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Seasonal High Road Mortality of *Incilius luetkenii* (Anura: Bufonidae) Along the Pan-American Highway Crossing the Guanacaste Conservation Area, Costa Rica

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Abstract.—**The Pan-American Highway in Costa Rica is currently undergoing expansion in capacity as a response to growth in vehicle traffic associated with growing international trade. This highway bisects the Pacific lowlands Tropical Dry Forest of the Guanacaste Conservation Area, a World Heritage site of the United Nations Educational, Scientific and Cultural Organization, with notably high biodiversity, including herpetofauna. As wildlife-vehicle collisions are one of the main direct causes of animal mortality, we quantified the species composition, seasonality, and location of amphibians and reptiles killed along a 30 km segment of the highway running through the conservation area. From August 2016 to February 2017, we mapped roadkill hotspots using Kernel Density Estimation (KDE) with KDE+ software. We detected 1,298 carcasses of 28 species, including seven anuran, one caecilian, three lizard, 15 snake, and two turtle species; the Neotropical Yellow Toad (*Incilius luetkenii*) comprised over half the total roadkill. The two most severe roadkill hotspots were short road segments near seasonally flooded depressional wetlands where *I. luetkenii* and other anurans breed. We urge construction of mitigation measures including barriers and subterranean passages to conserve amphibian populations, especially if the Pan-American Highway will be widened at these sites.**

Key Words.—amphibian conservation; KDE+; Neotropical Yellow Toad; road ecology; roadkill hotspots

Introduction

Costa Rica is renowned for its biological diversity and its extensive national system of protected areas known as Conservation Areas, comprising 26% of its territory (González-Maya et al. 2015). Included in this biodiversity are the herpetofauna of Costa Rica, which includes at least 207 amphibian and 241 reptile species (Leenders 2016). Despite the extensive protected areas, multiple synergistic factors including habitat loss and fragmentation, introduced pathogens, and climate change are resulting in amphibian and reptile population declines in Costa Rica (Savage 2002; Whitfield et al. 2007; Doherty et al. 2020).

Wildlife-vehicle collisions, one of the main direct causes of animal mortality worldwide (Forman and Alexander 1998), have also been associated with declines of amphibian and reptile populations (Fahrig et al. 1995; Gibbons et al. 2000). The density of the road network and the traffic volume it carries is increasing rapidly and is likely to threaten herpetofauna populations further, not only because of road mortality, but also because of degradation of roadside habitats, habitat fragmentation due to the barrier effect of roadways, and increased human access to wildlife habitats (Andrews et al. 2015; Hamer et al. 2015; Langen et al. 2015; Pinto et al. 2020). Anuran populations are particularly susceptible to mass road mortality while crossing heavily traveled roads during breeding migrations (Hamer et al. 2015). Numerous studies have found that amphibian and reptile road mortality is often highly aggregated at relatively short segments of roadway that are referred to as hotspots (Langen et al. 2009; Sillero et al. 2019). Hotspots are typically identified as useful locations for placing mitigation measures that reduce mortality and increase habitat connectivity (Jackson et al. 2015a).

There are very few studies of road mortality in Costa Rica, but those that have been published indicate that road mortality of reptiles and amphibians can be high in Costa Rica, especially for toads (Monge-Nájera 1996, 2018a; Rojas-Chacón 2011; Arévalo et al. 2017; reviewed in Monge-Nájera 2018b). For example, Arévalo et al. (2017) found that amphibian and reptile carcasses comprised 97% of the total roadkill along a highway at the Central Pacific Coast of Costa Rica. The contiguous Santa Rosa and Guanacaste national parks in northwestern Costa Rica are large, protected areas within the Guanacaste Conservation Area, a World Heritage Site of the United Nations Educational, Scientific and Cultural Organization (Janzen and Hallwachs 2016). These parks protect the largest expanse of Tropical Dry Forest in Mesoamerica, an ecosystem high in species richness that includes a large number of endemic herpetofauna and many other forms of biodiversity (Sasa and Solorzano 1995). The two parks are separated by the Pan-American Highway, the primary trans-national road corridor from North America through Central America. With ever-increasing international trade, traffic volumes on this transportation corridor have increased.

The Costa Rican Ministry of Public Works and Transportation has current project plans to double the number of lanes from Barranca to Peñas Blancas (Ministerio Obras Públicas y Transportes [MOPT]. 2008. Declaración de acciones de conveniencia para la rehabilitación de la carretera Interamericana Norte. MOPT. Available from http://www.pgrweb.go.cr/scij/Busqueda/Normativa/Normas/nrm_texto_completo.aspx?param1=NRTC&nValor1=1&nValor2=64572&nValor3=74979&strTipM=TC [Accessed 19 October 2021]), yet at the time of writing (2021) there is a possibility of excluding the segment that runs through the two national parks because of on-site low traffic (Kenneth Solano, pers. comm.). There have been no mitigation measures planned along this segment.

In an initial study of vertebrate road mortality along the Pan-American Highway, Hellen Lobo (unpubl. data) noted high numbers of anuran and snake mortality during the rainy season. Three species of toads (Dry Forest Toad, *Incilius coccifer*; Neotropical Yellow Toad, *I. luetkenii*; and Cane Toad, *Rhinella horribilis*) were especially numerous. In 2016–2017, we conducted Pan-American Highway road surveys during the rainy season through the transition to the dry season, with a focus on road mortality of herpetofauna. Our goal was to document the spatial and seasonal patterns of road mortality by identifying roadkill hotspots for herpetofauna, the most affected species, and mitigation measures that may reduce road mortality in stretches of highway where mortality is the highest.

Materials and Methods

Study site.—Our study focused on a 30 km segment of the Pan-American Highway, crossing Guanacaste and Santa Rosa national parks in Costa Rica (Fig. 1), which are located within the Guanacaste Conservation Area, a 163,000-hectare UNESCO World Heritage site (Janzen and Hallwachs 2016). The roadway is paved, has two-lanes, and is at the same elevation as the surrounding landscape. Adjacent to the roadway is a 5 m buffer of trimmed vegetation. Traffic volume is generally low (around 2,650 vehicles per day), with the heaviest volume occurring from dawn to dusk, and vehicle speeds exceed 80 km/h (Vargas-Alas and Agüero-Barrantes 2017). The landscape adjacent to the highway is primarily Tropical Dry Forest with segments of pastureland and small residential settlements. The climate consists of two distinct seasons: the dry season from December to April and the rainy season from May to November (Janzen and Hallwachs 2016; Langen and Berg 2016).

Data collection.—We surveyed the 30 km segment of the Pan-American Highway from beyond the southern boundaries of the national parks at the Tempisquito River bridge (10.815717°, -85.543958°) to the limits of Guanacaste National Park at the Santa Cecilia Exit (11.046349°, -85.626493°; Fig. 1). We surveyed by car, with at least two observers, while traveling at an average speed of 25 km/h. We recorded the GPS coordinates, date, and species for each roadkill observation. To avoid double-counting, we removed carcasses in every survey. Each sample date consisted of two complete road surveys, at dawn (between 0500 and 0700) and early night (between 2000 and 2200). We surveyed the road once or twice a month, for three consecutive days, between August 2016 and February 2017 (n = 10 survey periods, 30 survey dates including 30 dawn and 30 night road surveys). We recorded weather conditions (minimum and maximum temperature, precipitation, and relative humidity) for each survey (Santa Rosa Meteorological Station, Costa Rica National Meteorological Institute).

Because driving surveys are known to be poor at detecting small herpetofauna (Langen et al. 2007), we resurveyed portions of our highway transect using a walking transect protocol after each morning driving survey. We delineated and numbered the road into 300 plots of 100 m long and 8 m wide and surveyed using a systematic random sampling scheme (Angulo et al. 2006). We chose the starting plot randomly between plots 1 and 30, and we surveyed the following nine plots every 3 km. We surveyed plots by walking slowly while searching for small carcasses (8,000 m^2 total area searched).

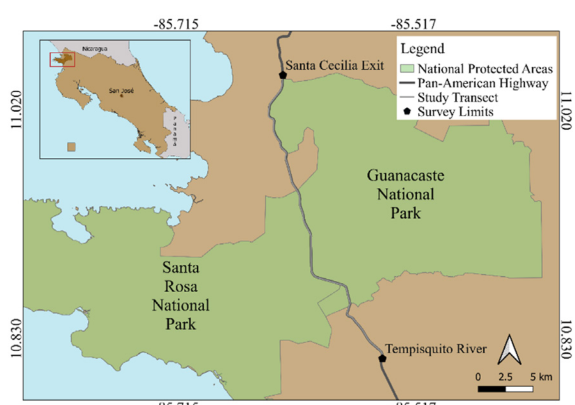

Figure 1. Location of the Guanacaste Conservation Area in Costa Rica, and the 30 km transect surveyed for herpetofauna roadkill along the Pan-American Highway as it passes through and along the two national parks (Santa Rosa and Guanacaste) that comprise the Guanacaste Conservation Area.

Data analysis.—We used the JGR package (Helbig et al. 2013) of R programming (R Development Core Team 2018) for data visualization, descriptive statistics, and statistical hypothesis testing. We used the Wilcoxon Rank Sum Test to evaluate statistical differences between dry and rainy seasons. We used the KDE + software (Bíl et al. 2013, 2015) to perform Kernel Density Estimation for identifying spatial clusters of roadkill. The core function width we used was 150 m, which we judged as a reasonable scale commensurate with the scope of potential mitigation measures (c.f. Malo et al. 2004; Ramp et al. 2005; Langen et al. 2012). We made a cluster map of roadkill hotspots using QGIS and ranked the severity of the hotspots according to the strength parameter, which quantifies the degree of clustering from zero to one (Bíl et al. 2013). Our severity scale, made after pooling all herpetofauna and all surveys, ranged from low roadkill density corresponding to color green (0.16–0.29), to moderate density – yellow (0.30–0.43), to high density – orange (0.44–0.57), to very high density – red (0.58–0.71).

Results

We detected 1,298 carcasses, equivalent to a rate of 0.7 carcasses/road-km/survey-day. We identified 28 species, including seven anuran, one caecilian, three lizard, 15 snake, and two turtle species. Species with the highest number of records included *I. luetkenii* (n = 695, 53.5% of all records) and *R. horribilis* (n = 420, 32.4% of records). Species classified by Costa Rican legislation as threatened include the Mesoamerican Boa Constrictor, *Boa imperator* (n = 16), the Purple Caecilian, *Gymnopis multiplicata* (n = 1), and *I. luetkenii* (La Gaceta. 2017. Article 5. Grupos taxonómicos de vida silvestre. Available from http://www.pgrweb.go.cr/scij/*úsqueda*/Normativa/Normas/nrm_texto_completo.aspx?param1=NRTC&nValor1=1&nValor2=84592&nValor3=109223 [Accessed 8 August 2021]). We found no species classified by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2020) as being of Conservation Concern.

Roadkill mortality was far higher during our rainy season surveys (August-October; 1,212 carcasses over 30 surveys) than dry season surveys (November-February; 86 carcasses over 30 surveys; $W = 8.51$, df = 29, $P < 0.050$). The mortality of most species occurred in the rainy season; the only species with more than 10 records that had similar dry and wet season mortality was the Forrer's Grass Frog (*Lithobates forreri*; Table 1). There were 32 clusters of roadkilled herpetofauna, including 12 low, 17 moderate, two high, and one very high severity hotspots (Fig. 2). The most severe hotspots corresponded to *I. luetkenii* mass-mortality sites.

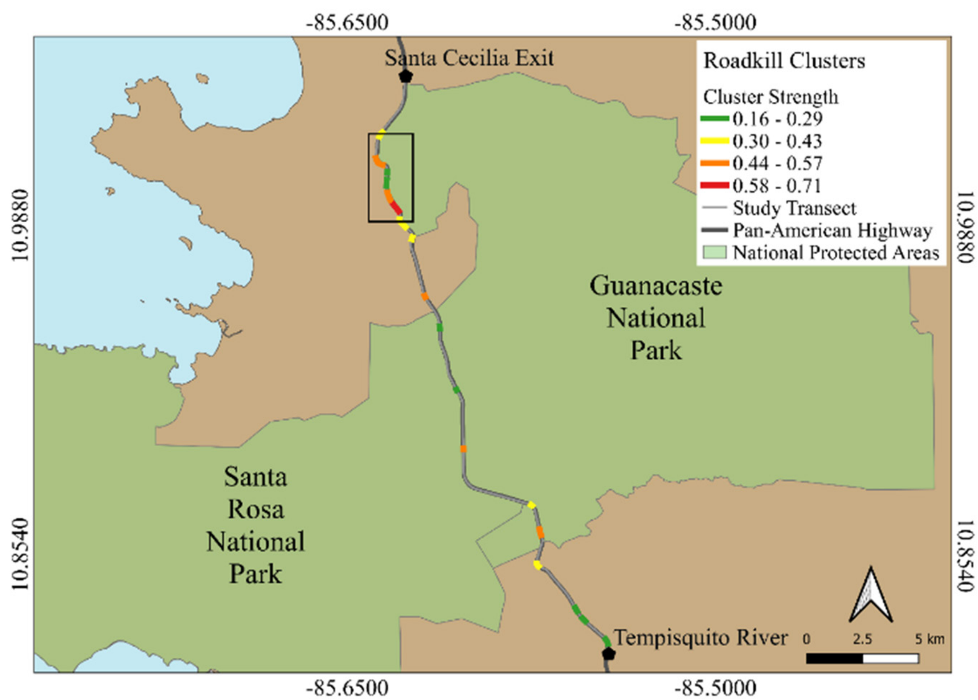

Figure 2. Herpetofauna roadkill hotspots using Kernel Density Estimation, classified from low roadkill density (corresponding to color green) to very high density (corresponding to color red) along the Pan-American Highway, Guanacaste, Costa Rica. Rectangle indicates region highlighted in Figure 3.

Table 1. Species identity and total carcass counts (#) of herpetofauna found during dry and rainy season surveys of roadkill within a 30 km road transect and 30 survey dates along the Pan-American Highway in the Guanacaste Conservation Area, Guanacaste, Costa Rica. These counts can be converted into rates by dividing the counts by 900 road-km (i.e., 30 km road × 30 road surveys, morning and night combined).

Taxon	Common name (Leenders 2016)	Dry Season (#)	Rainy Season (#)
Amphibians			
Anura			
Incilius coccifer	Dry Forest Toad	3	20
Incilius luetkenii	Neotropical Yellow Toad	0	695
Leptodactylus fragilis	White-lipped Foam-nest Frog	0	2
Lithobates forreri	Dry Forest Leopard Frog	12	11
Rhinella horribilis	Cane Toad	36	384
Smilisca sp.	tree frogs	0	2
Trachycephalus typhonius	Milk Frog	11	36
Unidentified		7	29
Caecilians			
Gymnopis multiplicata	Purple Caecilian	0	1
Reptiles			
Lizards			
Aspidoscelis deppei	Deppe's Racerunner	1	2
Ctenosaura similis	Common Spiny-tailed Iguana	3	4
Iguana iguana	Green Iguana	1	0
Unidentified		0	1
Snakes			
Boa imperator	Mesoamerican Boa Constrictor	1	1
Coniophanes piceivittis	Stripped Spotbelly	0	1
Crotalus simus	Central American Rattlesnake	0	1
Geophis hoffmani	Common Earth Snake	1	0
Imantodes gemnistratus	Banded Blunt-headed Vine Snake	0	1
Leptodeira nigrofasciata	Banded Cat-eyed snake	2	1
Leptophis mexicanus	Mexican Parrot Snake	0	1
Masticophis mentovarius	Neotropical Coachwip	0	2
Micrurus nigrocinctus	Central American Coralsnake	2	0
Ninia maculata	Banded Coffee Snake	0	1
Oxybelis aeneus	Brown Vine Snake	0	1
Porthidium ophryomegas	Dry Forest Hognosed Pitviper	4	2
Sibon sp.	Snail eaters	0	1
Stenorrhina freminvillei	Northern Scorpion-eater	0	1
Trimorphodon quadruplex	Central American Lyre Snake	1	2
Unidentified		1	6
Turtles			
Kinosternon leucostomum	White-lipped Mud Turtle	0	2
Kinosternon scorpioides	Scorpion Mud Turtle	0	1
Total Herpetofauna		86	1,212

Discussion

Our results indicate that herpetofaunal road mortality along the Pan-American Highway within the Guanacaste Conservation Area occurs primarily during the rainy season (93%) and is spatially concentrated at a small number of hotspots that comprise a relatively short (30km) segment of roadway. Two species of explosively breeding toads comprised the vast majority (86%) of carcasses found. That road mortality is associated with rainfall and high humidity is well-documented in amphibians (Hamer et al. 2015; Jackson et al. 2015b); 95% of our amphibian roadkill records occurred in the rainy season. In Guanacaste Province, *R. horribilis* and most other amphibians estivate during the dry season (Savage 2002; Hilje and Arévalo 2012). Reptile road mortality was also higher in the rainy season (65% of records); reptile activity is known to be higher in the rainy season of Costa Rican Tropical Dry Forest (Sasa and Solórzano 1995). In addition to favorable moisture conditions, the rainy season is also a period of more abundant prey, including amphibians, nesting birds, and insects (Wolda 1978; Langen and Berg 2016).

We must note that our roadkill records very likely underestimate the mortality that occurs on the Pan-American Highway. Small vertebrates are difficult to detect, especially at night, and only persist a short time on a roadway before disintegrating or being scavenged (Langen et al. 2007; Santos et al. 2011). Persistence of carcasses on roadways is especially brief under wet conditions, and anuran remains disappear particularly rapidly (Santos et al. 2011; pers. obs.).

Seasonally flooded depressional wetlands are important breeding habitats for many anurans in the Pacific lowlands dry forest (Leenders 2016), so it is not surprising that the two most severe roadkill hotspots were within 50 m and 450 m of large seasonally flooded depressional wetlands. We observed mass-breeding events, as evidenced by loud choruses of vocalizing toads and frogs, which coincided with peaks in mortality, and the roadkill records of the three toad species, especially *I. luetkenii*, occurred within road segments near these wetlands (Fig. 3). Such depressional wetlands are rare in this segment of the Pan-American Highway, and because of their rarity, amphibians that breed in these habitats likely migrate long distances to use them. The mortality trend near breeding sites is likely maintained over time as this same roadkill hotspot location was documented by Hellen Lobo in 2007–2008 (unpubl. data).

The high number of *I. luetkenii* roadkill is particularly concerning. This relatively large toad is endemic to the Pacific Lowlands Dry Forest of Mexico and Central America and is currently classified in the IUCN Red List as Least Concern (Bolaños et al. 2004) but is legally classified as Reduced Populations - Threatened (PR/A) in Costa Rica. *Incilius luetkenii* is notable as a species that is sexually dichromatic, males briefly turn bright yellow during breeding (Doucet and Mennill 2010; Rehberg-Besler et al. 2016). Toad breeding migrations

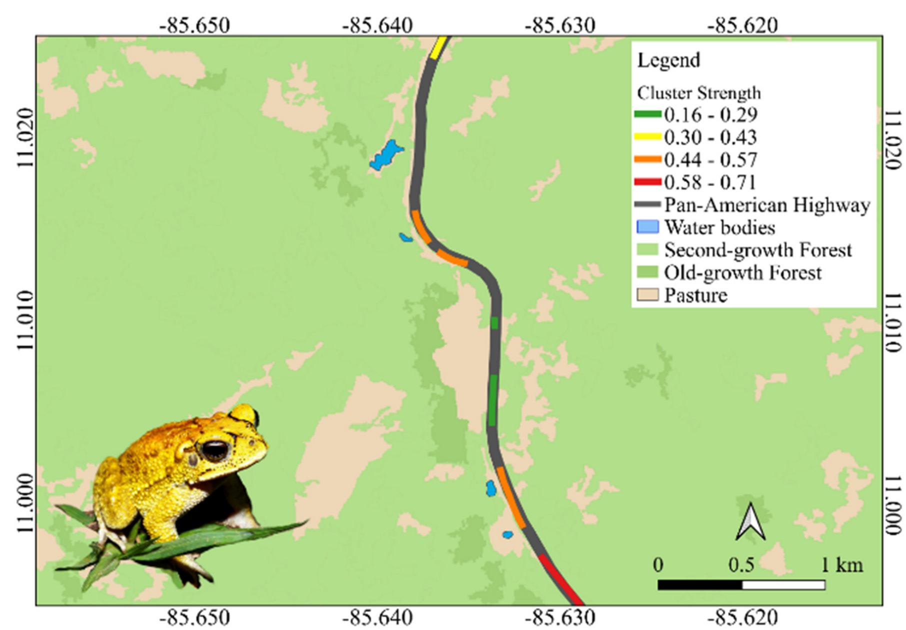

Figure 3. Landscape features adjacent to the principal roadkill hotspots of herpetofauna along the Pan-American Highway, Guanacaste, Costa Rica. High and intermediate severity hotspots are indicated in red and orange, respectively. Seasonal wetlands shown in blue; small, ephemeral pools that form in pastures after rainfall not shown. Inset map species: Neotropical Yellow Toad *(Incilius luetkenii*). (Photographed by Emmanuel Morán).

can result in road mortality high enough to place their populations at risk of local extirpation (Hamer et al. 2015; Jackson et al. 2015b). Considering the expected impact of increased traffic along the Pan-American Highway to toad breeding migrations in our study area, it is plausible that *I. luetkenii* and other depressional wetland breeding amphibians are at risk of local extirpation. If these roadside wetlands are population sinks with occupancy maintained by migration from sites distant from the highway, the wetlands will serve as what might be described as attractive nuisances and the problem of roadkill will be ongoing.

Increasing the number of lanes on the Pan-American Highway as it passes through the Guanacaste Conservation Area and the resulting traffic volume will almost certainly increase road mortality and make the roadway a greater barrier to dispersal and prevent access to amphibian breeding habitats. Even if this segment remains two lanes, improvements elsewhere on the highway will increase traffic flow, thus increasing harmful road effects on reptiles and amphibians. We recommend, at a minimum, considering installation of barriers that direct wildlife to culverted passage-ways under critical segments of the highway. For amphibians at the hotspots that we documented near the seasonally flooded depressional wetlands, this mitigation can be effective (Jackson et al. 2015a; Rytwinski et al. 2016) and practical because these hotspots are in relatively short segments of road (Langen et al. 2009). We suggest using designs and specifications for barriers and passage structures that have been tested for durability and found to be effective at preventing reptile and amphibian road mortality and improving habitat connectivity.

We also stress the importance of implementing best practices for monitoring and maintaining mitigation structures in perpetuity, e.g., Woltz et al. (2008), Gunson et al. (2016), and Helldin and Petrovan (2019). Warning signage, though less expensive, is generally judged to be ineffective (Jackson et al. 2015a), and we see little likelihood that this measure would yield better results along the Pan-American Highway, especially because motorists cannot possibly avoid amphibians during mass-crossing events on rainy nights. Another mitigation measure that may be worth considering is constructing depressional wetlands distant from the highway on either side of the roadway. If strategically located to intercept migrating toads on their path towards historically used breeding sites, constructed wetlands could provide breeding habitat that compensates for losses caused by the roadway (Lesbarreres et al. 2010). Although barriers, subterranean passages, and constructed wetlands are mitigation measures that have not been implemented or tested on Costa Rican roads, they have been found to be effective for toads and other anurans elsewhere (Jackson et al. 2015a; Hamer et al. 2015; Helldin and Petrovan 2019) and would be justified for conservation of native herpetofauna in a UNESCO Biosphere Reserve like the Guanacaste Conservation Area.

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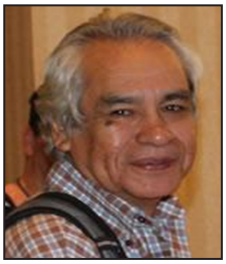

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