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# Estimating the effects of road-kills on the Fire Salamander population along a river

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# ABSTRACT

A number of factors contribute to the process of amphibian decline, among them population fragmentation and road-kills. In this work we evaluated the effects of a road that separates the hibernation area of the Fire Salamander population from their breeding site on the demography and movement behavior. For that we monitored the population for four years using transects along the road and along a river that runs in parallel to the road and serve as the breeding site. We found that the estimated percent of the population killed on the road steadily increased minimally from 2.56 % to 10.78 % over a four year period of increasing vehicular activity. Interestingly, only a small number of individuals were documented on both sides of the road, suggesting there is a potential for population fragmentation due to the road. Additionally, we used geostatistics to reveal that the spatial distribution pattern of the population and road-kills along the road is not random. Thus, conservation efforts that focus on preventing salamanders from accessing the road surface should focus on the areas where the population and road-kills aggregate.

# 1. Introduction

Amphibians are the most threatened group of vertebrates, with a third of known species classified as endangered with extinction by the IUCN (Stuart et al., 2008). A number of factors contribute to the process of extinction of amphibians including habitat loss, exotic predators, infectious diseases and road-kills (Collins, 2010; Cushman, 2006; Garriga et al., 2012; Gilioli, Bodini, Baumgärtner, Weidmann, & Hartmann, 2008; Manenti, De Bernardi, & Ficetola, 2009). In addition, local geography and dimensionality affect the migration pattern and isolation of a population. Landscape features, such as mountain ridges, wide rivers and roads were identified as primary causes of genetic population differentiation (Blank et al., 2013; Kershenbaum et al., 2014; Marsh, Milam, Gorham, & Beckman, 2005; Sinai et al., 2019). Landscape features may strongly affect genetic structure, especially in amphibians, because they are considered poor dispersers (Blaustein, Wake, & Sousa, 1994; Zeisset & Beebee, 2008).

Roads and traffic affect animal populations in diverse ways in different species (Jaeger et al., 2005). Among these effects are mortality due to roads kills (Ashley & Robinson, 1996; D'Amico, Román, De los Reyes, & Revilla, 2015), damage to the habitat and its quality, fragmentation due to avoiding crossing roads that can lead to restricted access to food sources or breeding areas, and splitting populations into small and vulnerable subpopulations (Beebee, 2013; Forman & Alexander, 1998; Goff, 2015). In addition, there are some reports that salamanders are active on roads, thus increasing the risk of been killed by traffic. For example, Briggler, Johnson, and Rambo (2004) found that male salamanders were slow at crossing roads as they stop for conspicuous posture displays. Other studies found that males prefer roads as there they can easily spot females, and indeed much more males were present on roads at the beginning of the breeding season (Vincenz & Reyer, 2005). A better understanding of the impact of roads on the distribution, fragmentation, and long-term survival of amphibian populations will help in estimating the conservation state of amphibians.

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Fig. 1. The location demarcated with blank point (left) and the map (right) of the study area.

There is little doubt that the roads directly damage individual animals. Rytwinski and Fahrig (2015) in a literature review of 75 studies found that all the 23 reviewed amphibian species were prone to negative road effects. Amphibians can represent as much as 90 % of vertebrates killed on roads and are particularly vulnerable to the effects of roads and the associated disturbance (Beebee, 2013). Glista, DeVault, and DeWoody (2008) performed a road-kill survey and found 10,515 road mortality events, 95 % of them were amphibians. The authors concluded that road-kill may be a major factor in the global decline of amphibian.

In this study, we explore the Near Eastern Fire Salamander populations (*Salamandra infraimmaculata*) in northern Israel (Blank & Blaustein, 2012, 2014; Sinai et al., 2019, 2020). This species is considered endangered in Israel (Dolev & Perevolotsky, 2004). Salamander populations in Israel occupy the southernmost, and most xeric habitats of this genus worldwide (Blank & Blaustein, 2012; Sinai et al., 2020). The study was conducted along the Dishon river- the longest river in the Upper Galilee in northern Israel. A paved road that runs parallel to the river was expanded and modified over the years and in the last two decades the traffic intensity on the road increased (Oron, 2012). Concerns were raised by the Israel Nature and Parks Authority over the potential fragmentation of the salamanders' populations due to widening of the road and due to a road cuts that were created on the southern side of the road that might limit the salamanders ability to cross the road and to access the river.

The aims of this work were to evaluate the effects of the road on the percentage of the population killed on the road and spatial distribution patterns of the species along the road. The surveyed road separates the hibernation area of the Fire Salamander population from their breeding site, thus provide an ideal opportunity to explore the effects of roads on population fragmentation and estimate the movement rate of this species. We use this information to identify the optimal sites along the road for constructing road passages.

# 2. Materials and methods

#### 2.1. Study site and data collection

The study was conducted along a 2 km stretch of the Dishon river in the Upper Galilee (Fig. 1). The Upper Galilee climate is Mediterranean, characterized by 714  $\pm$  163 mm of an average annual precipitation, which mostly falls (79  $\pm$  10 %) between December and March (Lövy, Sumbera, Heth, & Nevo, 2020). Activity of adult *S. infraimmaculata* is largely restricted to rainy nights. In Israel, *S. infraimmaculata* females emerge from their summer estivation site beginning with the fall or winter rains. To estimate the probability of a road-kill and other demographic parameters, transects were performed by walking along the road and the stream (Fig. 1). These surveys were performed during rainy nights over four years (2012–2015), with around 10 transects in each year. Santos et al. (2015), suggest that widely spaced surveys at weekly or longer intervals may produce poor estimates of road-kill hotspots,

particularly for small-bodied species as amphibians. We minimized this problem in this study by surveying after every rainy night - 11 nights in 2012, 9 nights in 2013, 15 nights in 2014 and 17 nights in 2015. During each visit, the same two scouts during the entire study period walked from west to east along the road (approximately 2 km) and searched for adult salamanders along both sides of the road while scanning a swath of approximately 5-10 m. On the way back, the scouts walked along the Dishon river again for an approximately 2 km transect. All salamanders found in the transects were photographed and their location coordinates were recorded with a GPS. In addition, the gender of each individual was identified (Segev, Hill, Templeton, & Blaustein, 2010). Salamander individuals are easy to identify as each individual has a unique spot pattern on the dorsal side of the body, thus no marking is needed (Goedbloed et al., 2017; Segev et al., 2010). We assigned each encountered individual to one of three different locations: stream bed (SB), southern part of the road (SR), and northern part of the road (NR).

Data on traffic volume was obtained from the Central Bureau of Statistics for the road segment that is the study area. We summed the average number of cars between 20:00 and 4:00 as these are the main activity hours of the fire salamander. Unfortunately, in 2015 and 2016 the census was not performed.

# 2.2. Survey of salamander road-kills

Salamanders that were killed on the road were surveyed along the same transect in the early morning in order to find salamanders that were killed the previous night. When dead salamanders were found, they were photographed and their coordinates were recorded using a GPS. Identifying individuals after being run-over was not always possible.

# 2.3. Data analysis

#### 2.4. Estimating the percent of road-kill

The total number of road-kills, observed in season i, Ni was recorded, as was the number of road-kills in that season that were marked, n<sub>i</sub>. By marked, we mean that the salamander had been photographed and identified in a previous encounter that year or earlier years. The probability of being killed on the road is estimated by  $p_i = n_i / N_i$ . This simple estimator is most likely an underestimate. First, not all marked animals that are killed on a road are identifiable. We minimized this by doing surveys almost every rainy night during each season, but it is still possible that some marked animals were not identifiable, which would decrease n<sub>i</sub>. Second, some of the marked animals may have died from other causes off the road or dispersed out of the area of study. In either case, such animals would no longer be at risk for becoming a road-kill in our surveyed area. Hence, a subset of  $N_{\rm i}$  could have a zero probability of becoming a marked road-kill in our survey. Both of these factors would cause  $p_i$  to be biased downwards. Therefore, p is a conservative estimator of the amount of road-kill.

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### Table 1

Marked and road-kill data of the Dishon salamander population over four years.

Year	Marked individuals	Road- killed	Number of identified Road-killed individuals	Probability of Road-kill (p <sub>i</sub> )
2012	117	37	3	0.0256
2013	105	32	5	0.0476
2014	122	28	7	0.0574
2015	102	44	11	0.1078

We are specifically interested in testing the hypothesis that the probability of road-kill increases with years as traffic intensity also increased. This hypothesis is tested with a Jonckheere-Terpstra test on an ordered 4 (seasons) by 2 (marked road-kill; marked but not road-kill) contingency table as implemented in the program StatXact9 (Cytel Studio, Cambridge, MA, www.cytel.com/software/statxact). An exact probability was determined under the null hypothesis of no effect of years on the  $p_i$ 's against the alternative one-sided hypothesis of an increasing  $p_i$  with increasing i.

#### 2.5. Estimating the Fire Salamander's focal area on the road

We used the convex hull estimate for calculating individual's focal area on the road (Yalcin & Leroux, 2017). This area was calculated from the number of observations points, as measured by GPS in the field, (see the methods of the salamander survey). Individuals with at least three observations, for at least two years, were examined using this approach. Because of the longitudinal transect, most movement approximated linear movement. Hence, we used (for each convex hull polygon) the distance between the two most distant points, instead of the total area. The differences between the genders was calculating by using an exact permutation test of the null hypothesis of homogeneity using the program StatXact9 (Cytel Studio, Cambridge, MA; www.cytel.com/softw are/statxact).

# 2.6. Quantifying the distribution of individuals and road-kill along the road

To analyze spatial patterns and explore spatial clustering of individuals and road-kill in the survey area, nearest neighbor analysis was performed using spatial statistics tools in ArcGIS 10.5 (ESRI, Redlands, CA). We used a heatmap to visualize individuals and road-kill density OGIS v. 2.12.0 software (http://www.ggis.org/) (Blank, Birger, & Eizenberg, 2019). As a first step, we tested the null hypothesis that individuals and road-kill were randomly distributed throughout our four plots using nearest neighbor analysis. Nearest neighbor analysis was performed using the average nearest neighbor function in ArcGIS to evaluate the degree of clustering. The analysis calculates the ratio of observed average nearest neighbor distance and expected average Euclidean distance based on random distribution. If the ratio differs significantly from zero, the null hypothesis is rejected and the spatial pattern is either dispersed (>1) or clustered (<1) (Mitchel, 2005). Heatmaps are a visualization tool for dense point data. Heatmaps are used to easily identify clusters where there is a high concentration of certain features. The heatmap uses Kernel density estimation to create a density raster of an input point vector layer. The density is calculated based on the number of individuals in a location, with larger numbers of clustered points resulting in larger values. To check whether road-kill and surveyed live individuals were correlated we quantified the number of live individuals found on the road and road-kill in a 200 m segments along the road.

# 3. Results

A total of 677 salamanders were captured during the study. The number of individuals that were recaptured at least once were 366 (54



Fig. 2. Estimated probability of road-kill over four years (solid line) and the number of cars at the study area (black circles and dash line).

Table 2

Dinstance between convex hulls of the same individual between years (convex hulls overlap = 0).

Salamander ID	Year 1	Year 2	Distance of convex hull between years (overlap = $0$ )
1	2012	2014	0
14	2012	2014	0
16	2014	2015	0
19	2012	2014	0
19	2014	2015	0
20	2012	2013	19
20	2013	2014	0
419	2014	2015	7
459	2014	2015	0
517	2013	2014	0
574	2014	2015	0
722	2014	2015	0

%). The number of individuals found each year were fairly constant and ranged from 102 (2015) to 122 (2014) (Table1).

#### 3.1. Estimated probability of road-kill

The number of total road-kills, marked road-kills, marked individuals, and probability of road-kill ( $p_i$ ) are given in Table 1. Fig. 2 shows a plot over time of the probability of road-kill. Both Fig. 2 and Table 1 indicate a steady increase in the amount of road-kill over time, going from 2.56 % in 2012 to 10.78 % in 2015. The exact Jonckheere-Terpstra test yields a 1-tailed probability of 0.006, indicating that this ordered trend was highly significant. Between 2012 and 2017 there was a sharp increase in traffic, and the number of cars increased by ~22 % (Fig. 2).

#### 3.2. Salamander movement rate between regions

Only 14 individuals were recaptured in more than one of the three predefined areas (SB, RS, RN). Of these, 10 were captured near the stream (SB) and on the southern road (RS), and 4 were captured on the southern and northern part of the road (RN). There were no individuals captured both in the stream and on the northern road. Overall, movement in and out the three regions was minimal to zero between years. Only 14 individuals were captured in more than one region. This result implies that movement out of the surveyed area may also be minimal, thereby minimizing the potential bias in the probabilities of road-kill due to this factor.

# 3.3. Focal area on the road

The longest distance between recaptures was relatively short. In 32 cases, the distance was smaller than 100 m, in six cases over 100 m, in



Fig. 3. Heatmap showing absolute density of presence individuals.



Fig. 4. Heatmap showing absolute density of road-killed individuals.

 Table 3

 Results of the Average Nearest Neighbor analyses in the four seasons.

Season	Captured individuals		Road-killed	
	Average Nearest Neighbor ratio	Р	Average Nearest Neighbor ratio	Р
2012	0.6	>0.001	0.49	>0.001
2013	0.63	>0.001	0.53	>0.001
2014	0.51	>0.001	0.47	>0.001
2015	0.39	>0.001	0.48	>0.001

five cases over 200 m and only in two cases over 300 m., In addition, although most of the captured salamanders (with at least three captures) were males (27) and only five were females, two of these females showed the longest distances (339 and 441 m) (Table A1, Appendix). The distances differences between the genders were significant with a 2-sided p-value of 0.0241.

Table 2 shows the distance of "convex hulls" between years of the same individuals ("0" indicating partly overlapping hulls). There are only two individuals (20 and 419) with no overlap between years, indicating that a salamander's range remains in the same areas from year to year (Table 2). The observed short movement distances and stable focal area on the road both indicate restricted movement, thereby minimizing the potential bias in the probabilities of road-kill due to marked animals leaving the entire surveyed area.



Fig. 5. Correlation between the numbers of run over and captured individuals in 11 segments of 200 m along the road.

# 3.4. Quantifying the distribution of individuals and road-kill along the road

The distribution of the captured individuals was clustered and the highly dense areas located in several areas both in the Dishon river and along the road (Fig. 3). Similarly, the distribution of road-killed individuals was clustered and mostly located in the western part of the road (Fig. 4). The nearest neighbor ratio did not exceed 0.63 and 0.53 for the surveyed and road-killed individuals, respectively, in the four years survey, suggesting that their distribution along this road stretch was clustered (Table 3). There was relatively high correlation ( $r^2 = 0.56$ ) between the location of the live-surveyed and road-killed individuals (Fig. 5).

Roadside vegetation is unlikely to be a cause of clustering because it was homogeneous throughout this stretch of road, with the sides of the road cleared of vegetation and then forested both north and south of the road. We made an analyses to find a significant relationship to the slope near the road, but failed to find any such relationship.

#### 4. Discussion

#### 4.1. Estimating the danger of road-kill, dispersal and fragmentation

Roads are known to be a dominant factor affecting amphibians, including high mortality risk for individuals trying to cross the roads (Fahrig & Rytwinski, 2009; Garriga et al., 2012), or avoidance of roads that restricts dispersal and migration (Ray, Lehmann, & Joly, 2002), particularly when restricted dispersal affects the metapopulation dynamics (Pittman, Osbourn, & Semlitsch, 2014). The steady and significant increase in the probability of road-kill over time, reaching over 10 % of the population by the end of this study, in parallel of the increase in traffic, supports our assumption that the road and increased traffic might negatively affect the salamander population.

Only a very small number of individuals were found on both sides of the road, and individuals tend to remain in the same locations between years. These findings support the hypothesis of road avoidance, but may reflect low movements of Fire Salamanders in general. Originally, it was thought that the Fire Salamander lived in discrete, isolated populations with little movement (Blaustein et al., 1994; Degani, 1996). However, like other amphibian species (Schmidt, Schaub, & Steinfartz, 2007; Smith & Green, 2005), evidence indicates that *S. infraimmaculata* can migrate longer-distances than thought before, and that subpopulations at various breeding sites are linked to varying degrees as metapopulations (Segev et al., 2010). Bar-David et al. (2007) showed that Fire Salamanders in the Carmel have moved up to 1.3 km between capturing points. However, our study indicated that in the vicinity of the road most of the movement of individuals was relatively short. This might be explained by the fact that in this study we documented the last stage of movement from the hibernation areas to the breeding site. Around the breeding site behavior is probably switching from movement to mating behavior. The longest distances between captures was associated with females, but only five recaptured female were found in the study (from 32 individuals captured more than twice). This phenomenon of a low ratio of females to males is known for Fire Salamanders, but it is also associated with the season in which individuals are examined and the males' behavior that tends to be more active in order to find females for reproduction (Degani, 1996; Segev et al., 2010). Within the limits of a four-year study, it seems that the combination of increasing road-kill and limited dispersal, particularly across the road, can lead to population fragmentation in the future.

### 4.2. Distribution maps

Maps of distribution are important for developing management programs to protect endangered species (Gaston & Williams, 1996). The distribution of the captured individuals and clustered and the highly dense areas located in several areas both in the Dishon river and along the road. Similarly, the distribution of road-killed individuals was clustered and mostly located in the western part of the road. We suspect that this is due to a permanent spring that is located north to the road on the west part of the study area. Because of the relatively high correlation between the number of road-killed and live individuals present along the road, such a relationship can be used to allocate safe passes that can facilitate the movement of individual across the road from their hibernation areas to the breeding sites while minimizing road-kills (Blaustein et al., 2017).

#### 5. Conclusion

While longer studies are needed in order to fully understand the demographic dynamics in this site, it is clear from our work that dozens of individuals, of this endangered species, are run over each year and there is a relatively low level of movement between both sides of the road. These effects on the population can be ameliorated by constructing road passages and fencing walls to keep individuals off the road and direct them to the tunnel. Experiments conducted with amphibians have found that underground passages characteristics (e.g., width, substrate type, and length) affect amphibian's use of underpasses (Bain, Cook, & Girman, 2017). However, studies highlight the fact that there is no unique solution, and underpasses characteristic need to be species-specific in order to function effectively (Woltz, Gibbs, & Ducey, 2008). In addition, we used geostatistics to identify areas along the road that are preferred by the species and thus might be the focus of such solution.

# **Declaration of Competing Interest**

The authors report no declarations of interest.

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### Table A1

The longest distance between recaptures.  $M=\mbox{male};\ F=\mbox{female};\ U.$   $I=\mbox{unidentified}.$ 

The longest distance (m)	Sex	Salamander ID
50.9	М	1
79.3	М	1
44.3	М	2
41.5	Μ	5
59.1	Μ	14
20.6	М	14
58.5	М	15
65.6	М	16
36.5	М	16
35.6	М	17
196.4	М	18
103.7	М	19
58.3	М	19
47.3	М	19
77	М	20
121.1	Μ	20
30.1	Μ	20
25.9	Μ	419
94.4	Μ	419
24.1	Μ	420
191.4	Μ	443
186.8	Μ	447
37.7	F	455
95	М	459
77	М	459
88.8	М	462
251.9	М	471
98.7	Μ	476
83.5	U.I	514
76.7	М	517
339	F	517
78.3	М	518
431.1	F	521
44.8	Μ	537
209.9	М	565
240.6	М	574
204.3	М	574
13.7	М	580
50.5	М	665
47.1	М	721
58.2	М	722
116.5	М	722
211.6	М	737
61.9	F	738
37.6	F	787

University permit number 033\_b9947\_6. To the Nature and Parks Authority in Israel for budget helping.

# Appendix A

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