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Anuran Responses to Natural Substrates Within Two Wildlife Underpasses

Dorian Pomezanski[1,2] *and Lorne Bennett*[1]

[1]*Department of Geography, University of Guelph, Guelph, Ontario N1G 2W1, Canada*
[2]*Corresponding author, e-mail: dorian.pomezanski@gmail.com*

***Abstract.*—As crossing structures such as underpasses become more common as a tool for reducing anuran road mortality, there is a need to understand how variations in design influence effectiveness. Of particular importance is the impact of substrate on use, behavior, and movement. To explore the effects of two natural underpass substrates (sod and smooth pea gravel) on anuran crossing behavior, we monitored two ACO® crossing structures in Guelph, Ontario, Canada, using high-interval time-lapse photography and microclimatic sensors between 24 April and 29 October 2016. Although there were more crossing events associated with the sod-lined underpass, both the total crossings times and pause times were longer. Furthermore, crossing events through the first 2 m of the underpasses were significantly more successful and less meandering in the gravel-lined underpass, suggesting that pea gravel may be a more suitable material for facilitating anuran passage in underpass crossing structures. Lower temperatures and higher humidity within the sod-lined underpass, as well as greater differences in temperature and humidity between the entrance and 2 m within the sod-lined underpass, suggest that microclimate is a contributing factor in the crossing behaviors of anurans. We emphasize the importance of incorporating design elements into crossing structures that maintain balances between internal and ambient conditions.**

Key Words.—amphibian behavior; trail camera monitoring; underpass design

Introduction

Roads networks have undergone remarkable expansion in the last century, increasing both in density and extent. As a result, adjacent wildlife populations often end up as the victims of wildlife-vehicle collisions (Forman and Alexander 1998). Long term negative impacts on these populations may be incurred if the mortality rates exceed the reproductive rates of the species (Forman and Alexander 1998). In some cases, levels of road mortality are high enough to result in extirpation (Gibbs and Shriver 2005). For particularly vulnerable amphibians, even low traffic densities can result in high mortality, especially if the roads are located next to wetlands (Aresco 2005). Anurans tend to be most heavily impacted by road mortality because they are slow-moving, slow to respond to moving traffic, and migrate/disperse in large numbers over short periods of time (Glista et al. 2007). In addition, juveniles tend to be the most vagile life stage of anurans, and high mortality rates can limit the dispersal of genetic information within pond networks (Lodé 2000).

Efforts to reduce anuran road mortality have been occurring for decades, and mitigation methods have frequently taken the form of underpass crossing structures. Ranging in size, shape, and porousness, these structures have been used by a variety of amphibian species (Dodd et al. 2004; Sparks and Gates 2012). Some underpass systems have been designed specifically for anurans by decreasing the diameter of the underpass and including slotted ceilings to allow light, air, and moisture to infiltrate the underpass depths, allowing the microclimate of the underpass to better resemble the ambient environment (Pagnucco et al. 2011). Of particular importance is ensuring that underpasses remain moist and humid to reduce the risk of desiccation for anurans that require moisture on their skin to properly absorb oxygen (Churchill 1995). These design elements should in theory reduce instances of anurans hesitating to use underpasses, wherein individuals that approach or enter the underpass pause before continuing forward or turning back (Pagnucco et al. 2011; Hamer et al. 2014). There have also been documented differences in underpass usage in response to underpass substrate materials, with anurans showing a preference for natural substrates, such as soil, sand, or gravel, over bare concrete (Mougey 1996; Lesbarrères et al. 2004; Woltz et al. 2008; Patrick et al. 2010). Though these studies have identified concrete as an unsuitable substrate, there has been little work done to explore preferences and/or differences in anuran usage between more appropriate natural substrates.

An understanding of the patterns and behaviors of organisms in response to underpass structures is integral to designing structures that are effective at facilitating passage beneath roads. Trail cameras have previously been deployed to monitor underpass systems, recording use by mammals, birds, reptiles, and amphibians

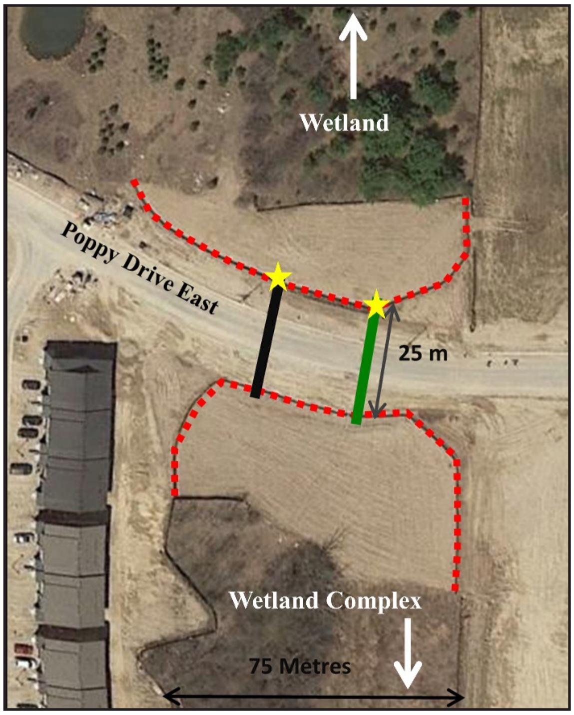

Figure 1. Placement of wildlife underpasses under Poppy Drive East, Guelph, Ontario, Canada (about 43°N, 80°W). The green underpass is lined with sod and the black underpass is lined with pea gravel. The red dotted lines represent the exclusion fencing. The locations of the cameras are denoted by the yellow stars. (Image taken from Google Earth, Google LLC, Mountain View, California, USA).

(Lapoint et al. 2003; Dodd et al. 2004; Pagnucco et al. 2011; Crosby 2014). Trail cameras have become a helpful tool in monitoring wildlife use of underpasses because they are passive and seldom influence animal behavior (Olsson et al. 2008; Ford et al. 2009; Pagnucco et al. 2011).

Two amphibian underpasses were installed beneath a road within a suburban development in Guelph, Ontario, in the fall of 2015 to maintain connectivity between a wetland complex and another wetland isolated by the development. Construction crews, under the guidance of ecological consultants, lined one of the underpasses with sod, and the other with smooth pea gravel. We installed a trail camera at each of the northern ends of the underpasses to monitor anuran behavior in response to substrate. We expected anurans to exhibit behaviors that favored the soil substrate because of its ability to facilitate higher humidity and better mimic natural ground cover.

Materials and Methods

Study area.—We monitored the movement of anurans in underpasses from 24 April to 29 October 2016 at the Dallan Lands, in Guelph, Ontario, Canada (about 43°N, 80°W). The 23.1 ha area is the site of a 409-unit residential development located on the margins of a provincially significant wetland complex. The development led to the isolation of one large wetland and necessitated mitigation to avoid impact to local wildlife populations. A 75-m wide wildlife corridor was set aside on the western boundary of the property to maintain connectivity between the isolated wetland and the rest of the wetland complex to the south. However, a residential road was built that bisected this corridor, prompting concerns of high rates of road mortality. Of concern were local anuran populations, which, prior to development, had been able to frequently migrate and disperse between the wetlands to the south and those situated in and/or around the Dallan Lands (Samantha Hughes and Sarah Mainguy, unpubl. report).

Two ACO® Polymer Products wildlife underpasses (ACO® Systems Ltd., Oakville, Ontario, Canada) were installed in December of 2015 (Fig. 1). The polymer concrete structures had an internal width of approximately 50 cm at their base, an internal height of 30 cm, and were approximately 25 m in length with slotted ceilings. Because of the requirement to install sidewalks, sections of the underpass were overlain with soil, grass, and concrete, sealing the slots for 7.5 m at either end of the underpass, leaving 10 m of the underpass with slotted ceilings. One underpass was lined with smooth pea gravel, the other with sod. Both substrates were noted in previous literature to be more effective than bare concrete at facilitating anuran movement through crossing structures (Lesbarrères et al. 2004; Woltz et al. 2008; Patrick et al. 2010). Construction included 250 m of exclusion fencing parallel to the roads within the wildlife corridors to ensure that wildlife did not cross onto the road surface. The installation of the fencing ensured that there were small to no gaps between the opening of the underpass and the fencing directly adjacent to it.

Trail cameras.—We used two Reconyx® PC900 (Holmen WI) trail cameras, which we positioned on the northern ends of each underpass. We used trail cameras as monitoring tools because they were non-invasive and limited our disruption of anuran movement behaviors. We chose the PC900 because it can take time lapse photographs in low light conditions using a covert infrared flash, and because of its ability to take time lapse photographs at frequent intervals. The cameras were also able to use motion sensing capture and time lapse simultaneously. We placed the cameras within steel enclosures bolted to the underside of the ACO entrance structures so that they pointed towards the inside of the underpass, capturing about 2 m of underpass length and the entire underpass width (50 cm). We collected the photographs from an Secure Digital (SD) card every

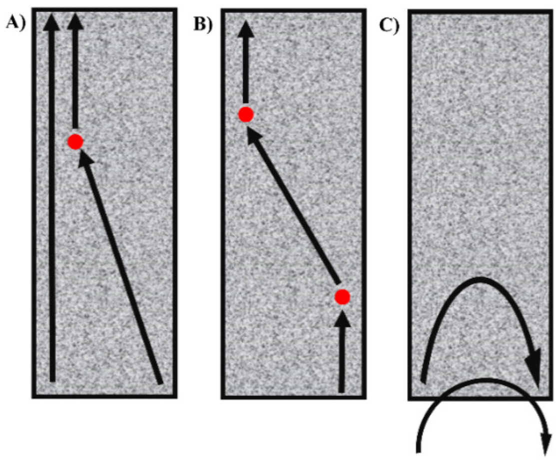

Figure 2. Categories of anuran crossing types considered in this research. Crossing type (A) is a straight crossing with one or fewer nodes (represented by the red dots), crossing type (B) is a meandering crossing (two or more nodes), and crossing type (C) is a turn around or a failed crossing.

fourth day and replaced the batteries daily. The infrared motion sensors were active continuously. A 15-s time lapse was active between the 0700 and 1000 from 24 April to 3 June. We briefly increased the frequency to 10 s from 3 June and 1 July between 2130 and 0730 to experiment with a schedule that was more focused on peak anuran movement times. From 1 July onwards, in expectation of major juvenile dispersal events, the time lapse interval was set to 15 s and was active 24 h/d.

Photographic analysis.—We sorted through the photographs manually looking for any detection of anurans. Nearly all the photographs were taken using the infrared no-glow flash due to the low light conditions and were therefore restricted to black and white coloration. We recorded each photograph capture of an anuran as a crossing event. For each crossing event, we recorded the species. In cases where it was not possible to identify the species of anuran, particularly among frogs of the family Ranidae, we limited identification to family group. We recorded the direction of movement of the individual as either moving north (exiting the underpass, moving towards the wetland) or moving south (entering the underpass, moving away from the wetland). We classified crossing events (i.e., movement through the first 2 m of the underpasses) as successful, unsuccessful, or uncertain. It must be noted that this was not a measure of the success of the anurans in crossing through the entire length of the underpass, but an exploration of their behaviors as they travelled through the first 2 m of the underpasses. We considered unsuccessful those crossings in which the individual turned around or merely passed by the entrance. In cases where there was no conclusive photographic evidence to suggest that the individual moved north or south from its previously recorded position, we classified the success of the crossing event as uncertain.

We defined the total time of a crossing event as the time that the individual stayed within the field of view of the camera, calculated using the time stamps provided on the photographs. Because anurans exhibit hesitancy crossing underpasses (Pagnucco et al. 2011; Hamer et al. 2014), we recorded the pause times exhibited by any crossing anurans in addition to the total crossing time. We defined pause times as any instance in which the anuran did not move between time lapse frames. Within the course of one crossing event, an anuran may have paused several times.

We identified an anuran crossing event as a straight, meandering, or a turn around crossing (Fig. 2). We determined the type of crossing by tracing the path of the anuran through the field of view of the camera. We classified any path that had more than one node as a meandering crossing. We classified an individual who unsuccessfully crossed through the underpass by passing through the entrance or turning back as a turn around.

Climate sensors.—We used two HOBO U23 Pro temperature/relative humidity sensors (Onset®, Bourne, Massachusetts, USA) to monitor microclimate within the first 2 m of the underpasses. Between April and August, one sensor was placed 2 m inside the northern end of each underpass, attached onto a steel angle bar. The sensors recorded temperature and relative humidity every 5 min. Beyond August, we used the sensors to record temperature and humidity differences at the entrance and within each underpass by placing them at 0 m and 2 m inside each underpass. Cameras captured measurements for about 10 d (back to back) in each underpass.

Statistical analyses.—We used chi-square tests of independence to determine whether the proportion of successful crossings in each underpass differed based on direction of travel (i.e., north or south, and whether the proportions of successful crossings (grouped by direction of travel) differed based on composition of the underpass substrate. We used a two sample Kolmogorov-Smirnoff Goodness-of-Fit Test to determine whether the distributions of total crossing event times and pause times of all anurans (regardless of family) differed between underpasses. We also used chi-square tests of independence to determine whether the proportions of crossing types differed between underpass. We used Shapiro Wilks Tests to verify normal distribution of the data. We used paired *t*-tests to test for differences in internal mean temperature and humidity between the

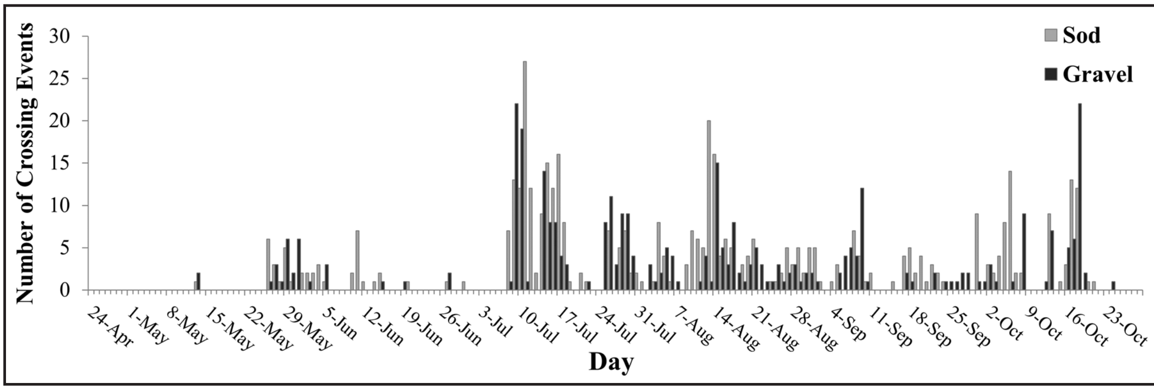

Figure 3. Seasonal timing of crossing events by anurans from 24 April to 30 October 2016 in sod and gravel underpasses at Poppy Drive East, Guelph, Ontario, Canada.

two underpasses, and to test for differences between internal and ambient temperature and humidity in each underpass. For all tests, $\alpha = 0.05$.

Results

Substrate conditions.—Once the underpasses were opened in April 2016, the sod continued to survive for a few weeks. By late May, the sod started to die, and by the end of our monitoring period, the substrate resembled a compacted sheet of soil and organic material. During significant rainfalls, the sod-lined underpass flooded for brief periods of time, with sustained saturation occurring in depressions in the substrate. The gravel-lined underpass rarely flooded, exhibiting better drainage than its sod counterpart. During intense rainfall events, streams of draining water carved channels into the sod substrate as it flowed away from the center of the road. By the end of the monitoring period in October, the erosion of sediment at the edge of the underpass had exposed several centimeters of underpass concrete. The smooth pea gravel substrate remained stable and experienced very little erosion.

Anuran usage patterns.—We recorded 795 crossing events of anurans between 24 April and 30 October 2016, with 42.4% (n = 337) of crossing events taking place in the gravel-lined underpass and 57.6% (n = 458) taking place in the sod-lined underpass. July and August yielded the highest number of crossing events for both underpasses, whereas April, May, and June yielded the lowest numbers (Fig. 3). Identification to species was often not possible due to photograph resolution and lack of color, so reliable grouping of species was limited to family. Of the 795 crossings, 650 were by individuals of the family Ranidae. We confirmed at least one individual of each of the following species in the underpasses: Green Frog (*Rana clamitans*), Northern Leopard Frog (*R. pipiens*), American Bullfrog (*R. catesbeianus*), and Wood Frog (*R. sylvaticus*). There were 119 crossings by individuals of the family Hylidae. Confirmed species included Gray Treefrog (*Hyla versicolor*) and Spring Peeper (*Pseudacris crucifer*). Another 17 crossings were by American Toads (*Anaxyrus americanus*) of the family Bufonidae, and a further nine were unidentified to family. Of the total 795 crossing events, 712 (89.6%) were by juvenile anurans.

Anuran crossing events took place throughout the day but were highest during late evening to early morning (Fig. 4). This difference was most pronounced from 1600 to 2200 during which time there was a higher number of crossings in the sod-lined underpass. From 2200 to 1600, the number of crossing events per hour were relatively similar between the two underpasses.

Anuran behavior.—One hundred and seventeen (34.7%) of the 337 crossing events captured in the gravel-lined underpass, and 258 (56.3%) of the 458 crossing events captured in the sod-lined underpass were heading south (i.e., entering the underpasses). Two hundred and twenty-one (65.2%) crossing events in the gravel-lined underpass, and 200 (43.7%) crossing events in the sod-lined underpass were heading north. The proportion of crossing success for north-bound crossing events (i.e., exiting the underpasses) was significantly higher in the gravel-lined underpass (91%) compared to the sod-lined underpass (84%; $\chi^2 = 10.13$, $P = 0.006$, n = 421). The proportion of crossing success for south-bound crossing events in the sod underpass (53%) was also significantly lower than for the gravel underpass (82%; $\chi^2 = 17.51$, $P < 0.001$, n = 339). There was a significant difference in the proportion of crossing success for anurans travelling south in the sod-lined underpass (53%) and individuals travelling north (84%; $\chi^2 = 48.6$, $P < 0.001$, n = 408). The gravel south-bound crossing success (82%) was also significantly different than the north-bound crossing success (91%), but the magnitude of the difference is less pronounced ($\chi^2 = 7.72$, P = 0.009, n = 334).

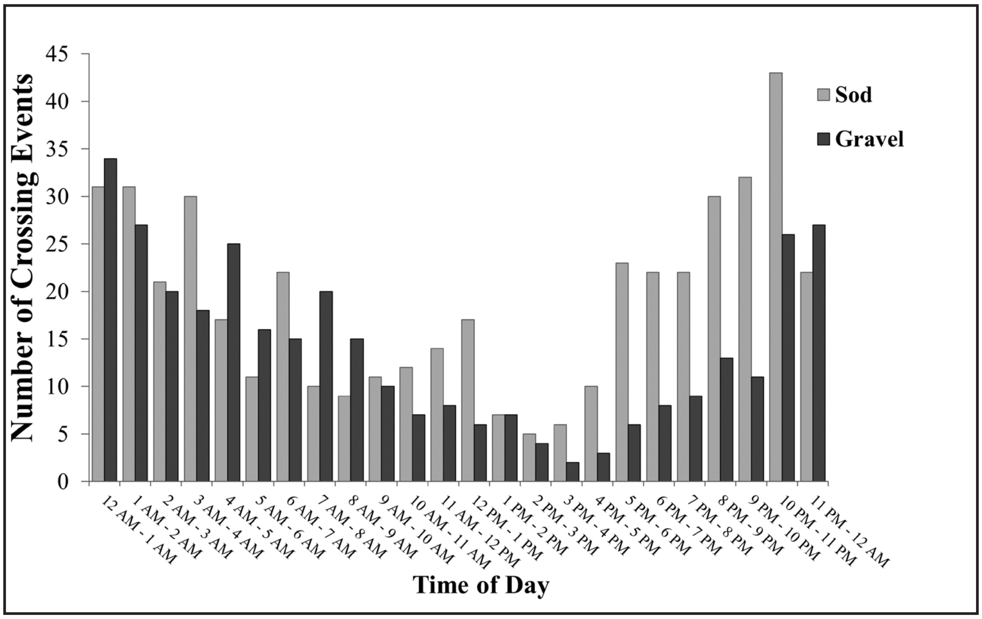

Figure 4. Distribution of starting times of crossing events by anurans in sod and gravel underpasses at Poppy Drive East, Guelph, Ontario, Canada.

Total crossing times were as short as 15 s (a crossing event captured with only a single photograph), and as long as 45,000 s (12.5 h). The longer crossings were generally characterized by long periods of what appeared to be resting. The distribution of total crossing times differed significantly between the underpasses ($Z = 4.199$, df = 795, $P < 0.001$), with a higher number of longer crossings in the sod-lined underpass. Pauses in movement were common in both underpass but averaged higher in the sod (212.7 ± [SE] 12.3 s) than in the gravel (115.4 ± 8.0 s). The distributions of pause times were significantly different ($Z = 2.558$, df = 590, $P < 0.001$), with higher numbers of longer pause times in the sod-lined underpass (Fig. 5). Average pause times were lowest during periods of darkness and tended to be higher during dawn and dusk (i.e., transitions from dark to light or light to dark; Fig. 6). There was a significantly higher proportion of meandering crossings in the sod-lined underpass (36%) compared to the gravel-lined underpass (14%; $\chi^2 = 70.75$, $P < 0.001$, n = 795). Straight crossings were made 51.2% of the time in the sod-lined underpass and 80.1% of the time in the gravel-lined underpass. We classified the remaining crossing events as passing through.

Underpass microclimate.—Relative humidity and temperature 2 m inside of both underpasses fluctuated daily in response to diurnal changes in ambient temperature, sunlight, and atmospheric moisture levels. Microclimatic differences within the underpass were lowest during periods of sunrise and sunset (Fig. 6). Internal temperature ($t = 251.7$, df = 18,680, $P < 0.001$) and relative humidity ($t = -174.6$, df = 18,680, $P < 0.001$) in the underpasses were significantly different between the underpasses over the course of the monitoring period. The magnitude of this difference was consistently 0–20% ($\mu = 9.256$) for relative humidity and 0–2.5° C ($\mu = 1.04$) for temperature.

In the sod-lined underpass, the means of the differences in temperature ($\mu = 1.57$) and relative humidity ($\mu = 5.76$) between the entrance of the underpass and 2 m into the underpass were both significantly different (temperature: $t = -7.41$, df = 1,895, $P < 0.001$; humidity: $t = -6.98$, df = 1,895, $P < 0.001$). The same was true for temperature ($\mu = 1.77$, $t = -31.8$, df = 2,879, $P < 0.001$) and relative humidity ($\mu = 7.01$, $t = 31.04$, df = 2,879, $P < 0.001$) in the gravel-lined underpass. Lower temperatures and higher humidity levels were recorded 2 m inside the underpass during the daytime compared to the ambient conditions at the underpass entrance. Conversely, higher temperatures and lower humidity levels were recorded 2 m inside the underpass during the nighttime. Mornings and evenings experienced brief periods of time when these differences approached zero.

Discussion

Effects of substrate on anuran behavior.—The significantly higher proportions of meandering crossings and longer crossing times in the sod-lined underpass suggest that the sod may have had some effect on the olfactory cues of the anurans (which are responsible for orientation and navigation), or that the anurans did not recognize the structure as a method to

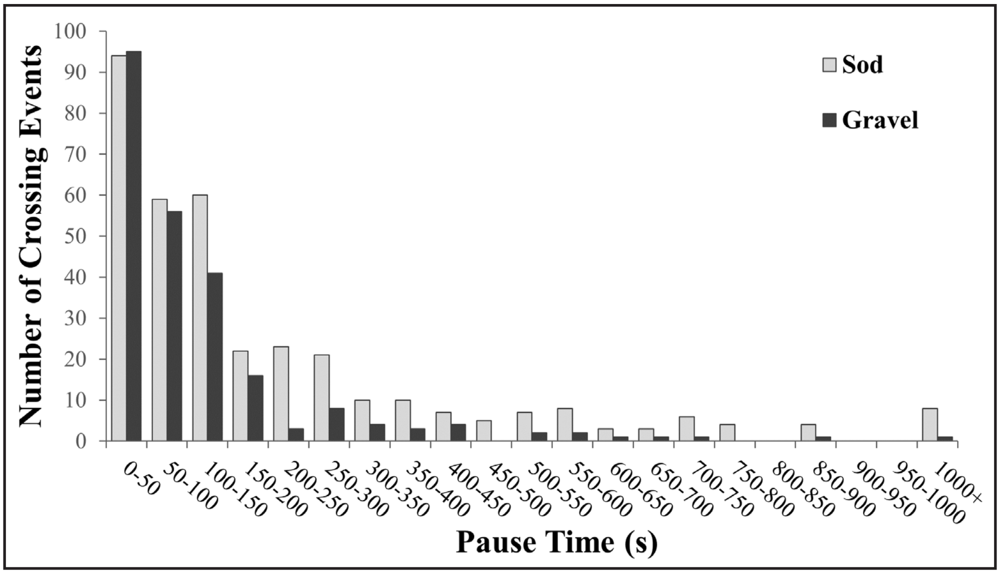

Figure 5. Distribution of pause times of crossing events by anurans in sod and gravel underpasses at Poppy Drive East, Guelph, Ontario, Canada.

facilitate passage, but rather as the destination they were seeking (Dall'Antonia and Sinsch 2001; Lesbarrères et al. 2004). We suspect that anurans, particularly dispersing juveniles who were searching for wetlands to colonize, may have experienced some level of confusion upon reaching the sod-lined underpass, whose interior conditions in many ways resembled that of a wetland bank (i.e., damp, humid, and muddy). A higher number of longer pausing events coupled with a lower proportion of successful crossing events suggest that many anurans found the sod-lined underpass more attractive not as a crossing structure, but as a refuge. In many instances, anurans who paused in the underpass for long periods of time did not continue through the underpass but turned back the way they had entered.

On the other hand, the gravel-lined underpass had higher proportions of successful crossing events, shorter pause times, shorter total crossing times, and a higher proportion of straight crossings. These significant behavioral differences between the underpasses suggest that the gravel substrate is better at encouraging passage. Because these structures were designed and installed specifically to facilitate passage, many of these behavioral observations favor the use of gravel over sod as an underpass substrate. The question then becomes: should the underpass be lined with a substrate that is more attractive to encourage higher rates of usage (whether they be successful crossing events or not), or a substrate that facilitates quicker and more successful crossing events? We believe that although anurans might favor a more natural sod substrate, it requires maintenance and is susceptible to erosion and flooding. This may become problematic in the future, as the alkalinity of concrete substrates has been suggested to be far less attractive for crossing anurans (Mougey 1996). Additional sheets of sod or soil may be placed in the underpass to replace the eroded material. Alternatively, a thin layer of gravel may be overlain with sod to promote better drainage and avoid erosion. However, the sod substrate seems to also reduce crossing event success. A more practical and ultimately more effective underpass substrate may be smooth pea gravel (i.e., gravel with no jagged edges to avoid injury and abrasion), as suggested by the behavioral discrepancies we observed in our study.

Microclimatic influences.—Sod-like substrates with soil and vegetation are surely more capable than gravel-like substrates at retaining moisture. It is this characteristic that might have led to increased relative humidity inside the confined micro-environment of the sod-lined underpass, acting as a source of moisture for

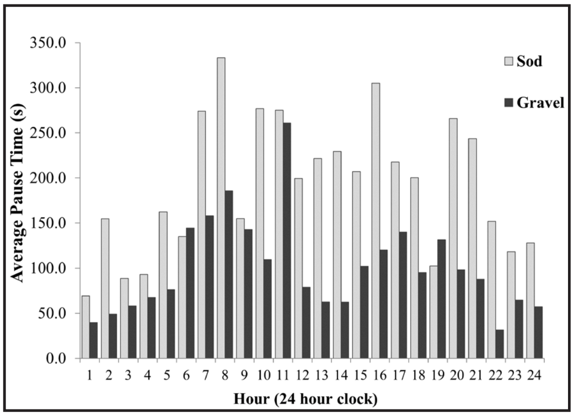

Figure 6. Average pause times per hour of crossing events by anurans in sod and gravel underpasses at Poppy Drive East, Guelph, Ontario, Canada.

anurans that were travelling during periods that exposed them to desiccation. As expected, the warmest hours of the day (1200 to 1600) saw the lowest number of crossings, as anurans move most often during the nighttime when there is a lower risk of desiccation. Between 2300 and 1000, crossing events were similar in number between the two underpasses, but between 1600 and 2200 there were more events observed in the sod-lined underpass. The higher humidity levels in the sod-lined substrate may have been the cause of this pattern, as individuals would likely have favored a cooler and moister environment as relief from the intensive insolation of late afternoons in mid-summer.

Our temperature and relative humidity sensor readings also revealed diurnal fluctuations in the microclimate within the underpasses. The magnitude of these differences between the entrance and 2 m into the underpasses may have played some role in determining anuran behavior. Longer pause times and a greater proportion of meandering crossings in the sod-lined underpass coupled with greater daily differences in temperature and relative humidity (compared to the differences observed in the gravel-lined underpass) between the entrance and 2 m into the underpass point to some degree of microclimate induced hesitancy to cross underpasses. The ACO AT500 wildlife crossing structures were designed with slotted ceilings to allow for moisture and air to penetrate the underpass depths, a design that is meant to reduce the magnitude of microclimatic discrepancies between ambient and internal conditions. However, because the sidewalks and associated green areas on top of the underpasses closed off these slots for 7.5 m on either end, their effects were surely diminished. Municipal requirements for these areas of grass and sidewalk concrete are therefore an issue for the installation of these types of underpasses in suburban settings.

Sudden changes in air moisture and temperature should have contributed to some response by crossing anurans due to the sensitivity of their skin to moisture (i.e., humidity) and temperature. Our results show that many anurans paused as they crossed through the underpass entrances/exits. Regardless of substrate, if it was the differences in microclimate that caused anurans to pause, we would have expected to see the shortest pause times during periods of the day when these differences were at a minimum (i.e., approaching zero between 0 and 2 m into the underpass). Our sensor data showed that microclimatic differences within the underpass were lowest during periods of sunrise and sunset. However, the average pause times were actually higher during these transitional periods compared to overnight when relative humidity and temperature were more stable (a result of the absence of sunlight). Coincidently, these transitional times corresponded to times when the rate of change in microclimate conditions were the greatest. It may be, then, these rapid shifts in microclimate that caused longer pauses during those times of day. If the ceiling slots were exposed and able to function as they were originally designed, then these changes would likely have been far less pronounced, dampened by a more natural mimicry of diurnal shifts in atmospheric conditions, leading to potentially shorter pause times and increased rates of crossing success.

Summary.—Our comprehensive monitoring system revealed behavioral responses of anurans to different substrates. The willingness of anuran individuals to use these underpasses is promising in the long-term conservation of urban anuran populations within the Dallan Lands development. Unfortunately, our study design was limited because we did not have cameras on both ends of the underpasses, which could have allowed us to confirm passage across the entire underpass length (an undertaking which would have introduced its own set of challenges due to photograph quality, and the near impossible task of distinguishing and identifying individual anurans). Another limitation of our study is that we did not have replicates of underpasses, so we do not know how much variation exists in the measures we gathered.

We identified significant differences in behavioral responses of anurans to underpass substrate, which will help inform future underpass installations. Higher proportions of successful crossing events and shorter pause times suggest that a smooth gravel substrate may promote and facilitate movement through underpass crossing structures. We also emphasize the importance of ensuring the openness of the underpass slots to prevent fluctuations in microclimatic conditions, which may in some part be contributing to patterns of pausing and lowered crossing success. Some effort must be made to explore anuran behavior farther within these underpass structures where our cameras were not able to record. Our focus on the first 2 m was meant to explore the responses of anurans to initial entry or exit, but anurans are dynamic and complex creatures, and there is certainly much to be learned about their movement patterns as they cross through the entirety of the structures.

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Dorian Pomezanski recently completed his M.Sc. degree in Geography at the University of Guelph, Guelph, Ontario, Canada. His M.Sc. research used wildlife cameras to investigate small animal usage of wildlife underpass crossing structures in a suburban development. He received his B.Sc.Env in Environmental Geography at the University of Guelph in Guelph, Ontario. Dorian is currently working as an ecological field technician. (Photographed by Dorian Pomezanski).

Lorne Bennett is currently an Associate Professor in the Department of Geography at the University of Guelph, Ontario, Canada, specializing in biophysical processes, environmental monitoring, and landscape ecology. He received his M.Sc. from the University of Guelph in 1981, and his Ph.D. in Geology from the University of Ottawa, Ontario, Canada, in 1989. His recent work has focused on the long-term monitoring or avian and anuran populations in southern Ontario in the context of landscape ecology. (Photographed by Department of Geography, University of Guelph).