitations that influence design choice and may restrict the feasibility of options. This leads to many wildlife crossing structures with reduced effectiveness (Glista et al. 2009).

A key assumption for this study was that long dry tunnels would inhibit *A. californiense* movement and that added moisture would enhance their effectiveness. However, by conducting research on this internal variable and crossing structure design, we found that this assumption was incorrect and that *A. californiense* will use a solid dry tunnel during its migration. This finding should encourage further study of wildlife crossing structure designs to identify features that enhance or impact the usefulness of tunnels.

The tunnel system constructed at this site successfully connected the fragmented *A. californiense* habitat across Stony Point Road. However, 56% of the salamanders used in the experiments rejected the tunnels on their first attempt. Internal moisture was found to have little effect on salamander movement through the tunnels, so it is likely another variable dissuaded them. In this study, 97% of the rejections occurred during precipitation levels of < 25 mm, and so it is plausible that lower levels of rainfall accounted for many of the tunnel rejections. Other potential variables include airflow, internal temperature, vehicular sound, ambient light, substrate, or handling effects. For example, it is possible that handling salamanders prior to use in behavioral experiments could have a negative impact on their natural migratory behavior. Future studies on these variables, and the role of precipitation, should be conducted to assess the impact on crossing success at this site and others.

Overall this study showed that wildlife crossing structures can be a valuable strategy in decreasing road effects on migratory amphibians. The tunnels were successful at providing a conduit for *A. californiense* to cross under the road, and the fencing effectively guided many salamanders to the tunnel entrances after project hours. Although the roadside geography and property restrictions limited the number of tunnels to three in the migration corridor, they were positioned in the location of highest salamander crossing density to provide clear routes for the majority of migrating salamanders. Similarly, the fencing was not installed along the entire migration corridor due to property restrictions and to ensure there were no impediments to migration outside of the tunnel area. Construction of additional tunnels and directional fencing within the Stony Point migration corridor would provide increased opportunities for salamanders to avoid the roadway,



limit vehicular exposure, and reduce mortalities for this endangered species.

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#### LITERATURE CITED

- Allaback, M.L., and D.M. Laabs. 2003. Effectiveness of road tunnels for the Santa Cruz Long-toed Salamander. *Transactions of the Western Section of the Wildlife Society* 38:5–8.
- Andrews, K.M., J.W. Gibbons, and D.M. Jochimsen. 2008. Ecological effects of roads on amphibians and reptiles: a literature review. *Herpetological Conservation* 3:121–143.
- Barry, S.J., and H.B. Shaffer. 1994. The status of the California Tiger Salamander (*Ambystoma californiense*) at Lagunita: a 50-year update. *Journal of Herpetology* 28:159.
- Beebee, T.J.C. 2013. Effects of road mortality and mitigation measures on amphibian populations. *Conservation Biology* 27:657–668.
- Bennett, A.F., J.T. Garland, and P.L. Else. 1989. Individual correlation of morphology, muscle mechanics, and locomotion in a salamander. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 256:R1200–R1208.
- Bissonette, J.A., and W. Adair. 2008. Restoring habitat permeability to roaded landscapes with isometrically-scaled wildlife crossings. *Biological Conservation* 141:482–488.
- Carr, L.W., and L. Fahrig. 2001. Effect of road traffic on two amphibian species of differing vagility. *Conservation Biology* 15:1071–1078.
- Cash, W.B., R.L. Holberton, and S.S. Knight. 1997. Corticosterone secretion in response to capture and handling in free-living Red-eared Slider turtles. *General and Comparative Endocrinology* 108:427–433.
- Clevenger, A.P., and N. Waltho. 2000. Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. *Conservation Biology* 14:47–56.
- Coffin, A.W. 2007. From roadkill to road ecology: a review of the ecological effects of roads. *Journal of Transport Geography* 15:396–406.
- Cook, D.G., P.C. Trenham, and P.T. Northen. 2006. Demography and breeding phenology of the California Tiger Salamander (*Ambystoma californiense*) in an urban landscape. *Northwestern Naturalist* 87:215–224.
- Corlatti, L., K. Hackländer, and F. Frey-Roos. 2009. Ability of wildlife overpasses to provide connectivity and prevent genetic isolation. *Conservation Biology* 23:548–556.
- Davidson, C., H.B. Shaffer, and M.R. Jennings. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conservation Biology* 16:1588–1601.
- Finkler, M.S., M.T. Sugalski, D.L. Claussen, and S.J. Beaupre. 2003. Sex-related differences in metabolic rate and locomotor performance in breeding Spotted Salamanders (*Ambystoma maculatum*). *Copeia* 2003:887–893.
- Ford, A.T., and A.P. Clevenger. 2010. Validity of the prey-trap hypothesis for carnivore-ungulate interactions at wildlife-crossing structures. *Conservation Biology* 24:1679–1685.
- Forman, R.T. T., and L.E. Alexander. 1998. Roads and their major ecological effects. *Annual Review of Ecology and Systematics* 29:207–231.
- Forman, R.T.T., and R.D. Deblinger. 2000. The ecological road-effect zone of a Massachusetts (U.S.A.) suburban highway. *Conservation Biology* 14:36–46.
- Gibbs, J.P., and W.G. Shriver. 2005. Can road mortality limit populations of pool-breeding amphibians? *Wetlands Ecology and Management* 13:281–289.
- Glista, D.J., T.L. DeVault, and J.A. DeWoody. 2009. A review of mitigation measures for reducing wildlife mortality on roadways. *Landscape and Urban Planning* 91:1–7.
- Gloyne, C.C., and A.P. Clevenger. 2001. Cougar Puma concolor use of wildlife crossing structures on the Trans-Canada highway in Banff National Park, Alberta. *Wildlife Biology* 7:117–124.
- Goosem, M., N. Weston, and S. Bushnell. 2006. Effectiveness of rope bridge arboreal overpasses and faunal underpasses in providing connectivity for rainforest fauna. Pp. 304–316 *In* Center for Transportation and the Environment. Irwin, C.L., Garrett P., and K.P. McDermott (Eds). North Carolina State University, Raleigh, North Carolina, USA.
- Grilo, C., J.A. Bissonette, and P.C. Cramer. 2010. Mitigation measures to reduce impacts on biodiversity. Pp. 73–114 *In* Highways: Construction, Management, and Maintenance. Jones, S.R. (Ed).



- Nova Science Publishers, Hauppauge, New York, USA.
- Hels, T., and E. Buchwald. 2001. The effect of road kills on amphibian populations. *Biological Conservation* 99:331–340.
- Jackson, S.D., and C.R. Griffin. 2000. A strategy for mitigating highway impacts on wildlife. Pp. 143–159 *In* *Wildlife and Highways: Seeking Solutions to an Ecological and Socio-economic Dilemma*. Messmer, T.A., and B. West (Eds). The Wildlife Society, Bethesda, Maryland, USA.
- Jackson, S.D., and T.F. Tynning. 1989. Effectiveness of drift fences and tunnels for moving Spotted Salamanders *Ambystoma maculatum* under roads. Pp. 93–99 *In* *Amphibians and Roads*. T.E.S Langton (Ed). ACO Polymer Products, Shefford, England.
- Lesbarrères, D., T. Lodé, and J. Merilä. 2004. What type of amphibian tunnel could reduce road kills? *Oryx* 38:220–223.
- Little, S.J., R.G. Harcourt, and A.P. Clevenger. 2002. Do wildlife passages act as prey-traps? *Biological Conservation* 107:135–145.
- Loredo, I., and D. van Vuren. 1996. Reproductive ecology of a population of the California Tiger Salamander. *Copeia* 1996:895–901.
- Mata, C., I. Hervás, J. Herranz, F. Suárez, and J.E. Malo. 2005. Complementary use by vertebrates of crossing structures along a fenced Spanish motorway. *Biological Conservation* 124:397–405.
- Mata, C., I. Hervás, J. Herranz, F. Suárez, and J.E. Malo. 2008. Are motorway wildlife passages worth building? Vertebrate use of road-crossing structures on a Spanish motorway. *Journal of Environmental Management* 88:407–415.
- Mazerolle, M.J., M. Huot, and M. Gravel. 2005. Behavior of amphibians on the road in response to car traffic. *Herpetologica* 61:380–388.
- Mccollister, M.F., and F.T. Van Manen. 2010. Effectiveness of wildlife underpasses and fencing to reduce wildlife-vehicle collisions. *The Journal of Wildlife Management* 74:1722–1731.
- Ng, S.J., J.W. Dole, R.M. Sauvajot, S.P.D. Riley, and T.J. Valone. 2004. Use of highway undercrossings by wildlife in southern California. *Biological Conservation* 115:499–507.
- Orloff, S.G. 2011. Movement patterns and migration distances in an upland population of California Tiger Salamander (*Ambystoma californiense*). *Herpetological Conservation and Biology* 6:266–276.
- Pagnucco, K.S., C.A. Paszkowski, and G.J. Scrimgeour. 2012. Characterizing movement patterns and spatio-temporal use of under-road tunnels by Long-toed Salamanders in Waterton Lakes National Park, Canada. *Copeia* 2012:331–340.
- Patrick, D.A., C.M. Schalk, J.P. Gibbs, and H.W. Woltz. 2010. Effective culvert placement and design to facilitate passage of amphibians across roads. *Journal of Herpetology* 44:618–626.
- Searcy, C.A., and H.B. Shaffer. 2011. Determining the migration distance of a vagile vernal pool specialist: how much land is required for conservation of California Tiger Salamanders? Pp. 73–87 *In* *Research and Recovery in Vernal Pool Landscapes*. Alexander, D.G., and R.A. Schlising (Eds). California State University, Chico, California, USA.
- Searcy, C.A., E. Gabbai-Saldade, and H.B. Shaffer. 2013. Microhabitat use and migration distance of an endangered grassland amphibian. *Biological Conservation* 158:80–87.
- Shaffer, H.B., G.B. Pauly, J.C. Oliver, and P.C. Trenham. 2004. The molecular phylogenetics of endangerment: cryptic variation and historical phylogeography of the California Tiger Salamander, *Ambystoma californiense*. *Molecular Ecology* 13:3033–3049.
- Tigas, L.A., van Vuren, D., and R.M. Sauvajot. 2002. Behavioral responses of Bobcats and Coyotes to habitat fragmentation and corridors in an urban environment. *Biological Conservation* 108:299–306.
- Trenham, P.C. 2001. Terrestrial habitat use by adult California Tiger Salamanders. *Journal of Herpetology* 35:343–346.
- Trenham, P.C., and D.G. Cook. 2008. Distribution of migrating adults related to the location of remnant grassland around an urban California Tiger Salamander (*Ambystoma californiense*) breeding pool. Pp. 9–16 *In* *Urban Herpetology, Herpetological Conservation*. Mitchell, J.C., and R.E. Jung Brown (Eds.) Urban Herpetology, Herpetological Conservation. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, USA..
- Trenham, P.C., H.B. Shaffer, W.D. Koenig, M.R. Stromberg, and S.T. Ross. 2000. Life history and demographic variation in the California Tiger Salamander (*Ambystoma californiense*). *Copeia* 2000:365–377.
- Trocmé, M. 2006. The Swiss defragmentation program – reconnecting wildlife corridors between the Alps and Jura: an overview. Pp. 144–149 *In* *Proceedings of the 2005 International Conference on Ecology and Transportation*. Irwin, C.L., P. Garrett, and K.P. McDermott (Eds.). Center for Transportation and the Environment, North Carolina State University, Raleigh, North Carolina, USA.
- U.S. Fish and Wildlife Service (USFWS). 2003. Endangered and threatened wildlife and plants; determination of endangered status for the Sonoma County distinct population segment of the California Tiger Salamander, final rule. 53:13498–13520.

- van der Ree, R., N. Gulle, K. Holland, E. van der Grift, C. Mata, and F. Suarez. 2007. Overcoming the barrier effect of roads-how effective are mitigation strategies? Pp. 423–431 *In* Proceedings of the 2007 International Conference on Ecology and Transportation. Irwin, C.L., D. Nelson, and K.P. McDermott (Eds). Center for Transportation and the Environment, North Carolina State University, Raleigh, North Carolina, USA.
- van der Ree, R., J.A.G. Jaeger, E.A. van der Grift, and A.P. Clevenger. 2011. Effects of roads and traffic on wildlife populations and landscape function: road ecology is moving toward larger scales. *Ecology and Society* 16:1–9.
- Waye, H.L. 2013. Can a tiger change its spots? A test of the stability of spot patterns for identification of individual Tiger Salamanders (*Ambystoma tigrinum*). *Herpetological Conservation and Biology* 8:419–425.
- Woltz, H.W., J.P. Gibbs, and P.K. Ducey. 2008. Road crossing structures for amphibians and reptiles: informing design through behavioral analysis. *Biological Conservation* 141:2745–2750.



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