

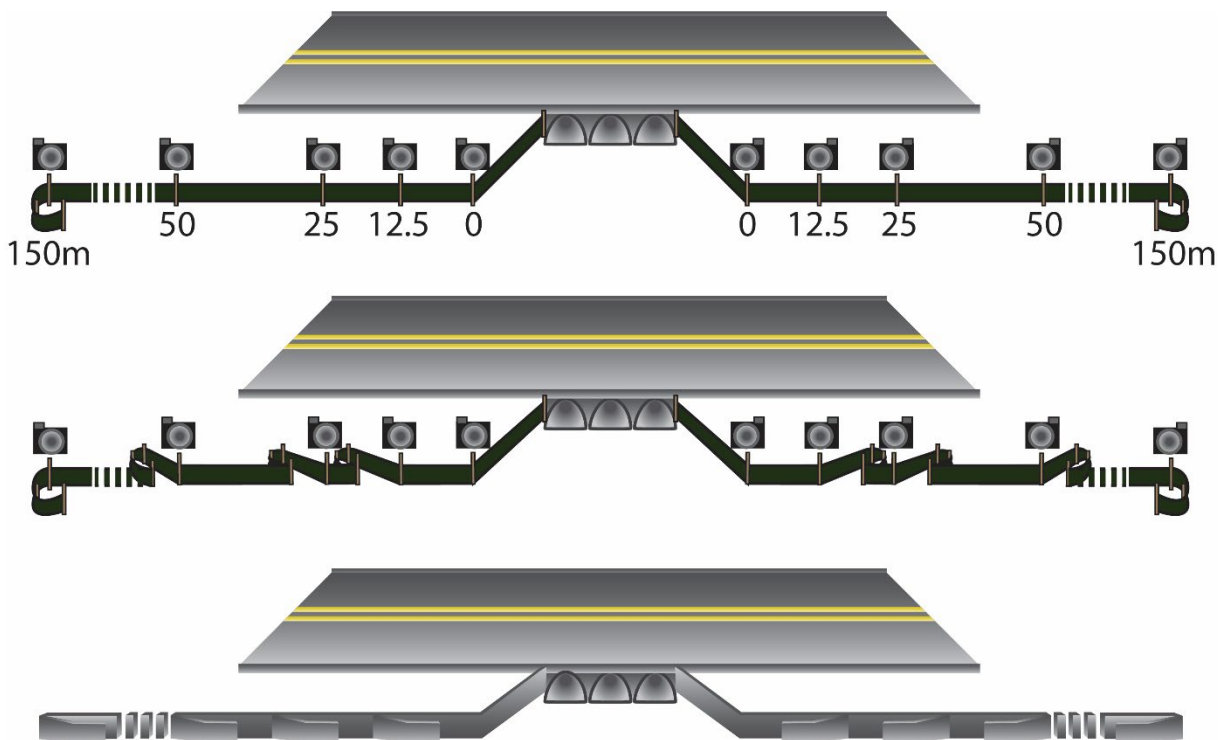
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16. ABSTRACT Many migratory amphibians make annual population-level migrations among breeding wetlands and over-wintering and/or summer foraging upland terrestrial habitats. To reduce the negative impacts from road mortality on these vulnerable populations, it has been standard practice to build safe crossings in the form of small passages connected by barrier fencing as mitigation. The permeability of crossing structures is dependent upon the proportion of migrating animals that even reach the passages. In Phase 1 of this project, California tiger salamanders (<i>Ambystoma californiense</i> ; CTS) were shown to move an average of approximately 40m along a barrier fence before giving up (90% tolerance interval of 12.5m). CTS that came in contact with fencing and initially moved the 'wrong' way (away from a passage) had a very low probability of reaching the passage system. In another study, turnarounds, often placed at fence ends, were shown to be effective in changing the trajectory of amphibians and reptiles. In this Phase 2 study, we tested if multiple turnarounds along the length of barrier fencing would increase the probability reaching the passage system. At the study site in Stanford, CA, we installed turnarounds every 25m, with an additional turnaround 12.5m from the passage system. Individual CTS movements were monitored using active-trigger cameras, documenting speed, direction, use of turnarounds, and success at reaching the passage system for 3 years prior and 2 years after the multiple turnarounds were in place. Our results showed that an average of 36% of CTS initially turned in the 'wrong' direction. Prior to installation of multiple turnarounds, 5% of CTS that initially turned in the 'wrong' direction made it to the passage system. After installation of the turnarounds, 96% of CTS that initially turned in the 'wrong' direction interacted with one or more turnarounds and their probability of reaching the passage system increased to that of CTS that initially moved in the 'right' direction (mean 66% success rate); with probabilities increasing in relation to initial distance from passage. To our best knowledge, this is the first study of multiple turnarounds and their impacts on passage system permeability. In addition to increasing the number and quality of passages along migratory pathways, we believe this is promising and cost-effective method to increase overall permeability of passage-barrier systems to migrating amphibians.		
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Roadway Crossings for Sensitive Amphibians and Reptiles: Phase II



Multiple Turnarounds Increase the Proportion of California Tiger Salamanders Reaching a Road Passage System in Stanford, CA



Prepared for:
California Department of Transportation (Caltrans)
Division of Research, Innovation and System Information (DRISI)

Roadway Crossings for Sensitive Amphibians and Reptiles: Phase II

Multiple Turnarounds Increase the Proportion of California Tiger Salamanders Reaching a Road Passage System in Stanford, CA

Cheryl Brehme, Brittany Ewing, and Robert Fisher

U.S. GEOLOGICAL SURVEY, WESTERN ECOLOGICAL RESEARCH CENTER

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San Diego Field Station

USGS Western Ecological Research Center

4165 Spruance Road, Suite 200

San Diego, CA 92101

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Abstract

Many migratory amphibians make annual population-level migrations among breeding wetlands and over-wintering and/or summer foraging upland terrestrial habitats. To reduce the negative impacts from road mortality on these vulnerable populations, it has been standard practice to build safe crossings in the form of small passages connected by barrier fencing as mitigation. In addition to crossing success within the passage(s), the permeability of crossing systems to amphibian population movements is also dependent upon the proportion of migrating animals that even reach the passage. In Phase 1 of this project, California tiger salamanders (*Ambystoma californiense*; CTS) were shown to move an average of approximately 40m along a barrier fence before giving up (90% tolerance interval of 12.5m). CTS that came in contact with fencing during their migration and initially moved the ‘wrong’ way (away from a passage) had a very low probability of ever reaching the passage system. In another study, turnarounds, often placed at fence ends, were shown to be effective in changing the trajectory of amphibians and reptiles. In this Phase 2 study, we tested if multiple turnarounds along the length of barrier fencing would increase the probability that migrating CTS would reach the passage system.

At the study site in Stanford, CA, there is a 3-tunnel system with 150m of fencing on each side to support safe crossings of CTS to and from their breeding habitat. We installed turnarounds every 25m, with an additional turnaround 12.5m from the passage system. Individual CTS movements were monitored using active-trigger cameras, documenting speed, direction, use of turnarounds, and success at reaching the passage system for 3 years prior and 2 years after the multiple turnarounds were in place. Our results showed that an average of 36% of CTS initially turned in the ‘wrong’ direction. Prior to installation of multiple turnarounds, 5% of CTS that initially turned in the ‘wrong’ direction made it to the passage system. After installation of the turnarounds, 96% of CTS that initially turned in the ‘wrong’ direction interacted with one or more turnarounds and their probability of reaching the passage system increased to that of CTS that initially moved in the ‘right’ direction (mean 66% success rate); with probabilities increasing in relation to initial distance from passage. For CTS at Stanford, the overall probability of CTS reaching the passage system increased from 14-33% prior to installation of the turnarounds to 57-71% after turnarounds were in place. To our knowledge, this is the first study of multiple turnarounds and their impacts on passage system permeability. In addition to increasing the number and quality of passages along migratory pathways, we believe this is promising and cost-effective method to increase overall permeability of passage-barrier systems to migrating amphibians.

Introduction

Many migratory amphibians make annual population-level migrations among breeding wetlands and over-wintering and/or summer foraging upland terrestrial habitats. This requires high levels of connectivity among these habitats (Semlitch 2008, Hamer and McDonnell 2008, Hamer et al. 2015). Because roads often intersect these migratory pathways, all California migratory salamanders, toads and some frogs ranked in the highest risk categories for potential negative road effects (Brehme et al. 2018).

There is substantial evidence that habitat fragmentation and mortality due to roads negatively affect many of these amphibians. For instance, newts regularly migrate long distances over land between breeding ponds and terrestrial foraging habitats (2 km; Trenham 1998). Large numbers are found dead on roads during dispersal periods and newt species are often the first to disappear in fragmented landscapes (Gibbs 1998, Trenham 1998, Shields pers. comm.). Similarly, road mortality and habitat fragmentation are primary threats to the California tiger salamander and other Ambystomid salamanders because the terrestrial habitat is used for interpond migration and overwintering (Semlitsch 1998, Trenham et al. 2001, Bolster 2010). There is also evidence that migrating bufonid toads are particularly susceptible to negative impacts from roads (Trenham et al. 2003, Orłowski 2007, Eigenbrod et al. 2008).

To reduce the negative impacts from road mortality on these vulnerable populations, it has been standard practice to build safe crossings in the form of small passages (e.g., culverts, tunnels, etc.) connected by barrier fencing as mitigation. There are a wide variety of small passages and barrier materials that have been constructed with varying degrees of success, although post mitigation monitoring is relatively rare (see review by Langton and Clevenger 2017).

However, in addition to crossing success within the passage(s), the permeability of crossing systems to amphibian population movements is also dependent upon the proportion of migrating animals that even reach the passage opening. After 2 years of monitoring the movements of a population of California tiger salamanders (CTS: *Ambystoma californiense*) in Stanford, CA, we found that CTS moved an average of 40m along barrier fencing before “giving up” (i.e. going back into the habitat without ever encountering a passage; Brehme et al. 2021). Their probability of reaching the passage system was highly dependent upon distance from the passage where CTS initially encountered the barrier fencing, as well as their initial movement direction (toward vs. away from passage system). This resulted in only 16-33% of CTS in reaching the passage system in 2017-18 and 2018-19 seasons. Similar responses were documented for migrating Common toads and Yosemite toads (Ottberg and van der Grift 2019, Brehme et al. 2022).

Concurrent to these studies, we also studied the effectiveness of turnarounds at fence ends in changing the trajectory of reptiles and amphibians. We found turnarounds to be highly effective in changing the trajectory of 92% of species tested, with most headed back along the original fence line (Brehme and Fisher 2020). At the fence end turnarounds at Stanford, we documented 3 CTS, of which 2 were subsequently documented moving back along the fence and toward the passages.

These studies led to our current research question: Will addition of multiple turnarounds along the length of barrier fencing increase the probability that migrating amphibians reach the

passage system? We tested this research question with the CTS population at Stanford, CA. Rather than having to move up to 150m to reach the turnarounds at the fence ends, we hypothesized that CTS moving the ‘wrong’ way would interact with turnarounds sooner, and potentially have a higher chance of reaching the passage system before “giving up”. If so, this would result in an overall increase in proportion of migrating CTS that reached the passage system to access their breeding habitat on the other side of the road.

Methods

Field Study

We studied the movement of CTS adjacent to three existing underpasses in Stanford, CA (Stanford University) in the winter breeding seasons of 2017/18, 2018/19, 2020-21, 2021-22, and 2022-23. In this location, a busy two-lane paved road (Juniper Serra Blvd: average daily vehicle count is 17,300; (City of Menlo Park 2017)) transects upland CTS habitat and Lagunita water detention basin (hereto referred to as Lake Lagunita), a historic CTS breeding site (Figure 1). Large rates of CTS road mortality spurred the construction of a 3-tunnel system in 2003. The concrete polymer tunnels (ACO Wildlife®; 63 cm wide x 52 cm height) are 14 m in length and spaced 5 m apart with approximately 5 m of barrier fencing on each side (Figure 1). . Before our studies in 2017, we expanded the footprint of existing barrier fencing on the south side of the road by 150 m in each direction (Figure 2).



Figure 1. Underpass photos; A) Aerial image of 3 tunnels across road and B) View of south entrance of westernmost tunnel.



Figure 2. Map of Barrier and Tunnel Study System at Stanford University Between Upland and Breeding Habitat for CTS.

One portion of the fencing installed was semi-transparent mesh (ERTEC ® rigid polymer matrix) and the other portion was solid (Animex ® high-density polyethylene (HDPE-2)). To minimize the potential for vandalism, the fencing was placed within existing security fencing present on site. Jump-outs (ERTEC® cones and high berms) were installed a minimum of every 25 m along the fence to provide CTS and other small vertebrates a way to get back into the habitat if they ended up on the roadside of the barrier fencing. At the outer fence ends, turnarounds were installed to redirect animals away from the road and back toward the upland habitat in a U-shaped fashion. The turnarounds were approximately 2 m long and 1 m in width. Fencing was installed with the bottom buried in the ground according to manufacturers' guidelines. In 2018, fencing was also expanded along the north side of the road.

For monitoring, we focused on breeding migrations to Lagunitas lake that typically occur in large pulses associated with rain events in November through January, whereas return migrants are sporadic and can occur intermittently through the summer months (Trenham 1998, Trenham et al. 2001). We did not monitor CTS along the north side fencing due to funding constraints and a limited number of cameras.

For this, HALT ® camera systems (Hobbs and Brehme 2017) were placed every 25 m along the new fence lines from 0 to 125 m from the existing tunnel system (Figure 2). Each 0 m camera was approximately 5–8 m from the closest tunnel opening where our newly installed fencing intersected with the existing barrier fencing. Before 2020-21, a 15 cm (6 in.) visual barrier was added to the lower portion of the mesh fencing, basically creating a solid barrier for CTS (e.g. Brehme and Fisher. Extra cameras were placed at 12.5m to increase the precision of movement

estimates near the passage system. We also installed cameras at the fence end turnarounds to record CTS entrances and exits.

Prior to the 2021-22 season, fence turnarounds were installed at 12.5m, 25m, 50m, 75m, 100m, and 125m for the multiple turnaround study (Figures 2 and 3). At fence turnarounds, HALT camera systems were placed at the entrance/exit of turn-around to record animals' entrance and exit time and their movement trajectory after coming out of the turn-around. Half of the trigger spanned the entrance into the turnaround, while the other monitored CTS moving along the fence-line (Figure 2). Cameras were placed within the tunnels as funding would allow. In 2018-19 and 2022-23, we also placed these camera systems within each tunnel opening and exit to record tunnel permeability (Figure 2). In 2020-21, cameras were only placed on one side of the tunnel. Cameras were set whenever rain was predicted and checked weekly during the winter adult migration season from the uplands toward the pond (Nov.–Mar.). Each time we set and checked the cameras, we took a photo of a battery-powered atomic clock to calibrate exact minutes and seconds upon processing. All work was performed under Stanford University Habitat Conservation Plan (Federal incidental take permit # TE182827-0) and California State Consistency Determination (2080-2016-001-03).



Figure2. Photo of turnaround along fence line (A), HALT camera and trigger spanning turnaround entrance and fence line (B), HALT camera inside ACO tunnel passage (C).

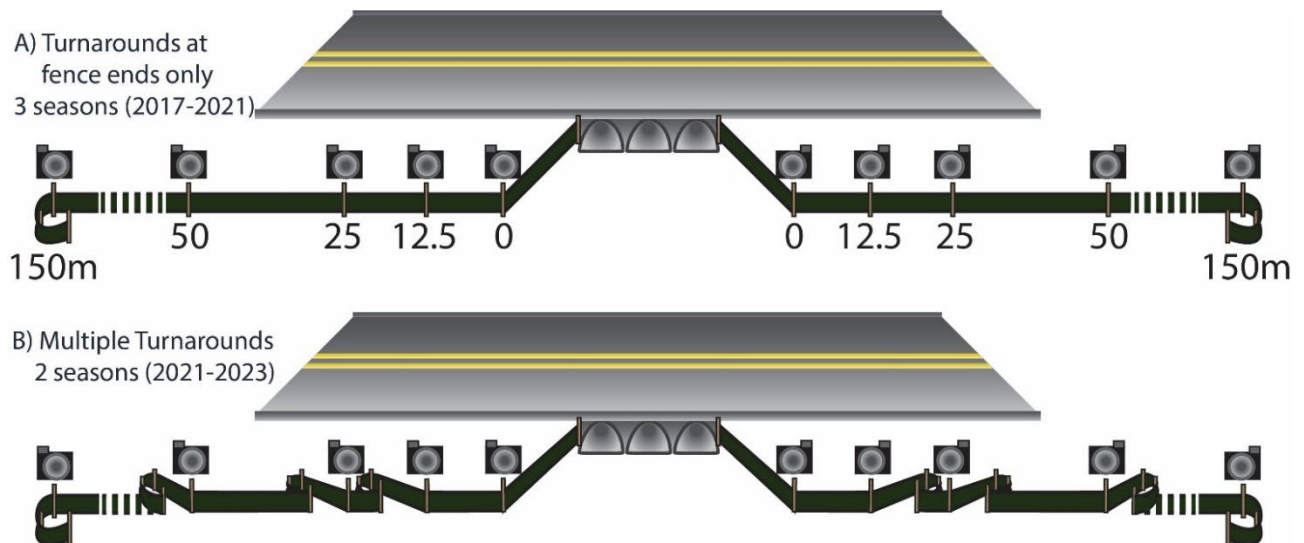


Figure3. Diagram of CTS movement study with (A) camera placement in 2017-18, 2018-19, and 2020-21, (B) multiple turnarounds added and monitored in 2021-22 and 2022-23.

Analysis

Photos of all CTS were analyzed using pattern recognition software to identify individuals by their unique spot patterns (I³S Spot; Van Tienhoven et al. 2007; Figure 34). Camera location, time, and direction of movement were recorded for each individual. Snout to vent length was measured with Program ImageJ (Rasband 1997–2018) using the 1 cm grids from the HALT trigger for calibration.

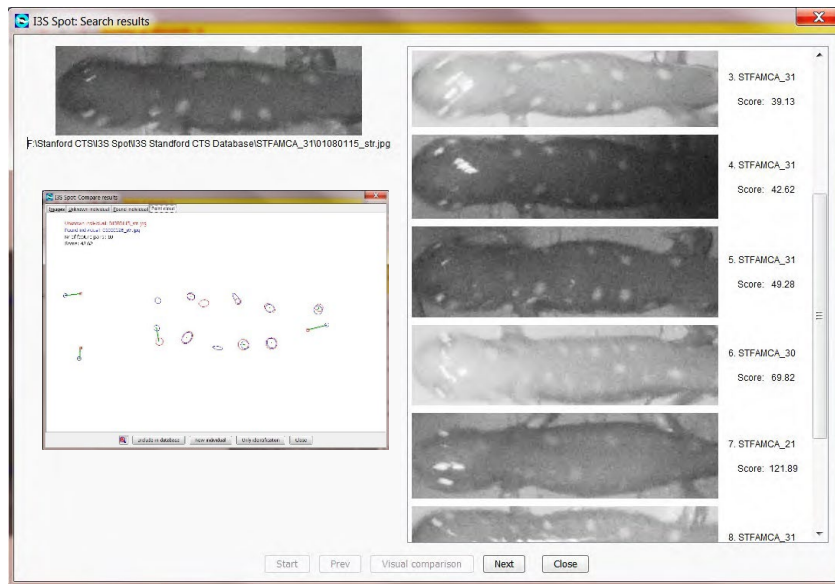


Figure 4. Example of CTS Identified to Individual Using I³S Software to Distinguish Spot Patterns (top 3 on right are same individual)

For individual CTS, we then calculated movement distances along the fence lines, numbers of turn arounds, speed, and “success” at reaching 0 m cameras next to underpass system. Because cameras were placed 25 m apart, our margin of error for estimating fence movement distance was largely between 0 and 25 m. For instance, if an animal was only detected at a single camera between 25 m and 125m, then our average estimated distance was 25 m (12.5 m before reaching the camera and 12.5 m after exiting the camera). Similarly, if an individual was detected at multiple consecutive cameras moving in the same direction, our margin of error was typically 25 m. In the instances where individuals were detected at consecutive cameras, we also calculated the movement speed between segments. If such an individual then turned around and was re-detected at a camera while moving in the other direction, we were able to estimate the distance traveled along the fence before turning around by multiplying the time between detections by its average speed. Because of this, if individuals traveled back and forth several times, we were able to more accurately estimate the total distance of fence line traversed (fence movement distance). If an individual reached the 0 m camera (where the experimental fence lines attached to the short length of the existing fence), this was considered a “success” at reaching the passage system with no added error for the distance moved afterward. An added camera system placed in 2021 at 12.5m

also allowed for greater precision of movement distance estimates nearer to the passage system (i.e., added error of 6.25m).

Finally, we removed individual CTS where there was evidence that they were not migrating to the lake (i.e., migrating back from Lagunitas to uplands). This was determined by co-monitoring of the passages and the direction of movement of CTS in years when passages were monitored (2018-19, 2021-22, 2022-23). For instance, in 2023, CTS were moving toward lake from Nov. 8, 2022, to Jan. 3, 2023; moving both ways between Jan.3-Jan 10, and moving away from lake after Jan.10. Thus, we removed all CTS moving along the fences after Jan.10 from the analyses. In 2017-18 and 2021-22, CTS moving away from the passage system first detected at the 0m camera were considered migrating back and removed. Numbers of CTS movements removed are presented in the Results section.

Analyses- Modeling

We modeled the probability of success of CTS in reaching the 0 m camera near the crossing opening using mixed logistic regression (package lme4; Bates et al. 2015) in R (v4.2.3; R Core Team 2023). Covariates included FenceType, InitLoc, InitAway, TurnaroundInteraction YN, and BarrierFenceDistance). FenceType is a binary variable where 0 indicates a mesh fence and 1 indicates a solid fence. InitLoc is the position along the fence where the animal was first detected in meters from the crossing opening (with error described in the previous paragraph), InitAway is a binary variable where 0 indicates that the animal was initially moving toward the crossing and 1 indicates it was initially moving away from the crossing, and TurnaroundInteraction YN is a binary variable indicating an individual CTS entered at least 1 turnaround. BarrierFenceDistance is the total linear distance of fence that each CTS moved during the study. Two and three-way interactions among covariates were evaluated with the most parsimonious model chosen using Akaike's Information Criterion (AIC; Burnham and Anderson 2002). Continuous covariates (i.e., InitLoc, BarrierFenceDistance) were standardized prior to modeling. We accounted for annual differences with year as a random variable in all models (Year). We also modeled the distance that CTS moved along the fence (BarrierFenceDistance) using mixed linear regression (package lme4; Bates et al. 2015) and the same set of covariates.

Tunnel System Permeability was calculated as the number of complete passes (individual detected at entrance and exit) divided by the number of attempts. Other summary data were also calculated in R (v4.2.3; R Core Team 2023).

Results

We documented 391 adult CTS over 5 winter seasons moving along the fence-line (Annual range 41-233; Table 1). Total precipitation during the meteorological rainfall season of July 1 to June 30 ranged between 5.3 in and 16.4 in. (ave: approx. 13.5 in.; ggweather.com summarized from California Nevada River Forecast Center, NOAA <https://www.cnrfc.noaa.gov>). The Stanford University Conservation Program observed no recruitment in 2018 but confirmed high recruitment of CTS in 2019 (A. Launer and E. Adelsheim, pers. comm.). CTS numbers recorded in 2022-23 were over 4 times greater than any previous season, indicating the CTS population expanded over this period. We did not compare individuals between years, and therefore, considered individual movements across years as independent in the analysis. A total of 32 CTS were removed from the analysis that were migrating back from the lake during the monitoring period. Since camera monitoring was restricted to the upland side, this analysis has inference to CTS migration toward breeding habitat.

Table 1. Annual CTS winter migration totals documented along the south road barrier fence.

Year	Rainfall (in.)	CTS Movements	Time Periods of Primary Migration			Total CTS	No. Removed (returning from lake)	Tunnels monitored?
			Moving to Lake	Both Ways	Away from Lake			
2017-18	9.2	1/3-1/9	1/3-1/9	UNK*	UNK*	41	2	NO
2018-19	16.4	11/22-3/5	11/22-2/25	2/16-2/25	2/26+	50	6	YES
2020-21	5.3	12/18-2/15	12/18-2/1	2/1-2/22	2/1-2/22	44	8	In only-TBD
2021-22	7.3	12/17-1/10	12/17-1/10	UNK*	UNK*	23	0	NO
2022-23	15.8	11/8-3/13	11/8-1/12	1/3-1/10	1/10-3/13	233	16	YES

Summarized Data

Over the period of monitoring, 64% of CTS were initially documented moving toward the passage system, while 36% initially turned in the ‘wrong’ direction and were moving away from the passage system. Distributions of CTS in relation to distance from the passage system, initial direction choice, and counts of CTS that interacted with turnarounds in 2021-22 and 2022-23 are presented in Figure 5. Although CTS numbers and distributions were somewhat variable, sample sizes across these covariates were relatively even, with all values well represented (Figure 5).

Density distributions of fence movement give-up distances for CTS that did not reach the passage were relatively similar across years (annual means: 25-45m, Figure 6). However, distributions of fence movement distances for CTS that did reach passages were skewed higher after turnarounds were in place (mean 13-19m (-TA) vs. 38-63m (+TA)). There was no apparent relationship to seasonal rainfall totals, however, large movements were observed during high rainfall events in 2022-23)

After turnarounds were installed, 32% of CTS initially moving toward the passage system entered 1 or more turnarounds (54/167), while 96% of CTS moving away from the passage system

entered 1 or more turnarounds (70/73). The proportion of CTS that reached the passage system was greater after the turnarounds were installed for both CTS that were initially moving toward the passage system (Before: 21-44%, After 61-74%) and those that were initially moving away from the passage system (Before: 4-8%, After 40-66%, Figure 7).

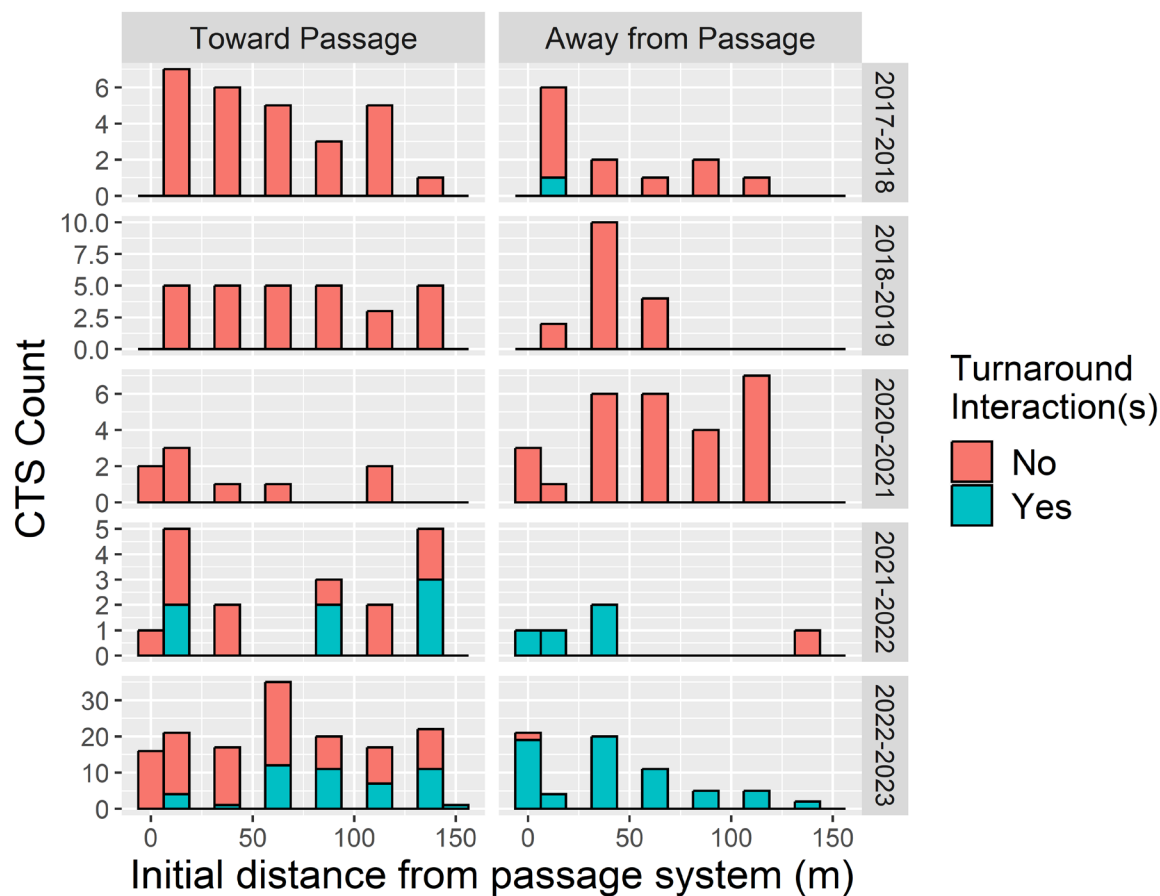


Figure 5. Histograms showing counts of CTS according to the initial distance from the passage system and initial direction of travel (toward or away from the passage system). Multiple turnarounds were installed before 2021-22 season. Here, the proportion of CTS that went into 1 or more of these turnarounds are shown in blue.

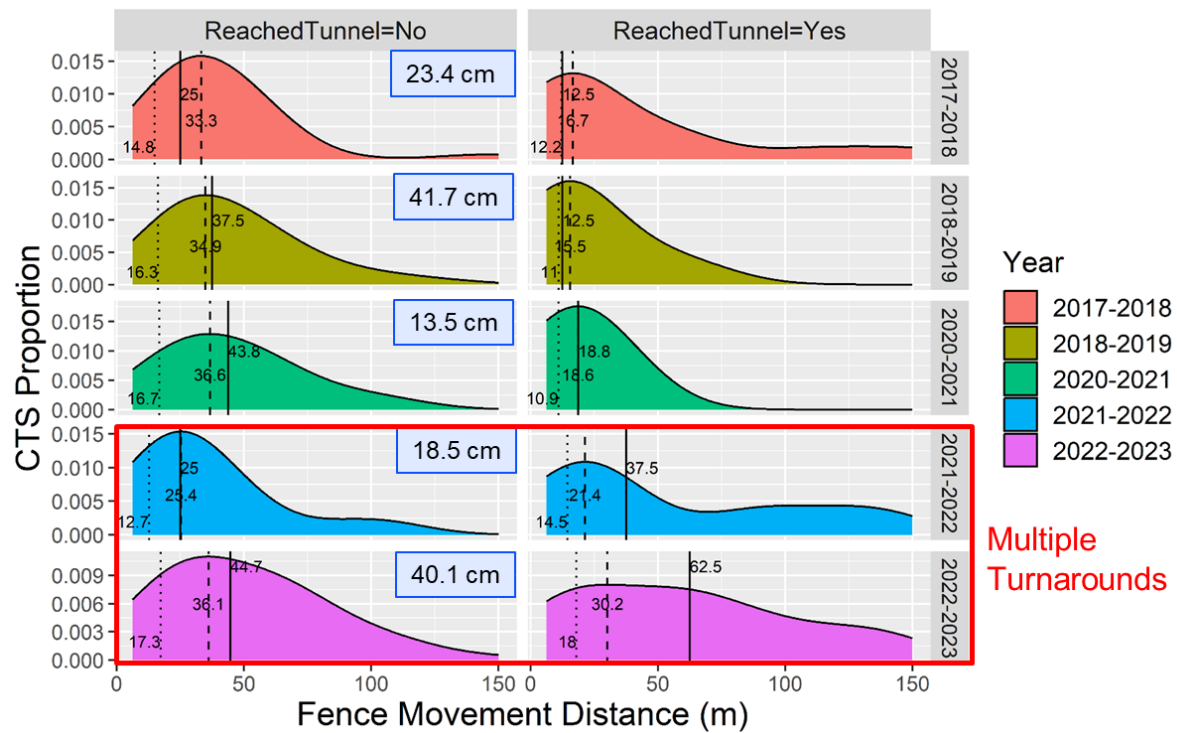


Figure 7. Density distributions of distances California tiger salamanders traveled along fence in relation to if they reached the passage system across years. Annual seasonal rainfall totals are shown in blue boxes. Means (solid line), medians (dashed line), and lower 90% tolerance intervals (dotted line) are shown

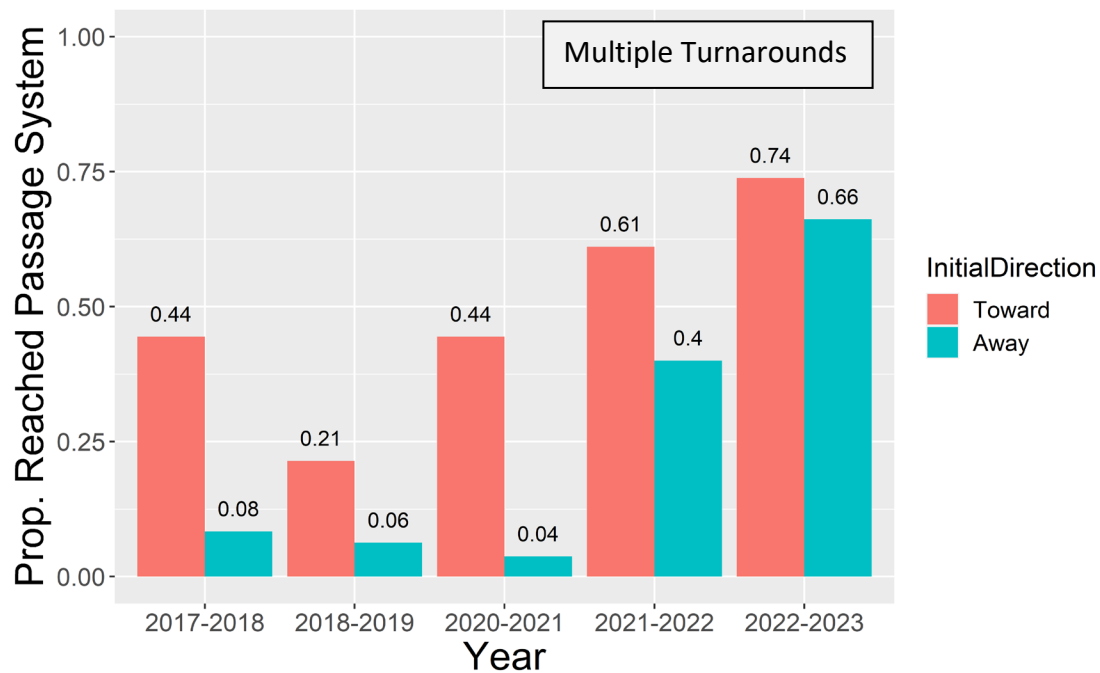


Figure 6. Proportion of California tiger salamanders that reached the passage system across years in relation to initial movement direction (toward vs away from passage system).

Table 2. Summary statistics for CTS movement behavior across years (cumulative 2017-2023)

General- All Years	Turnaround Interaction= YES						Turnaround Interaction= NO						Ratio mean	p <0.10
	Mean	Median	SD	Range	90% CI	N	Mean	Median	SD	Range	90%CI	N		
Initial Fence Encounter Location	63.2	62.5	44.2	0.0- 150.0	56.6- 69.8	125	58.4	62.5	41.3	6.3- 137.5	53.9- 63.0	234	1.1	NO
Fence Movement Distance (m)	64.6	62.5	40.6	12.5- 150.0	58.5- 70.7	125	45.0	37.5	33.3	6.3- 147.2	41.4- 48.6	233	1.4	YES
Total Fence Time (min)	103.7	80.9	86.4	7.5- 459.9	90.7- 116.7	124	41.9	29.4	39.5	0.3- 198.5	36.6- 47.3	152	2.5	YES
Speed along Fence (m/min) ¹ Total Distance	1.2	1.1	0.5	0.2- 2.5	1.1- 1.2	124	1.6	1.6	0.7	0.3- 3.6	1.5- 1.7	152	0.7	YES
Speed along Fence (m/min) ² Fence Distance	0.8	0.6	0.5	0.1- 2.4	0.7- 0.9	117	1.5	1.5	0.8	0.2- 3.7	1.4- 1.6	152	0.5	YES
Proportion that Reached Tunnel Initially moving away from Passage	0.66	1.00	0.48	0.0- 1.0	0.6- 0.8	71	0.05	0.00	0.23	0.0- 1.0	0.0- 0.1	57	12.6	YES
Proportion that Reached Tunnel Initially moving toward Passage	0.54	1.00	0.50	0.0- 1.0	0.4- 0.7	54	0.64	1.00	0.48	0.0- 1.0	0.6- 0.7	177	0.83	NO

Turnaround Specific (2021-22, 2022-23)

Total time in turnarounds (min)	19.2	11.2	21.9	0.0- 143.0	15.9- 22.5	123
No turnaround entries	3.6	2.0	3.5	0.0- 17.0	3.1- 4.2	124
Ave time in each turnaround (min)	6.0	4.4	4.7	1.1- 34.8	5.3- 6.7	119
<u>Exit Turnaround: Proportion</u> <u>Move toward passage</u>	0.82	1.00	0.27	0.0- 1.00	0.78- 0.86	123
<u>Exit Turnaround: Proportion</u> <u>Go back in turnaround</u>	0.08	0.00	0.15	0.0- 0.50	0.06- 0.10	123
<u>Exit Turnaround: Proportion</u> <u>Move away from passage</u>	0.10	0.00	0.22	0.0- 1.00	0.06- 0.13	123

¹calculated for samples with time and total movement distance observed >0- includes back and forth movements along same stretch of fence

²calculated for samples with time and fence distance observed >0- excludes extra distance for back and forth movements along same stretch of fence

Not accounting for variation across years, general summary statistics are presented in Table 2. CTS that interacted with one or more turnarounds moved an average of 40% farther along the fence line (65m vs. 45m), with their speed approximately 50% slower due to time interacting with turnarounds. CTS that interacted with turnarounds did so an average of 3.6 times and spent an average of 6 min. in each turnaround (Table 2). CTS leaving the turnarounds moved away from the passage system 10% of the time ('wrong direction'), moved back into the same turnaround 8% of the time ('wrong direction'), and moved toward the passage system 82% of the time ('right direction'). The cumulative proportion of CTS that were initially moving toward and reached the passage system (0m camera) was not significantly different with and without turnaround interactions (54 vs. 64%, respectively). However, the proportion of CTS that were initially moving away and subsequently reached the passage system (0m camera) was significantly higher if they interacted with turnarounds (66% vs. 5%; Table 2).

Mixed Regression Models

Linear and logistic regression models standardized for variation across years (Year as random factor), both showed significant effects from interaction with turnarounds on both fence movement distance and success at reaching the passage system (Figures 7 and 8).

Linear mixed regression modeling indicated CTS moved longer distances if they encountered the fence farther away from the tunnel system. However, this was only if their initial direction choice was toward the tunnel system (Figure 7A, see Brehme et al. 2021). There was no difference in predicted move distances for those CTS that encountered the fence and initially turned in the "wrong" direction. After the installation of multiple turnarounds, CTS that initially moved away from the passages moved longer distances, more similar to that of CTS that initially moved toward the passages (Figure 7B).

In the absence of any interactions with turnarounds, the probability that CTS reached the tunnel system (0 m camera) decreased with increasing distance from the passage system. However, CTS that were initially moving away from the passages had very low probabilities of finding a passage regardless of distance (Figure 8A). For CTS that interacted with one or more turnarounds after their installation, there was no significant difference in the probability of reaching a passage based on initial direction choice (Figure 8B).

Passage System Permeability

In 2022-23, 71% of CTS (155/217) migrating to Lagunitas that were documented along the barrier fence reached the passage system. Of these, 83% (129/155) were estimated to enter and move through the passages. Overall, we estimated a total of 193 CTS moved through the passages, so that 33% were not identified along the fence line and were presumed to enter the 20 m (65.6 feet) wide passage system without interacting with the fence.

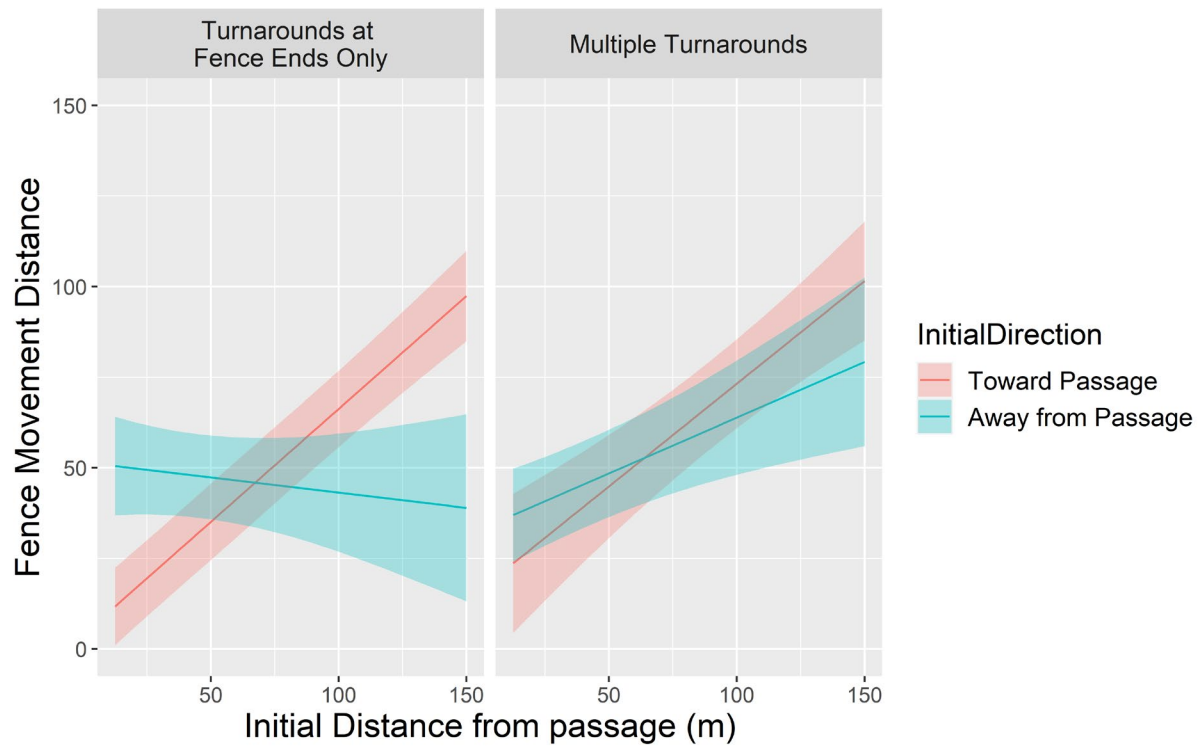


Figure 7. Distance California tiger salamanders move along the fencing in relation to their initial distance from the passage system, initial direction of travel, and whether they interacted with turnarounds. Colored bands represent 90% confidence intervals.

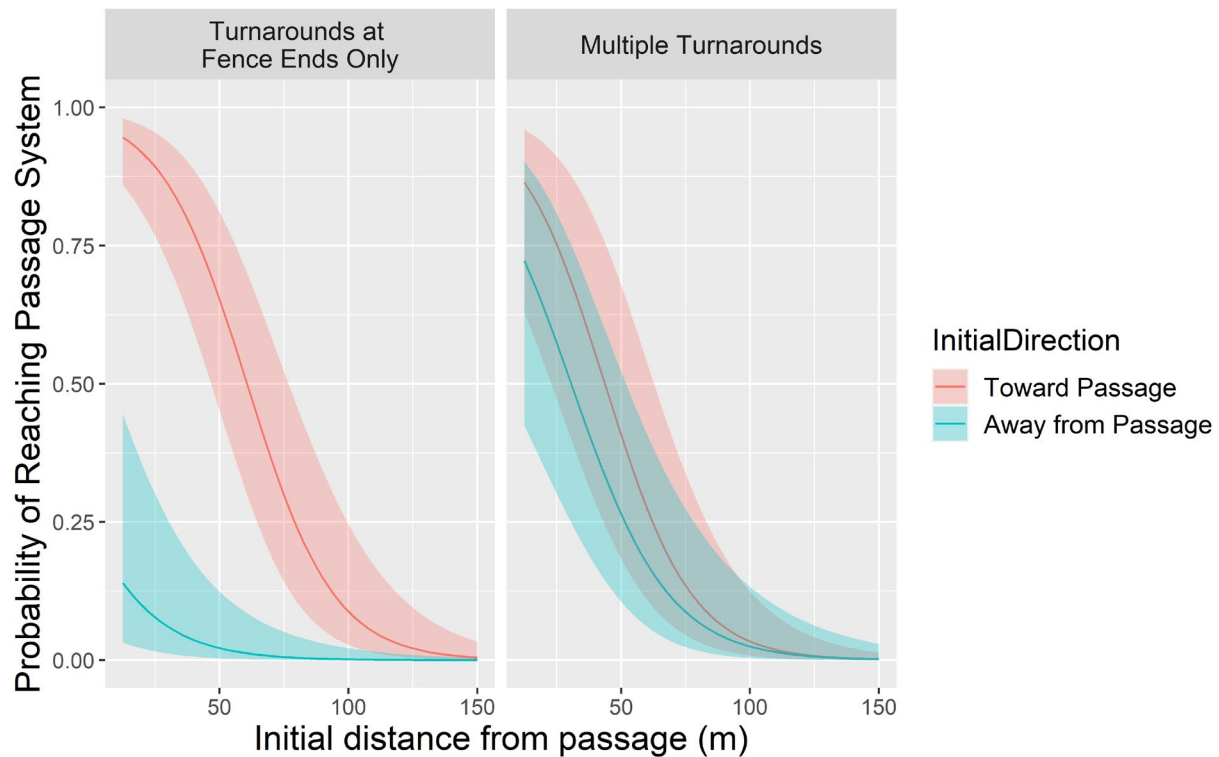


Figure 8. Probability of California tiger salamanders reaching the passage system (0 m camera) in relation to their initial distance from the passage system, initial direction of travel, and whether they interacted with turnarounds. Colored bands represent 90% confidence intervals.

Discussion

It is common practice and in many guidance manuals to put turnarounds at fence ends (e.g., Clevenger and Huijser 2011, Ontario Ministry of Natural Resources and Forestry 2016, Langton and Clevenger 2020). However, this requires that animals move all the way to the end of barrier fencing before potentially being redirected. Our results showed that addition of multiple turnarounds along road barrier fencing significantly and substantially increased the success of CTS reaching the passage system in Stanford, CA. To our knowledge, this is the first study of multiple turnarounds and their impacts on passage system permeability. We believe this is promising and cost-effective method to increase overall permeability of passage-barrier systems to migrating amphibians.

Prior to installation of multiple turnarounds, most all CTS that initially turned in the ‘wrong’ direction (away from the passage) never made it to the passage system (mean 5% success rate). After installation of the turnarounds, 96% of CTS that initially turned in the ‘wrong’ direction interacted with one or more turnarounds and their probability of reaching the passage system increased to that of CTS that initially moved in the ‘right’ direction (mean 66% success rate; with probabilities increasing in relation to initial distance from passage). For CTS at Stanford, the overall probability of CTS reaching the passage system increased from 14-33% prior to installation of the turnarounds to 57-71% after turnarounds were in place.

Thirty-two percent of CTS that were initially moving toward the passages also went into one or more turnarounds. We documented many instances where individuals initially moving toward the passages changed direction along the primary fence line and started moving away from the passages, then subsequently went into a turnaround and were redirected in the correct direction, enabling them to ultimately reach the passage system (Appendix). Additionally, there was evidence that CTS moved longer distances along the barrier fence with the presence of the multiple turnarounds. We conservatively used year as a random variable in our models to account for potential changes in CTS movements due to seasonal rainfall, climate, or other unknown annual changes. Because there was confounding of year and presence of multiple turnarounds, increases in movement distances and potential increased success of CTS that were initially moving toward the passages may be underestimated.

However, there was also a cost in time. We also observed many instances of CTS moving toward the passages that went into multiple turnarounds along their way. These may have been exploratory movements into the turnarounds and resulted in decreasing their overall speed to about half of that with no turnaround interactions (0.8 vs 1.5 m/sec). Thus, a proportion of CTS expended a higher amount of energy to make it to the crossing. It is possible that higher energy and time expenditures of these behaviors may have negative impacts on breeding success (Carr 2011, Navas et al. 2016). However, we believe the benefits of such a substantial increase in the proportion of the population that reached the passage system to access their wetland breeding habitat likely far outweigh the costs of increased time and energy to get there.

We continued to document that CTS that made the correct initial direction choice (toward passage system) were more likely to travel longer distances along the fence. Prior to installation of

the multiple turnarounds, CTS moving in the ‘wrong’ direction showed no difference in movement distances regardless of their initial distance from the passage. After turnarounds were installed, the behavior of these animals was more consistent with those that made the correct initial direction choice. The reasons for this are unknown but may be related to shorter distance orientation cues such as odors of ponds, conspecifics, and visual landmarks (Sinsch, 2006). The barrier fencing at Stanford was placed along a slightly curved road that created an approximate 20-degree angle leading to the passages and was perpendicular to the assumed main migratory path. Caltrans best management practices and others recommend installing barrier fencing at an angle into the habitat (“V” shaped toward the tunnel) in order to better lead migrating amphibians toward the tunnels (Federal Ministry of Transport 2000, Iuell et al. 2003, Schmidt and Zumbach 2008, Clevenger and Huijser 2011, Gunson et al. 2016, Langton and Clevenger 2020). The use of directional fencing at a greater angle is expected to reduce the proportion of individuals that turn away from road passages. This configuration would also be expected to increase movement distances along fencing because it is closer to the initial trajectory of the migrating amphibians. However, barrier systems are often limited to the road easement or there is concern interior barrier fencing would eliminate access to important habitats. There have not been any published studies we are aware of that directly compare the success of different fence configurations and this subject is in need of further study.

We and others have shown that migrating amphibians move an average of approximately 30-50m before “giving up” (Pagnucco et al. 2012, Ottberg and van der Grift 2019, Brehme et al. 2021). Give-up distances can be used to inform the spacing of passages to meet permeability goals. For instance, to target 90% of a population to reach passages, we used 90% tolerance intervals for “give up” distances to recommend 12.5 m and 20 m spacing of passages for CTS and Yosemite toads (Brehme and Fisher 2020, Brehme et al. 2021, 2022). Fence “give up” behavior also led us to design a novel elevated road passage (ERS; low terrestrial bridge) that could be made to any length and potentially negate the need for barrier fencing (Brehme and Fisher 2020, Brehme et al. 2022, 2023). However, adding passages to existing passage systems may not always be feasible. There also may be cost constraints in implementing closely spaced passages or ERS systems over long distances where migratory paths are wide (i.e., > 1km). Although properly designed closely spaced or open ERS passages are the best way to ensure high population permeability, in situations such as this, the addition of regularly spaced turnarounds is a relatively inexpensive and simple way to potentially increase the probability that target species find a safe road passage.

A potential permanent design for multiple turnarounds leading to a single passage system is presented in Figure 9. For this study where average CTS fence give-up distances are 40 m with a 90% tolerance limit of 12.5 m (Brehme et al. 2021), the first 2 turnarounds closest to the passage system were 12.5 m apart (to 25 m distance in each direction) and the remaining turnarounds were 25 m apart (to 150 m in each direction). We believe this was a reasonable design that resulted in no effect of initial direction choice. We were not able to test different spacing options or alternate turnaround designs. The choices of spacing and design for target species should consider migratory species give-up distances or non-migratory species home range diameters, permeability objectives, and site specific conditions. For migratory species, note that give-up distances are far shorter than

migration distances (see Brehme and Fisher 2020, Brehme et al. 2021). For turnaround design, small narrow turnarounds worked well in our studies (0.5m wide and 1.5 m in length). It would be expected that larger turnarounds would result in more time spent navigating within them. Care should be taken in design to not divert animals moving toward the passages out into the habitat, but back toward the primary fence line, so animals are more likely to continue moving along the primary fence line.

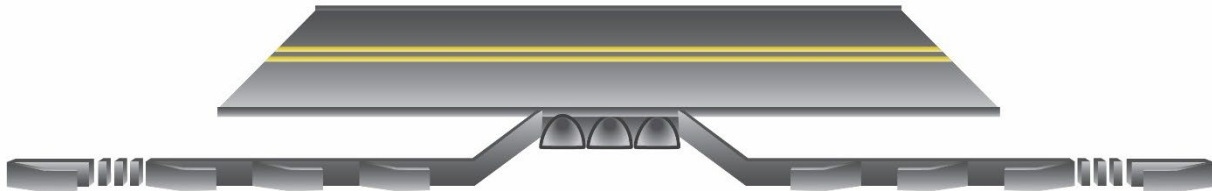


Figure 9. Diagram of potential design of multiple turnarounds along permanent barrier wall leading to existing passages.

Once individuals reach a passage, their probability of entering and moving all the way through is important in determining the overall permeability of the barrier-passage system. At Stanford in 2022-23, we estimated that 83% of CTS that reached the passage system (originally documented along the fence line) entered and moved their way through the tunnels toward their breeding site. The permeability of passages to amphibian and reptiles may be influenced by width, height, length, openness to sky (i.e., open vs. closed top), moisture and temperature conditions within the passage, noise and vibrations, accumulation of pollutants, and the correct placement of passages in the landscape (Jochimsen et al. 2004, Hamer et al. 2015, Langton and Clevenger 2017, Helldin and Petrovan 2019, White et al. 2023, Brehme et al. 2024). A supplementary memo of potential experimental designs to evaluate permeability of passages for differing herpetofauna species and species groups was submitted as part of this Phase 2 research. In addition, permeability of the system to CTS juveniles migrating from Lagunitas to the uplands is important to assess overall system permeability for the population and is recommended for future study (Petrovan and Schmidt 2019). Finally, maintenance is very important for long term success. This includes regular inspection and repair of fencing, maintenance of vegetation by the fencing to prevent climbing, and clearing of excess debris and pollutant accumulation from the tunnels (e.g., Schmidt and Zumbach 2008, van der Ree et al. 2015, Langton and Clevenger 2020). This was done prior to each field season at Stanford.

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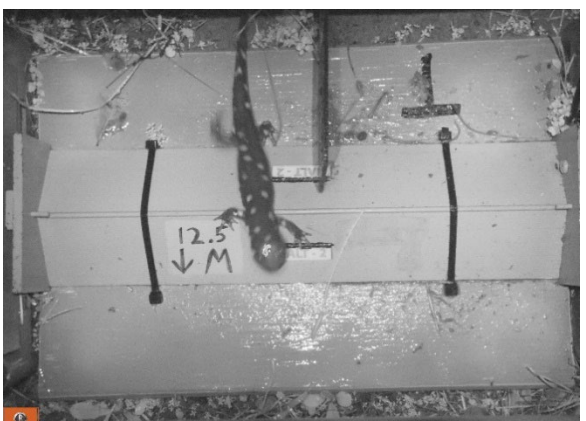
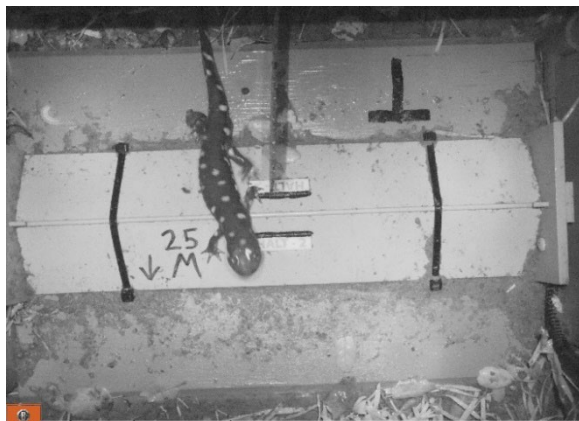
Appendix: Example Photo Sequences

Example 1: Single turnaround: CTS initially going in wrong direction

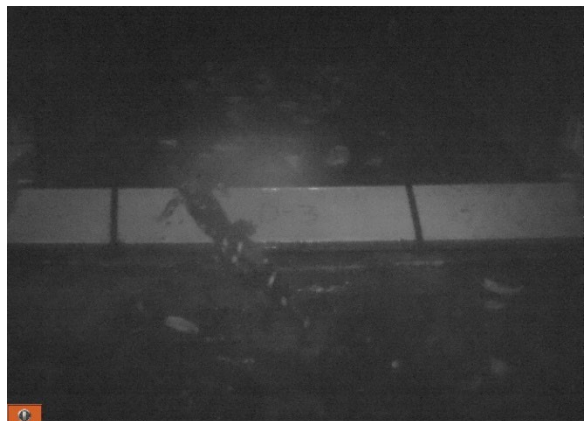
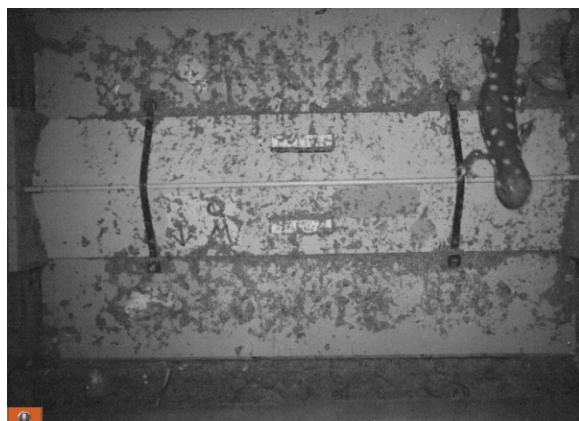
Individual	Cont	ImageDate	Time ch	DSH-3	LAK-3	DM000	DM012	DM025	DM050	DM075	DM100	DM125	DMEnd
AMCA_23_087	1	12/30/2022 23:29:56	0:01:43						12/30/22 23:29:56				
AMCA_23_087	1	12/30/2022 23:31:39	0:16:03					12/30/22 23:47:42	12/30/22 23:31:39				
AMCA_23_087	1	12/30/2022 23:47:42	0:08:16										
AMCA_23_087	1	12/30/2022 23:55:58	0:07:13				12/30/22 23:55:58						
AMCA_23_087	1	12/31/2022 00:03:11	0:02:57			12/31/22 00:03:11							
AMCA_23_087	2	12/31/2022 00:06:08	0:05:41	12/31/22 00:06:08									
AMCA_23_087	2	12/31/2022 00:11:49	end	12/31/22 00:11:49									



Initially moving away from passage system (50m) and entered turnaround “T”, turned around



Continued toward passage system



Continued toward passage system

Entered passage

Example 2: Single turnaround- CTS initially going in right direction

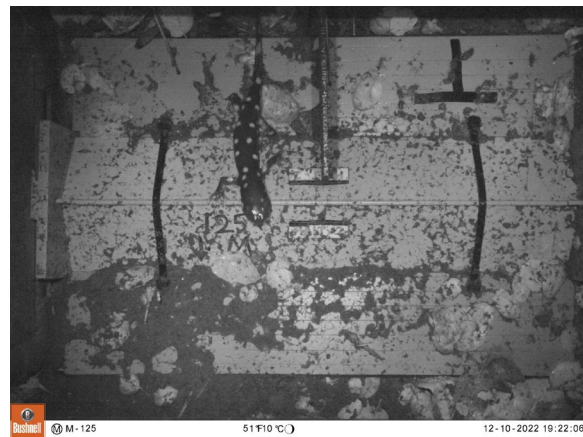
Individual	Cont	ImageDate	Time	D9H-3	LAK-3	DM000	DM012	DM025	DM050	DM075	DM100	DM125	DMEnd
AMCA_23_015	1	12/10/2022 19:22:19	0:13:07										
AMCA_23_015	1	12/10/2022 19:35:26	0:19:32										
AMCA_23_015	1	12/10/2022 19:54:58	0:20:37										
AMCA_23_015	1	12/10/2022 20:15:35	0:24:03										
AMCA_23_015	1	12/10/2022 20:39:38	0:08:53										
AMCA_23_015	1	12/10/2022 20:48:31	0:04:25										
AMCA_23_015	1	12/10/2022 20:52:56	0:03:06										
AMCA_23_015	1	12/10/2022 20:56:02	0:09:56										
AMCA_23_015	1	12/10/2022 21:05:58	0:04:30										
AMCA_23_015		12/10/2022 21:10:28	0:06:42										
AMCA_23_015		12/10/2022 21:17:10	end										



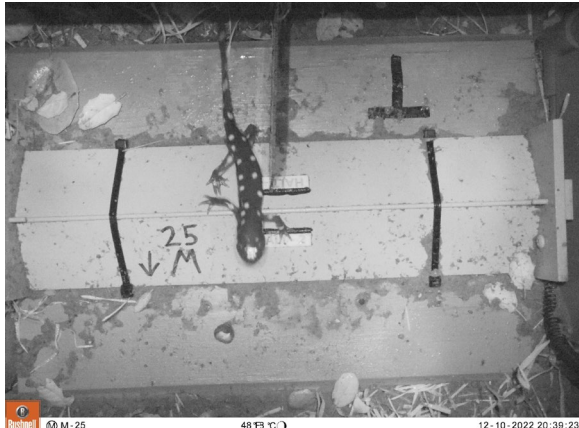
Initially moving toward passage system from 100m. Passes CTS exiting turnaround at 75m



Continues toward passage 50m



Continues toward passage 25m



Continues toward passage 25m



Continues toward passage 12.5m



Turned around somewhere between 12.5 and 0m
(4.4 min) Entered Turnaround "T"



Exited Turnaround



Continued toward passage system



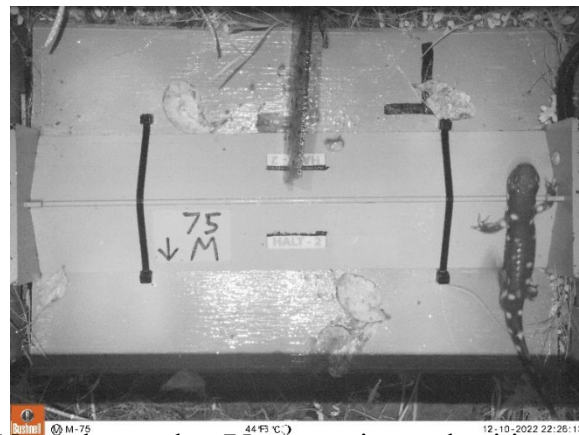
Entered passage

Example 3: Three turnarounds- CTS initially going in wrong direction

Individual	Cox	ImageDate	ImageCh	DH S	LAK S	DW000	DW012	DW025	DM050	DM075	DM100	DM125	DM150
AWKA_14_101	1	12/10/2022 21:36:44	010101						12/10/22 21:36:44				
AWKA_25_103	3	12/10/2022 21:51:25	010014						12/10/22 21:51:25				
AWKA_23_001	1	12/10/2022 21:57:47	010703						12/10/22 21:57:47				
AWKA_23_001	1	12/10/2022 21:54:50	010209						12/10/22 21:54:50				
AWKA_14_101	1	12/10/2022 21:51:04	010100						12/10/22 21:51:04				
AWKA_25_103	3	12/10/2022 22:06:27	010630						12/10/22 22:06:27				
AWKA_23_001	1	12/10/2022 22:01:07	010110						12/10/22 22:01:07				
AWKA_23_001	1	12/10/2022 22:01:19	010110						12/10/22 22:01:19				
AWKA_14_101	1	12/10/2022 22:01:01	010101						12/10/22 22:01:01				
AWKA_25_103	3	12/10/2022 22:06:02	010602						12/10/22 22:06:02				
AWKA_23_001	1	12/10/2022 22:05:04	010504						12/10/22 22:05:04				
AWKA_23_001	1	12/10/2022 22:06:04	010604						12/10/22 22:06:04				
AWKA_14_101	1	12/10/2022 22:06:01	010601						12/10/22 22:06:01				



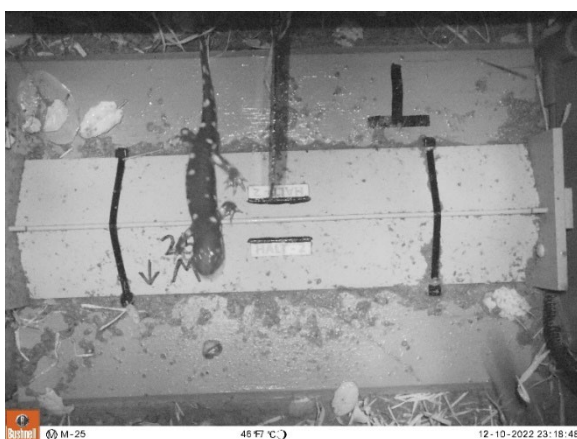
Initially moving away from passage system (50m), entered turnaround "T", turned around 2 times



Next observed at 75m: entering and exiting turnaround



Continued toward passage system (50m & 25m)



Continued toward passage system (12.5m & 0m)



Entered passage

Example of 7 turnarounds- CTS initially going in right direction

Individual	Con	ImageDate	Time	DSH-3	LAK-3	DM000	DM012	DM025	DM050	DM075	DM100	DM125	DMEnd
AMCA_23_0058	1	12/27/2022 01:31:01	0:45:09						12/27/22 01:31:01				
AMCA_23_0058	1	12/27/2022 02:16:10	0:00:56						12/27/22 02:16:10				
AMCA_23_0058	1	12/27/2022 02:17:06	0:00:40						12/27/22 02:17:06				
AMCA_23_0058	1	12/27/2022 02:17:46	0:14:12						12/27/22 02:17:46				
AMCA_23_0058	1	12/27/2022 02:31:58	0:04:31						12/27/22 02:31:58				
AMCA_23_0058	1	12/27/2022 02:36:29	0:00:53						12/27/22 02:36:29				
AMCA_23_0058	1	12/27/2022 02:37:22	0:00:55						12/27/22 02:37:22				
AMCA_23_0058	1	12/27/2022 02:38:17	0:01:59						12/27/22 02:38:17				
AMCA_23_0058	1	12/27/2022 02:40:16	0:01:05						12/27/22 02:40:16				
AMCA_23_0058	1	12/27/2022 02:41:21	0:00:42						12/27/22 02:41:21				
AMCA_23_0058	1	12/27/2022 02:42:03	0:05:07						12/27/22 02:42:03				
AMCA_23_0058	1	12/27/2022 02:47:10	0:02:34						12/27/22 02:47:10				
AMCA_23_0058	1	12/27/2022 02:49:44	0:01:20						12/27/22 02:49:44				
AMCA_23_0058	1	12/27/2022 02:51:04	0:01:23						12/27/22 02:51:04				
AMCA_23_0058	1	12/27/2022 02:52:27	0:17:41						12/27/22 02:52:27				
AMCA_23_0058	1	12/27/2022 03:10:08	0:06:26					12/27/22 03:10:08					
AMCA_23_0058	1	12/27/2022 03:16:34	0:07:25										
AMCA_23_0058	1	12/27/2022 03:23:59	0:03:32										
AMCA_23_0058		12/27/2022 03:27:31	0:03:42										
AMCA_23_0058		12/27/2022 03:31:13	end										



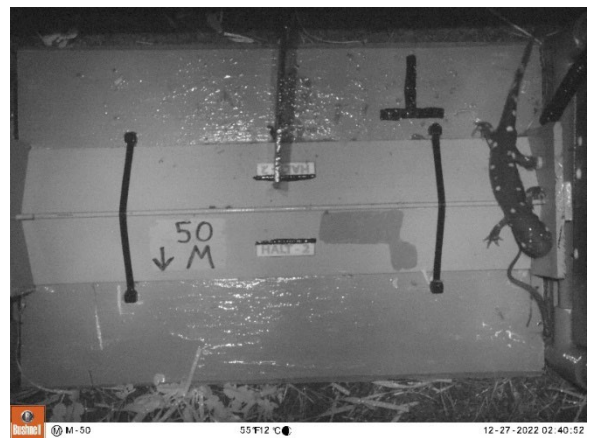
CTS moving toward passage (50m)



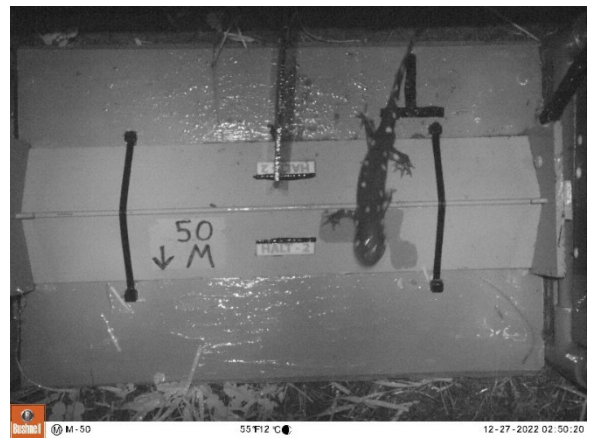
Turned around somewhere between 50m and 25m
35 min later entered Turnaround "T"



Exited Turnaround 1 min later



Entered and exited same turnaround 3 more times



Entered and exited same turnaround 3 more times
(Total of 7 times)



Continued toward passage (25m and 12.5 m)



Continued toward passage (0 m)

Entered passage

Note: After initial direction change along fence line, we expect that if there were no turnarounds, CTS likely would have not made it to passage system