

Urban conglomerates and Vertebrate roadkill on the verge of a Natura 2000 site

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ABSTRACT

Roadkill is one of the most striking ecological impact of the roads, that can have direct consequences on animal populations and sometimes pose danger to people. The paper gives the results of a 4-year monitoring campaign of vertebrate roadkill along a two-lane municipal road in the Eastern Po Valley. The road connects the city of Ferrara (Italy) with the border of the Natura 2000 site IT4060016, crossing arable lands, orchards, and residential villages. Mean mortality has been of 10.5 ind./km/year, like previous results from a similar road in the same landscape. Mammal mortality, with 4.1 ind./km/year, has resulted 27 times higher than on similar roads in the Western Po Valley. The most affected taxa have been the hedgehog *Erinaceus europaeus*, toads (*Bufo balearicus* and *Bufo bufo*), sparrows (*Passer italiae* and *Passer* sp.), Columbidae, *Athene noctua* and unidentified birds. Traffic volume was inversely correlated with mortality: traffic decreased from the city towards the Natura 2000 site while mortality increased. The vertebrate community close to the protected area was clearly distinct. Mortality has not changed among years but has changed among seasons, peaking in July both for number of killed animals and for number of involved taxa but also showing other peaks in early spring and autumn. Some particularly dangerous road stretches have been identified, where solutions for roadkill mitigation may be adopted. Climate warming can change the timing of reproductive migration, changing the exposure risk to roadkill.

Key words: Roadkill, Eastern Po Valley, *Erinaceus europaeus*, amphibians, *Passer italiae*.

RIASSUNTO

Conglomerati urbani e mortalità stradale dei Vertebrati in prossimità di un sito Natura 2000

La mortalità stradale degli animali è uno degli impatti ecologici più eclatanti delle strade, che può avere conseguenze dirette sulle popolazioni animali e rappresentare un rischio per le persone. L'articolo riporta i risultati di 4 anni di monitoraggio della mortalità stradale dei vertebrati lungo una strada comunale a due corsie senza barriera centrale nella Pianura Padana orientale. La strada collega la città di Ferrara (Italia) al confine del sito Natura 2000 IT4060016, attraversando seminativi, frutteti e villaggi residenziali. La mortalità media è stata di 10,5 ind./km/anno, come i risultati precedenti di una strada simile nello stesso paesaggio. La mortalità dei mammiferi, con 4,1 ind./km/anno, è risultata 27 volte superiore rispetto a strade simili nella Pianura Padana occidentale. I taxa più colpiti sono stati il riccio *Erinaceus europaeus*, i rospi (*Bufo balearicus* e *Bufo bufo*), i passerii (*Passer italiae* e *Passer* sp.), i colombidi, *Athene noctua* e uccelli non identificati. Il volume del traffico è risultato inversamente correlato alla mortalità: il traffico è diminuito dalla città verso il sito Natura 2000 mentre la mortalità è aumentata. La comunità di vertebrati vicina all'area protetta era chiaramente diversa. La mortalità non è cambiata tra gli anni ma è cambiata tra le stagioni, con un picco a luglio sia per numero di animali uccisi che per numero di taxa coinvolti, ma mostrando anche altri picchi all'inizio della primavera e dell'autunno. Sono stati individuati alcuni tratti stradali particolarmente pericolosi, dove potrebbero essere adottate misure di mitigazione del roadkill. Il riscaldamento climatico può cambiare i tempi delle migrazioni riproduttive, modificando il rischio di esposizione al roadkill.

Parole chiave: Mortalità stradale, Pianura Padana orientale, *Erinaceus europaeus*, anfibi, *Passer italiae*.

INTRODUCTION

Road ecology is a recent science that studies the impacts of roads on the environment. It is generally recognized that the discipline was born in 1998, when Forman first mentioned the term in his seminal paper. Roads are the largest human artifact on the planet (FORMAN *et al.*, 2003). They impact the ecosystems in many different ways: roads seal the soil, strongly modifying the hydrological cycle, reducing infiltration, evapotranspiration and groundwater recharge and increasing runoff

(EFTENE *et al.*, 2016), impeding the passage of air, polluting the environment through the substances released by the materials used to run the vehicles and to construct the roads itself (TIAN *et al.*, 2020), creating noise and light pollution (BOSIA *et al.*, 2003; SPALDING, 2019), polluting freshwaters and soil through the deicers used in cold countries in winter to prevent ice (TERRY *et al.*, 2019), creating barriers to the movements of the animals (SHEPARD *et al.*, 2008), favoring dispersal of alien species (JOLY *et al.*, 2011) and by roadkill.

Roadkill is one of the most striking impact of roads, that

can have direct consequences on animal populations. Demographic decline in fauna located near roads has occurred (JONES, 2000; MUMME *et al.*, 2000; GIBBS & SHRIVER, 2005; ROW *et al.*, 2007), even for common species that have, a priori, populations large enough to sustain high road mortality (FORMAN & ALEXANDER, 1998). The phenomenon has received public and scientific attention since the advent of the motor vehicles, at the beginning of the 20th century (STONER, 1925; BARNES, 1936), and now dead animals along roads are a common sight. Nevertheless, roadkill is generally overlooked by people using roads, unless it involves large animals such as deer, wolf, badger and so on, that can pose serious risks for drivers, too. Indeed, modern transportation has become one of the deadliest activities on the Earth, even for the humans (SEILER & HELLDIN, 2011). In Europe, about 194 million birds and 29 million mammals may be killed each year on roads (GRILLO *et al.*, 2020).

In Italy, a few projects have been carried out to specifically monitor roadkill, despite the fact that in the country most goods and passengers travel by road (UFFICIO STATISTICA M.I.T., 2016). Road accidents with damage to transport caused by wildlife represent 3% of the total number of claims (CEROFOLINI, 2006) and have an important impact on the economy, on society and biodiversity. In Italy, economic damages caused by the impact with medium-large sized animals range between 370 and 2,200 euro per vehicle (DINETTI *et al.*, 2008).

Among Italian projects, “Gufi e Strade” monitored—with citizens’ cooperation—the number of owls hit by vehicles (GALEOTTI *et al.*, 2001). The results of some monitoring programs were presented in 2008 at a meeting on road ecology (FABRIZIO, 2010). Other data can be inferred from atlases on regional or provincial distribution of species (BATTISTI *et al.*, 2012). The project “Italian Road Mortality” uses the online platform www.iNaturalist.org to record citizens’ voluntary reports of roadkill (GILIO, 2015). The same website has been adopted for the “Roadkill in Emilia-Romagna” and “Delta Road Kill” projects, that are driven by the authors of the present paper.

The first Italian wide-ranging project dealing with roadkill was “Life STRADE”, financially supported by the European Union. “Life STRADE” monitored roads in 3 regions of Central Italy (CIABÒ *et al.*, 2015) and identified places and techniques to prevent animal-vehicle collisions, with particular attention to large mammal species. “Life STRADE” evolved in the project “Life Safe Crossing” (<https://life.safe-crossing.eu/>), that takes care of four large, protected mammal species (*Ursus arctos marsicanus*, *Ursus arctos*, *Canis lupus* and *Lynx pardinus*) in four European countries, with the aim of promoting a safe use of roads both for the animals and for the people.

In the Po Valley, ALDROVANDI *et al.* (2018) were the first ones to systematically study the roadkill phenomenon along two important roads that connect the inner lowlands with the Adriatic Sea coast (Northeastern Italy). CANOVA & BALESTRERI (2018) studied mammal roadkill for fifteen years on two provincial roads of the Western lowlands. Recently, VALERIO *et al.* (2021) studied roadkill in Southern Italy.

This paper studies roadkill along a road close to a Natura 2000

site. Natura 2000 is an ecological network set in Europe since 1992 in force of the Bird EU Directive (1979, 79/409 and 2009, 09/147) and Habitat EU Directive (1992, 92/43) in order to protect biodiversity. It is a network of protected areas that are not integrally subtracted to human influence and intervention: productive activities such as agriculture or even hunting are often allowed, providing that these are made with respect to some rules. Natura 2000 sites can be crossed by high traffic roads and can be nearby or even include human settlements.

In Italy the banks of Po River, which is the main Italian river and crosses the most anthropized area of the country, are under the protection of many different Natura 2000 sites established by the different regional authorities touched by the river. In the Ferrara landscape (Emilia-Romagna region) the river marks the Northern limits of the municipality and it is protected under the Natura 2000 site IT4060016.

Through the centuries some small cities and villages have developed next to the riverbanks. In the last 40 years some of them underwent a quick urbanistic expansion, due to their attractiveness for people who work in the main cities but wish to live in a more peaceful and natural landscape.

Typically, in Italy the municipalities enjoy managing urban and spatial planning without being subject to almost any higher strategic supervision (ROMANO *et al.*, 2019): urban development often follows a sprinkling pattern (ROMANO *et al.*, 2017), with many sparse buildings that lead to over-urbanization and highly patchy, energy-intensive urban patterns that are destructive for ecosystems with bad environmental and urban quality.

Between 1980 and 1990 urbanized land-take in the province of Ferrara proceeded at the speed of about 1 hectare/day (ROMANO *et al.*, 2020); although it has slowed down, the process is still present. In the municipality of Ferrara, land uptake in 2018-2019 for exclusive residential use was of 0.02 ha/day (MUNICIPALITY OF FERRARA, 2021).

Increased anthropic presence close to the protected areas can be a threat to wildlife: we compared wildlife mortality by roadkill along a gradient of different urbanization, going from a village grown in contact with the main city to an ancient village originated in the 10th century and bordering the Natura 2000 site. In the past 40 years, the villages have undergone an increment in the number of inhabitants and almost doubled their urbanized surface.

The aim of the work was to identify the species most affected by roadkill, in the hypothesis that the phenomenon could affect protected species, and to identify the most dangerous stretches of the road where mitigation measures should be adopted.

MATERIALS AND METHODS

Study area

The study involved a 6.78 km municipal road in the Eastern Po Valley, with a constant width of 6 m and two lanes without central barriers. The road, named “Via dei Calzolari”, connects the Northeastern residential neighborhoods of the city of Fer-

rara (Emilia-Romagna, Italy) to the raised right bank of the Po River. The bank and the floodplain are protected under the EU Directive “Habitat” (97/43 CEE) coded as IT4060016 Natura 2000 site “Po da Stellata a Mesola e Cavo Napoleonico”.

Via dei Calzolari is an ancient road: it was first mapped with its present shape in the so called “Napoleonic map” drawn by the Austrian army in 1814, but it is possible to find its traces even in maps of the XVI and XVIII centuries (GEOPORTALE DELLA REGIONE EMILIA-ROMAGNA, downloaded on 2020/07/28).

The road crosses two residential villages with parks and gardens: the first one is close to the main city and hosts about 4500 inhabitants whilst the second one, with about 2000 inhabitants, is set on the opposite side, laying at the foot of the raised riverbank.

The two villages and the suburban parts of the road are surrounded by agricultural fields cultivated with wheat, orchards, corn, soy, and forage and with rare and scattered farm buildings. A bike lane runs alongside the road for all its length.

Truck traffic is allowed only in particular cases: the largest vehicles normally running across are the rare buses that serve the city. The river embankment hosts a bike path and it is generally forbidden to motor vehicles.

The Natura 2000 site IT4060016 is 3,240 ha large, including about 120 km of freshwater and riparian ecosystems. The site hosts species of European or regional interest: 5 species of bats, 21 bird species in Annex 1 of Bird directive, the reptiles *Emys orbicularis*, *Elaphe longissima*, the amphibians *Triturus carnifex*, *Bufo viridis* complex, *Hyla intermedia*, *Pelophylax esculentus* (REGIONE EMILIA-ROMAGNA, 2019). We directly also assessed the presence of *Bufo bufo* and *Rana dalmatina* among amphibians, *Lacerta bilineata* and *Hierophis viridiflavus* among reptiles.

Collection of data

From 26 March 2016 to 31 December 2019 Via dei Calzolari was monitored almost each day for Vertebrate roadkill, with a few interruptions in summer. The exact number of observational days per year is reported in Tab. 2. The animals were spotted while driving a car at a speed below the limits and then reached a few hours later with the bike lane to keep the observer safe. By doing so it has been often possible to collect photographs of the dead animals, useful for the proper identification of the species and the exact coordinates of the investment points.

Geolocated photographic observations were collected with a Samsung J5 smartphone and/or with a Sony DSCHX400VBC GPS camera. All the observations, with or without photographs, were uploaded to the web site www.inaturalist.org as part of the “Delta Road Kill” citizen science project: the project collects observations of roadkill animals (Vertebrate) from the territory of the Po River delta, creating a useful database to geolocate the processing of observations and taking advantage of the validation mechanism of iNaturalist. For some species (*Passer italiae* and *Bufo bufo*), which are considered “vulnerable to extinction” (BIRDLIFE INTERNATIONAL, 2018; ANDREONE *et al.*, 2013), a supplementary control of

the exact location of the observations was necessary since the website automatically obscures the coordinates of internationally protected or vulnerable species. Citizens’ observations on iNaturalist were used to describe the specific pool of vertebrates living in the surrounding of the road, in an about 2 km wide buffer.

The database in csv format was downloaded from the iNaturalist website and processed with Google Earth© and the QGIS software, Las Palmas release (QGIS DEVELOPMENT TEAM, 2017) to extract the observations made in Via dei Calzolari.

Taking into account the main infrastructural crossings (4 roads and a channel) that connect Via dei Calzolari to other suburban areas and potentially influence the total volume of traffic, we established 5 milestones that divided the road in 4 different sectors (Fig. 1) with different lengths and different environmental and structural conditions (Tab. 1). Every characterizing factor was standardized according with the length in meters of each sector. In that way, we had 4 statistical treatments to test for the main environmental and structural factors we supposed can influence roadkill.

The environmental and structural factors we measured are listed in Tab. 1. Since different conditions might occur on the left and on the right side of the roads, each variable was calculated adding up the values measured on both sides and then standardizing them according with the sector length in meters. Apart from two bridges above channels, the road has no underpasses.

According with ALDROVANDI *et al.* (2018), an important determinant of the deaths in the Eastern Po Valley are the habitats strictly adhering with the road. Thus, we characterized the different sectors of Via dei Calzolari by concentrating on the differential land use around the road (man-made areas with residential buildings and gardens, forests, channel surfaces, orchards, parks, arable lands, in square meters) within a 100m-wide buffer on both sides of the road and without considering other larger buffers. Our choice was supported by BUENO *et al.* (2015) who observed that a buffer model with enlarging radius was not significant to explain vertebrate mortality on roads, while habitats and matrix were.

The shape files of the land usage and of the massive built around the road were downloaded from the official topographic database of the Emilia-Romagna Region (<https://geoportale.regione.emilia-romagna.it/approfondimenti/database-topografico-regionale>) and processed with QGIS software, release 3.16.11.

Traffic was measured in 4 recording points and in 6 different days, equally divided among May and July 2018, that is before and after the summer closure of Italian schools, in the hypothesis that traffic could sensitively diminish after school closures. Traffic was measured separately for each lane but, since Via dei Calzolari is not wide and it does not have central Jersey barriers, we did not record dead animals separately for each lane: therefore, we pooled traffic data to and from the main city and in that way we got 12 replicates of traffic counts for each sector. Traffic was recorded on 6 working days from 8.20 to 10.55 AM. Each count lasted for 15 minutes and give

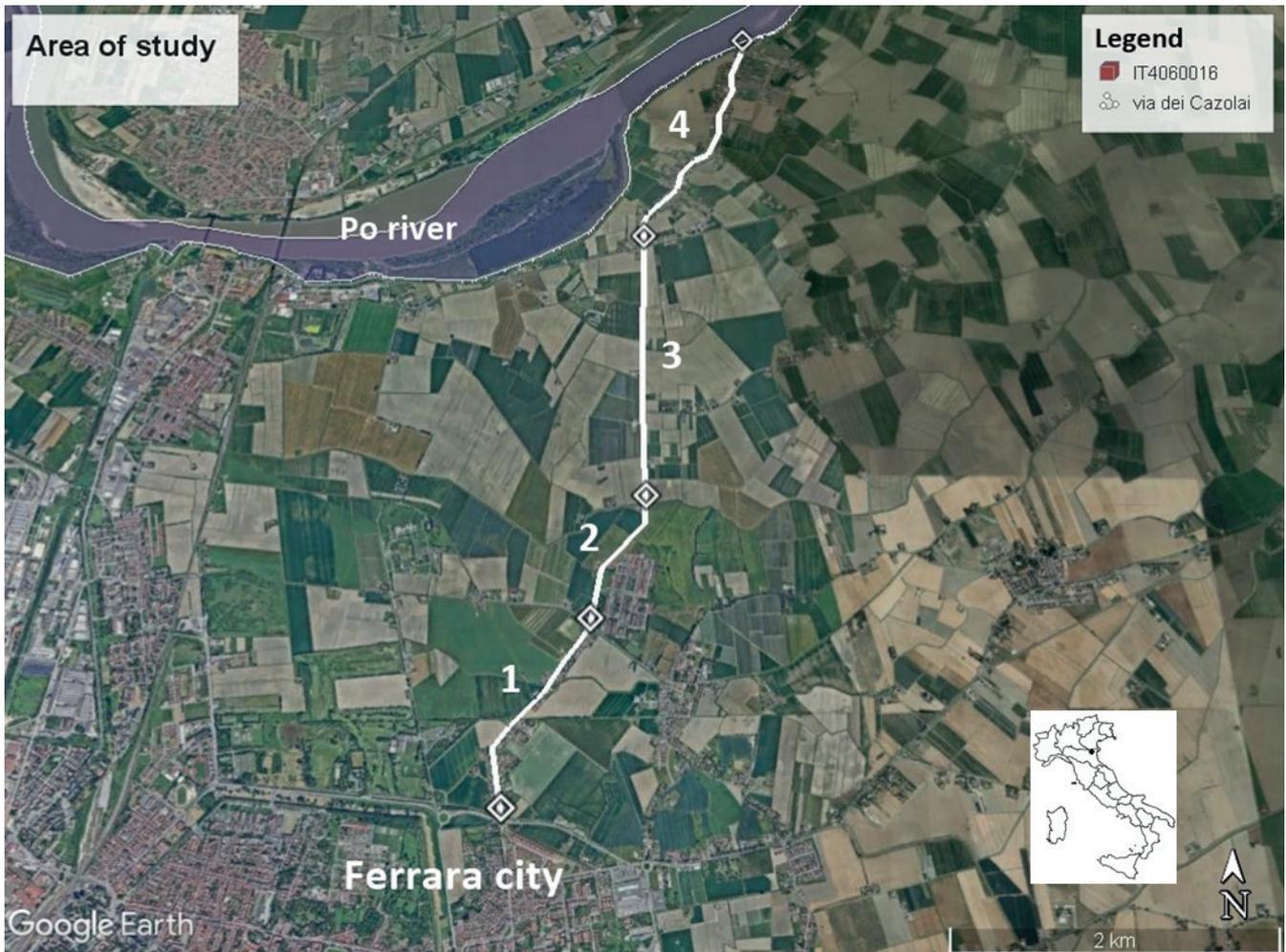


Fig. 1. Area of the study.

us an indication about the busiest sectors all along the day. By means of the QGIS heat-maps function we also detected the main crossing points used by the animals, collecting useful information to define roadkill mitigation strategies.

Statistical analyses

Roadkill counts were standardized per day and per km to compensate for differences in the total duration of monitoring and in the length of the road sectors. Traffic was expressed as the number of vehicles in one minute.

To detect significative trends in traffic intensity along the road and in roadkill events among years, months and sectors, counts were analyzed respectively with one-way (traffic) and two-way without replicates (roadkill) ANOVA (alfa = 0.05). We tested those taxa that totalized at least 3 casualties, the standardized number of taxa involved and the total standard mortality. Two-way ANOVA was also adopted to test for possible effects of summer school closures on traffic intensities. The analyses were performed with a MS Excel software extension. A correspondence analysis was performed with MVSP Statistical package (KOVAC, 2003) to highlight different structural

and environmental characteristics of the 4 road sectors that could be related to the roadkill phenomenon. All the analyzed predictors were free from collinearity.

Finally, since roadkill observation can be considered a way to sample Vertebrate communities in anthropized areas (CANOVA & BALESTRIERI, 2018; SCHWARZ *et al.*, 2020), we performed a cluster analysis based upon the standardized year counts, the Jaccard's coefficient and the UPGMA clustering algorithm, to analyze the Vertebrates communities settled in the different areas crossed by the road.

RESULTS

In 3.6 years of monitoring (1306 days) we found a total of 256 dead animals (Tab. 2, Fig. 2): 101 birds, 99 mammals, 40 amphibians, 8 reptiles, 2 specimens belonging to amphibia or reptiles and 6 vertebrates that was impossible to identify more in detail. Out of 66 animals which could not be identified at least to the genus level, almost the half (30) were unidentified birds. Via dei Calzolari proved to be lethal for at least 10.5 ± 0.9 Vertebrates per kilometer per year: 1.6 amphibians, 4.1 mammals

	Sector 1	Sector 2	Sector 3	Sector 4
Length (m)	1.480	1.000	2.140	2.160
Raised/m	0.00	1.70	1.73	0.00
N. crossing channels/m	0.01	0.02	0.01	0.00
N. crossing roads/m	0.03	0.02	0.01	0.08
Guardrail/m	0.06	0.39	0.01	0.00
Fences/m	1.86	0.58	0.91	1.67
Tree-lined/m	0.94	1.51	1.00	0.43
Parallel Hedgerows/m	0.00	0.22	0.00	0.00
Parallel Ditches/m	0.45	0.83	1.68	0.13
Parallel Channels/m	0.02	0.02	0.01	0.00
Man-made areas in buffer, m ² /m	114.98	50.67	21.07	154.99
Number of buildings in a 150 m buffer/m	0.11	0.09	0.02	0.24
Forests in buffer, m ² /m	0.00	4.60	0.00	1.85
Channel surfaces in buffer, m ² /m	1.13	1.80	0.84	0.00
Orchards in buffer, m ² /m	0.00	0.00	33.01	0.00
Parks in buffer, m ² /m	0.00	36.00	0.00	1.85
Arable lands in buffer, m ² /m	83.90	106.93	145.08	41.32
Speed 30 km/h per m	0.00	0.15	0.00	0.00
Speed 50 km/h per m	1.00	0.85	0.00	1.00
Speed 70 km/h per m	0.00	0.00	1.00	0.00

Tab. 1 – The different environmental and structural parameters collected to describe the four sectors of Via dei Calzolari.

and 4.2 birds, 0.3 reptiles and 0.3 unidentified Vertebrates. Citizen science data on inaturalist.org suggested that the vertebrate species pool around the road is made up by 7 amphibians, 7 reptiles, 7 mammals (not considering very small mammals such as Soricidae and bats, not easily spotted in the wild and never found among roadkill on our road), and 46 birds. Roadkill affected 100% of the mammal species living in the surrounding, 60% of the amphibians, 70% of the reptiles and 30% of the birds.

The most affected taxa were the hedgehog *Erinaceus europaeus* (74 casualties) and unidentified Aves (30 casualties). Birds involved were above all small-medium sized species living in gardens and urban parks, with a 15% of Columbidae species. The third affected taxon is the Balearic green toad *Bufo balearicus* (23 victims), to which we must add the common toad *Bufo bufo* (7) and unidentified Bufonidae (6). High mortality also struck Passeridae birds (17): *Passer italiae* was involved for sure in 7 casualties, while other 10 observations were assessed at the genus or family level. Bird diversity involved 14 species. Roadkill affected some protected or vulnerable species (Tab. 2): among Amphibians and Reptiles, the balearic green toad *Bufo-*

tes balearicus and the common toad *Bufo bufo*, the green whip snake *Hierophis viridiflavus*, the Western green lizard *Lacerta bilineata*, the barred grass snake *Natrix helvetica*, the common wall lizard *Podarcis muralis*; among mammals, the common hedgehog *Erinaceus europaeus* and, among birds, the little owl *Athene noctua* and the European green woodpecker *Picus viridis*. We underline that *Passer italiae*, considered to be a vulnerable species at the global level according with IUCN Red List criteria (BIRDLIFE INTERNATIONAL, 2018) and a near threatened species in Italy (GUSTIN *et al.*, 2019), still has no law protection.

Some alien and domesticated species were involved: bull frog *Lithobates catesbeianus*, pheasant *Phasianus colchicus*, cat *Felis catus*, coypu *Myocastor coypus*, rats *Rattus norvegicus* and *Rattus sp.* and pond slider *Trachemys scripta*.

Traffic from the main city to the Natura 2000 site significantly decreased ($p=0.0006$) (Tab. 3 and 4, Fig. 3a), going from an average of 4.6 vehicles per minute to 2.1 vehicles/min. However, traffic is rather similar in sector 2 and 3. ANOVA suggested a reduction trend in traffic after school closures ($p=0.12$, Fig. 3b). The effect was evident enough in sector 2 and 3 ($p=0.17$ and $p=0.14$), while no difference emerged in sectors 1 and 4.

Tab. 5 shows significant results in mortality. Taxa with less than 4 individuals never showed significant differences, so we omitted them from the table. Differential mortality was statistically ascertained among the different road sectors (Tab. 5

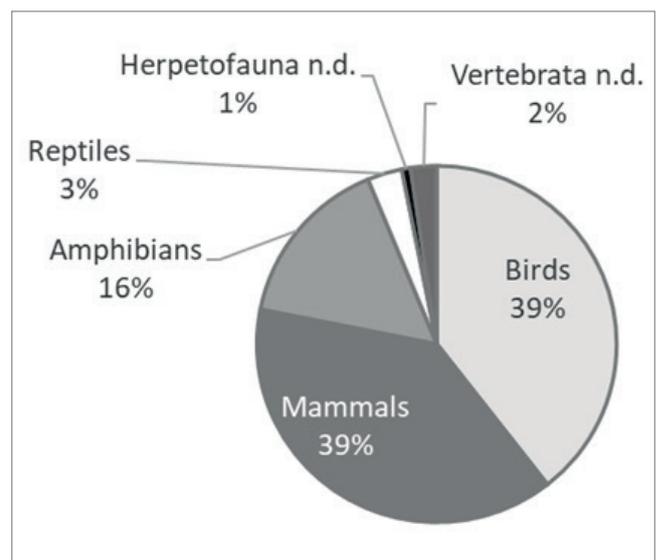


Fig. 2. Relative abundances of roadkill classes.

Roadkill monitoring days/year						
2016	2017	2018	2019			
260	344	365	337			
Vertebrate Class	Lower taxon	TOTAL	Roadkill/km/year n=4	IUCN Italian red list	Protection	
A	<i>Amphibia n.d.</i>	1	0.04±0.08			
A	<i>Bufo bufo</i>	7	0.29±0.20	VU	Annex 3 Bern convention, R.L. 2006/15	
A	Bufonidae n.d.	7	0.27±0.27			
A	<i>Bufo balearicus</i>	23	0.94±0.47	LC	Annex 2 Bern convention, Annex IV dir. Habitat 92/43/CEE, R.L. 2006/15	
A	<i>Lithobates catesbeianus</i>	1	0.04±0.08	-	-	
A	<i>Pelophylax sp.</i>	1	0.04±0.07	-	-	
	Amphibians tot.	40	1.62±0.36			
B	<i>Athene noctua</i>	6	0.25±0.08	LC	Italian law 92/157, Annex 2 Bern convention	
B	Aves n.d.	30	1.23±0.26			
B	<i>Columba livia domestica</i>	9	0.39±0.20	-	-	
B	<i>Columba palumbus</i>	2	0.08±0.09	LC	Annex 2 Bird Directive 2009/147/CE	
B	Columbidae n.d.	4	0.15±0.21			
B	Corvidae n.d.	1	0.04±0.08			
B	<i>Erithacus rubecula</i>	1	0.04±0.08	LC	Annex 2 Bern convention	
B	<i>Gallinula chloropus</i>	3	0.12±0.15	LC	Annex 2 Bird Directive 2009/147/CE, Annex 3 Bern convention,	
B	<i>Garrulus glandarius</i>	3	0.12±0.15	LC	Annex 2 Bird Directive 2009/147/CE,	
B	<i>Passer italiae</i>	7	0.27±0.25	NT	Annex 3 Bern convention, Italian law 92/157	
B	<i>Passer sp.</i>	5	0.19±0.15			
B	Passeridae n.d.	5	0.21±0.08			
B	Passeriformes	2	0.08±0.09			
B	<i>Phasianus colchicus</i>	1	0.04±0.08	-	Annex 2 Bird Directive 2009/147/CE	
B	<i>Pica pica</i>	8	0.34±0.31	LC	Annex 2 Bird Directive 2009/147/CE	
B	<i>Picus viridis</i>	3	0.11±0.14	LC	Annex 2 Bern convention, Italian law 92/157	
B	<i>Streptopelia decaocto</i>	8	0.36±0.32	LC	Annex 3 Bern convention, Annex 2 Bird Directive 2009/147/CE	
B	<i>Sturnus vulgaris</i>	1	0.04±0.08	LC	Annex 2 Bird Directive 2009/147/CE	
B	<i>Turdus merula</i>	2	0.08±0.09	LC	Annex 3 Bern convention, Annex 2 Bird Directive 2009/147/CE	
	Birds tot.	101	4.16±0.50			
M	<i>Erinaceus europaeus</i>	74	3.10±0.91	LC	Annex 3 Bern convention, Italian law 157/92	
M	<i>Felis catus</i>	1	0.04±0.07	-	-	
M	Leporidae n.d.	1	0.04±0.08			
M	Mammalia n.d.	6	0.24±0.25			
M	<i>Myocastor coypus</i>	3	0.13±0.16	-	-	

M	<i>Rattus norvegicus</i>	8	0.36±0.32	-	-
M	<i>Rattus</i> sp.	3	0.12±0.15	-	-
M	Rodentia n.d.	1	0.04±0.07		
M	<i>Talpa</i> sp.	1	0.04±0.08	LC/DD	-
M	<i>Vulpes vulpes</i>	1	0.04±0.08	LC	-
	Mammals tot.	99	4.14±0.80		
R	<i>Hierophis viridiflavus</i>	2	0.08±0.09	LC	Annex II Bern convention, Annex IV dir. Habitat 92/43/CEE, R.L. 2006/15).
R	<i>Lacerta bilineata</i>	1	0.04±0.07	LC	Annex II Bern convention, Annex IV dir. Habitat 92/43/CEE, R.L. 2006/15
R	<i>Natrix helvetica</i>	1	0.04±0.07	LC	R.L. 2006/15
R	<i>Podarcis muralis</i>	3	0.11±0.14	LC	Annex II Bern convention, Annex IV dir. Habitat 92/43/CEE, R.L. 2006/15
R	<i>Trachemys scripta</i>	1	0.04±0.07	-	-
	Reptiles tot.	8	0.30±0.42		
	Herpetofauna n.d.	2	0.08±0.09		
	Vertebrata n.d.	6	0.23±0.19		
	Total roadkill	256	10.53±0.89		

Tab. 2 – Monitoring days per year and total numbers of roadkills. A: amphibians, M: mammals, R: reptiles, B: birds.

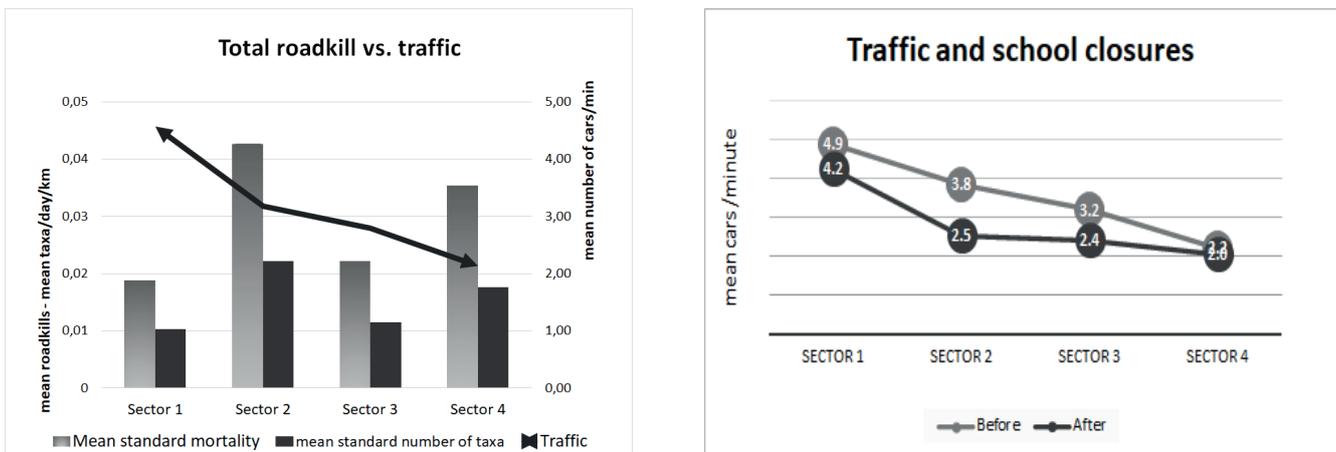


Fig. 3 – Traffic data. 3a: overall standard mortality and number of roadkilled taxa vs. traffic. 3b: trend of traffic before and after summer school closures.

and Fig. 3a), both for number of individuals and for number of taxa, but no significant difference was found among years.

In the hypothesis that high road mortality could influence the hedgehog population, the most affected species, we also tested its mortality among years with Spearman and Pearson coefficients, but we did not obtain consistent results. Analogous tests performed on other taxa and on total mortality gave similar results, so nothing we can say about the impact of roadkill on populations. Anyway, we suspect that the local population of *Athene noctua* in sector 3 was extinguished (6 victims). Roadkill significantly fluctuated while approaching the Natura

2000 riverbank: dead animals were a lot in sector 2 and 4, while mortality was lower in sector 1 and 3 (Fig. 3a, Tab. 3). In the same way, the number of involved taxa significantly fluctuated among sectors ($0.002 < p < 0.044$), being sectors 2 and 4 the richest in biodiversity.

Well sounded differences in mortality among sectors (Fig. 4, Tab. 5) were confirmed for toads (*Bufo balearicus*, *Bufo bufo*, *Bufo* n.d), sparrows (*Passer* sp., *Passer italiae* and *Passeridae* n.d.), rats (genus *Rattus* as a whole and *Rattus norvegicus*), and the little owl *Athene noctua*. *Bufo bufo* and *Passer italiae* were victims exclusively in sector 4. The little owl was

	Starting milestone (towards main city)	Ending milestone (towards riverbank)	Length km	Total deaths	Number of affected taxa	Deaths/km/year	Taxa/km/year	Average traffic/min n=12	Average May traffic/min n=6	Average July traffic/min n=6
Sector 1	44°50'55" N 11°38'19" E	44°51'37" N 11°38'43" E	1.48	36	12	6.8 ± 0.7	3.8 ± 0.8	4.6 ± 1.9	5.0 ± 2.2	4.2 ± 2.1
Sector 2	44°51'37" N 11°38'43" E	44°52'06" N 11°39'00" E	1.00	58	20	15.6 ± 7.5	8.1 ± 3.5	3.2 ± 1.6	3.8 ± 2.0	2.5 ± 1.6
Sector 3	44°52'06" N 11°39'00" E	44°53'15" N 11°38'60" E	2.14	61	18	8.1 ± 1.8	4.2 ± 1.1	2.8 ± 0.9	3.2 ± 1.8	2.4 ± 1.6
Sector 4	44°53'15" N 11°38'60" E	44°54'16" N 11°39'36" E	2.16	101	24	13.0 ± 1.9	6.4 ± 1.5	2.1 ± 0.4	2.2 ± 1.5	2.0 ± 1.4

Tab. 3 – The coordinates of the four road sectors, their length, the mean traffic/minute, the total and standard roadkills and number of taxa affected in each of them.

killed only in sector 3. *Aves n.d.* and *Erinaceus europaeus* statistically died in the same way in all the sectors, despite a large mortality of the hedgehog in sector 2.

Along the years (Fig. 5a) we found that deaths were at a minimum in winter, started to increase in March and April, decreased in May to recover in June and above all in July, when mortality peaked. Then, the number of victims decreased in August, as confirmed by unbiased data of 2018, it recovered in September and October to definitively crash in late autumn. The number of involved taxa (Fig. 5b) followed the same trend: the peaks of April and July were particularly rich in sectors 2 and 4. In August, sector 4 had the lowest number of individuals and taxa involved.

Fig. 6 shows the taxa significantly responsible for the described annual trend: almost all taxa peaked in July or anyway in summer, but toads (Bufonidae family, including *Bufo balearicus*, *Bufo bufo* and unidentified specimens) and *Erinaceus europaeus* had secondary peaks in early spring (March and April) and in September. For toads, the spring and September peaks are at least as important as the summer peak. Hedgehogs were spotted throughout the year except in the month of February.

Fig. 7 shows the results of the Correspondence Analysis performed on the different parameters through which we chose to characterize the road. The first two axes explained 98% of the total variability in data.

Sector 1 and sector 4, at the opposite extremities of the roads, resulted to be really similar: both are at ground level and surrounded by man-made habitats with townhouses and gardens. Maximum speed allowed is 50 km/h in both sectors and the road is not upraised. At the side of the two sectors, fences are diffusely present but there are also many “crossroads” (more abundant in sector 4: Tab. 1) made by townhouses gates opened to access the road and by small private roads reaching rural buildings among fields. The main difference between the two sectors is traffic, that we found being high in sector 1 and minimal in sector 4: sector 1 is close to the main city, while sector 4 is in touch with the riverbank and the Natura 2000 site. In both sectors there was no reduction in traffic after school closure (Tab. 4, Fig. 2).

Sector 2 is surrounded by parks with trees, bushes and meadows, forest habitats and hedgerows. Tree lines are a consistent presence on both sides of the road. A long tract allows a 30 km/h maximum car speed. Moreover, sector 2 shows a consistent presence of freshwater habitats made of channels of different width, both crossing and paralleling the road. A large part of the road in sector 2 is raised above the ground level. Traffic tended to decrease after school closures.

Sector 3 crosses arable lands and orchards with only a few rural buildings. As in sector 2, the road is upraised and it encounters very few crossroads but it is the only sector where maximum allowed car speed reaches 70 km/h. Traffic tended to decrease after school closures.

Vehicles crossing over the road often exceed the maximum speed limits, but true speeds are proportional to the limits established taking in account structural aspects of the road.

Cluster analysis showed that the roadkill community of sector 4 has always stayed different during the entire survey (Fig. 8), whilst the communities of the other three sectors of the roads mixed up.

That highlighted the different structure of the Vertebrate community inside the village closest to the Natura 2000 site: sector 4 was characterized by the exclusive presence of *Bufo bufo*, *Hierophis viridiflavus*, *Podarcis muralis*, *Natrix helvetica* and by the highest number of Bufonidae, including *Bufo balearicus*, and other unidentified herpetofauna. Among birds we found *Passer italiae*, *Passer sp.*, *Erithacus rubecula*, *Sturnus vulgaris* and the unidentified Passeridae and Corvidae.

Traffic	
Among sectors	Before/after school closures, p=
p = 0.000605	Entire road: 0.12
Sector 1	0.59
Sector 2	0.17
Sector 3	0.14
Sector 4	0.48

Tab. 4 – Traffic trends. Traffic was monitored only in 2018.

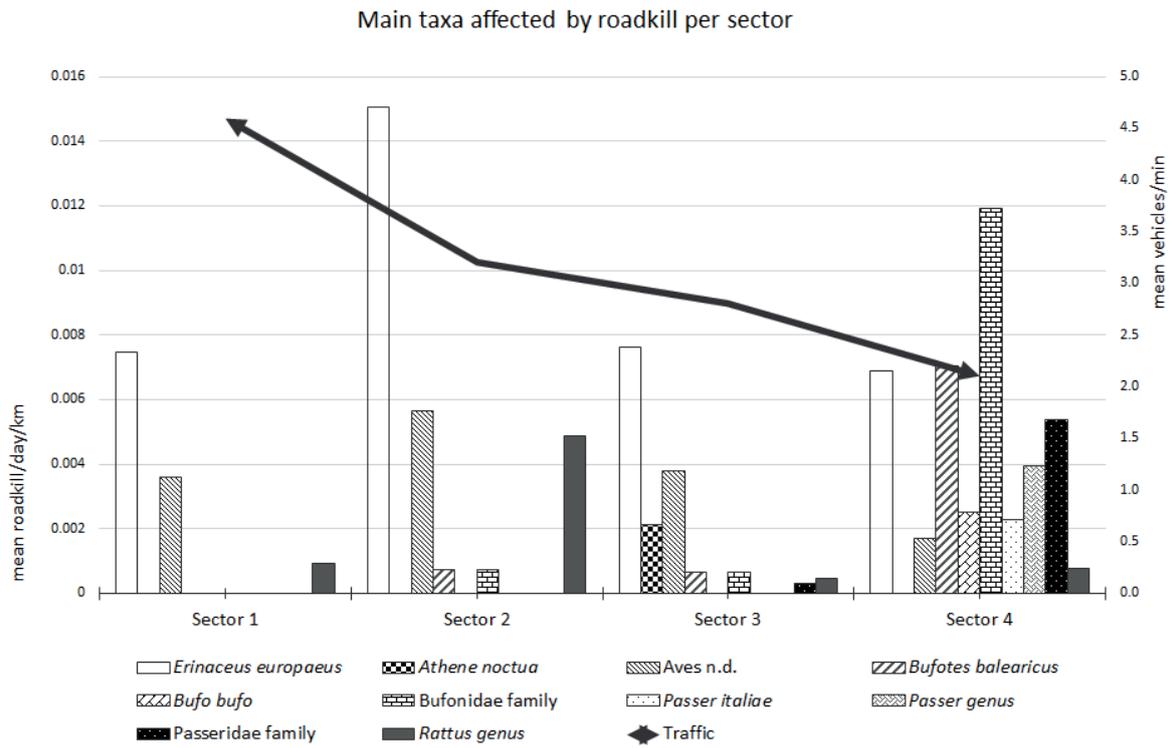


Fig. 4 – Traffic and mortality trends for the most abundant taxa.

The main hotspots for general roadkill are shown in Fig. 9: lethal points were not exactly the same among years, as found by LIMA SANTOS *et al.* (2017), indicating that wide stretch of the road must be considered for mitigation measures. The most critical stretches for hedgehogs (sector 2), toads and genus *Passer* (sector 1) were highlighted.

DISCUSSION

We found a total mortality of 10.5 ± 0.9 individuals/km/year: a similar result was found by ALDROVANDI *et al.* (2018) on a similar provincial road in the Eastern Po Valley. Via dei Calzolari, with its 4.1 ± 0.8 mammals killed per km/year, resulted 27 times more lethal for mammals than two provincial roads in the Western Po Valley (CANOVA & BALESTRIERI, 2018, 0.15 roadkill/km/year).

A complex relationship among traffic and mortality was found: as a whole, a decrease in traffic meant increase in deaths, both in number of individuals and in number of taxa involved, but intermediate traffic levels were related both to the highest mortality (sector 2) and to a moderate mortality (sector 3). Other factors must be involved: sector 2 is surrounded by hedgerows, parks and has low speed limits, while sector 3 is surrounded by arable lands and speed limit is 70 km/hour- often exceeded by drivers.

For sure sector 1, crossed over by the largest volume of ve-

hicles, had the lowest mortality, despite an environmental structure alike to the deadful sector 4, where traffic was at its minimum.

In literature, many cases of inverse correlation among traffic and mortality are reported. ALDROVANDI *et al.* (2018) proved it for two roads in the Eastern Po Valley and it has been recorded for badgers (CLARK *et al.*, 1998) and bats (ZURCHER *et al.*, 2010) in other countries. HUSBY (2016) found that birds were less prone to roadkill as the speed limit increased: high loads of fast-moving vehicles may discourage animals from attempting to cross. Passerine birds are disturbed by noise produced by vehicles and try to react going high on trees to better launch and receive calls (POLAK, 2014). In sector 1 we had heavy traffic even without fast moving vehicles, since the speed limit was 30-50 km/h, but there were often long queues of cars going to the main city: that may be enough to discourage birds both to cross and to build nests close to the road.

For our road, the traffic threshold that keeps animals far from the road must be between 3.2 and 4.6 vehicles/min.

The deaths encompassed all the seasons of the year but they were strongly reduced in the coldest months of January and February and very high in the month of July, when also the number of involved taxa increased. The increase of victims in July occurred in sectors 2 and 3, where traffic tended to decrease due to school closures, but also in sector 1 and 4,

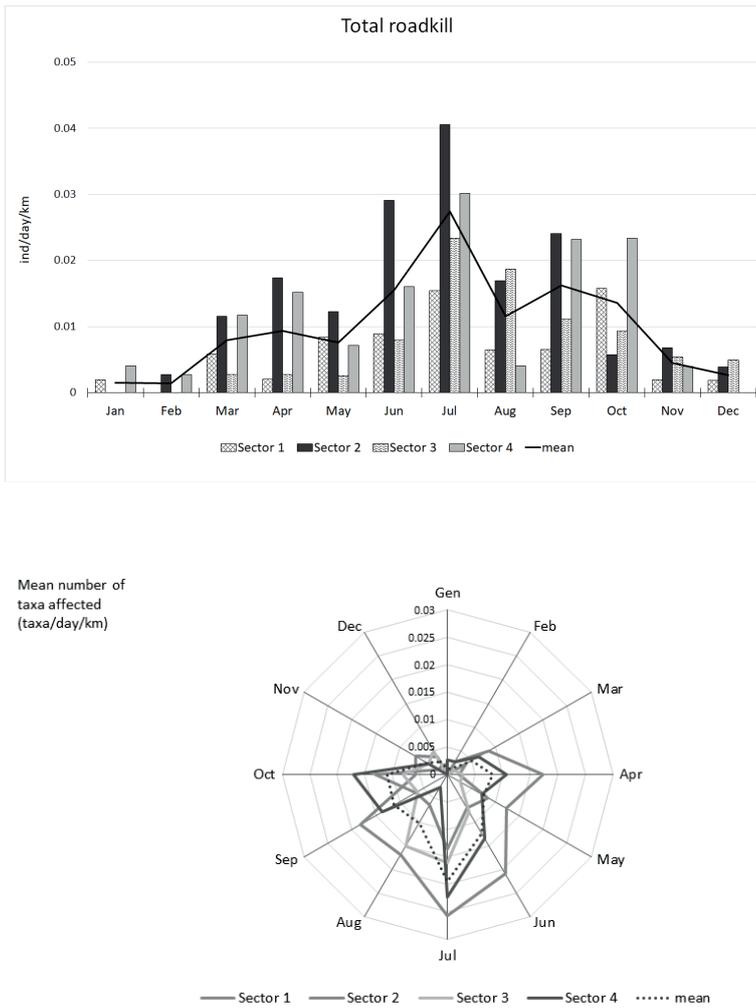


Fig. 5 – Above: total and mean mortality per month and sector (standardized). Below: mean number of taxa affected by roadkill in the different months and sectors (standardized).

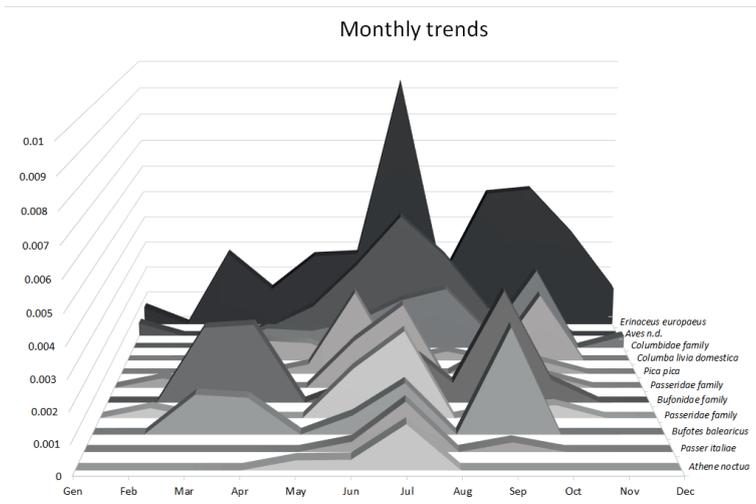


Fig. 6 - Taxa with significant monthly trends.

where traffic did not change: our results seem to relate more to a higher dispersal rate of individuals of a larger number of taxa in summer months than to the reduction in traffic volume. A slight decrease in May could be related to a rest in animal movements just after reproductive activity.

The absence of amphibians among roadkills in sector 1 points to the absence of eligible habitats in the neighborhood: as SILERO *et al.* (2019) wrote, the number of amphibians roadkills depend on the proximity to agricultural land, forests, water bodies and wetland areas, while the number of hotspots additionally depend on the proximity to urban settlements.

Roadkill in Via dei Calzolari involved 100% of the mammal species recorded in the close landscape, 70% of reptiles, 60% of amphibians and 30% of birds. In general, birds and mammals can avoid roads if these are visible from large distance in open landscapes, but their ability reduces in forested areas, where roads are less visible (BENITEZ-LOPEZ *et al.*, 2010). That applies probably to birds able to fly at high altitude and to large mammals such as deers but in our study we found only small sized bird and mammal species. Even raptors were represented only by the little owl, *Athene noctua*: raptors – unlike the majority of birds - do not avoid roads (BENITEZ-LOPEZ *et al.*, 2010), since carcasses of dead animals can be a source of food, but we believe that the only raptor species we found (a species that, in Italy, feeds itself mainly on entomofauna: ARCIDIACONO *et al.*, 2007) was killed because of the proximity of some rural buildings probably used as roosts. Moreover, the road is almost completely lined by tall *Platanus* sp. and *Celtis australis* trees: ALDROVANDI *et al.* (2018) found that bird mortality was lower just in stretches paralleled by tall trees, probably forcing birds to fly high to reach the tree crowns or above.

Via dei Calzolari is an ancient road built together with other landscape transformations more than 200 years ago. In general, the depressive effect of infrastructures on bird populations (except raptors) extends over distances up to about 1 km, and for mammal populations up to about 5 km (BENITEZ-LOPEZ *et al.*, 2010). The landscape transformations occurred together Via dei Calzolari construction have already deeply expressed their ecological impact on bird and mammal populations, excluding from victims the species most conspicuous in size.

While in the Western (CANOVA & BALESTRIERI, 2018) and in the Eastern (ALDROVANDI *et al.*, 2018) Po Valley the main victim among mammals was the alien *Myocastor coypu* (68-72%), the mammal community involved in Via dei Calzolari was largely different, since the dominating species was *Erinaceus europaeus* (75% of mammals, 29% of total victims, 3.1 ind/km/year).

Significance levels	Among Sectors	Among Years	Among Sectors	Among Months	Among Years	Among months
Degrees of freedom	3	3	3	11	3	11
Total number of roadkill	0.05	0.61	0.00	0.00	0.56	0.00
Number of Taxa***	0.04	0.42	0.00	0.00	0.47	0.00
<i>Erinaceus europaeus</i>	0.21	0.32	0.15	0.02	0.32	0.04
<i>Athene noctua</i>	0.00	0.44	0.12	0.47	0.91	0.00
<i>Bufotes balearicus</i>	0.00	0.23	0.04	0.45	0.16	0.00
<i>Bufo bufo</i>	0.01	0.44	0.01	0.47	0.61	0.20
Bufonidae n.d.	0.05	0.44	0.01	0.47	0.45	0.68
Bufonidae family	0.00	0.24	0.00	0.47	0.83	0.01
<i>Passer italiae</i>	0.03	0.44	0.14	0.47	0.25	0.00
Passeridae n.d.	0.06	0.89	0.21	0.18	0.95	0.43
<i>Passer sp.</i>	0.01	0.44	0.03	0.47	0.77	0.65
<i>Passer</i> genus	0.02	0.44	0.06	0.47	0.48	0.11
Passeridae family	0.00	0.34	0.11	0.21	0.46	0.01
Aves n.d.	0.56	0.79	0.23	0.00	0.69	0.01
<i>Columba livia domestica</i>	0.98	0.70	0.96	0.01	0.45	0.01
<i>Pica pica</i>	0.21	0.13	0.42	0.05	0.44	0.19
<i>Rattus norvegicus</i>	0.09	0.25	0.16	0.79	0.26	0.57
<i>Rattus</i> genus	0.10	0.67	0.08	0.49	0.57	0.29
Columbidae family	0.99	0.70	0.99	0.03	0.54	0.01
Mammalia n.d.	0.22	0.39	0.02	0.15	0.15	0.30

Tab. 5 – Significance values of ANOVAs performed on different variables. Significant values are in bold.

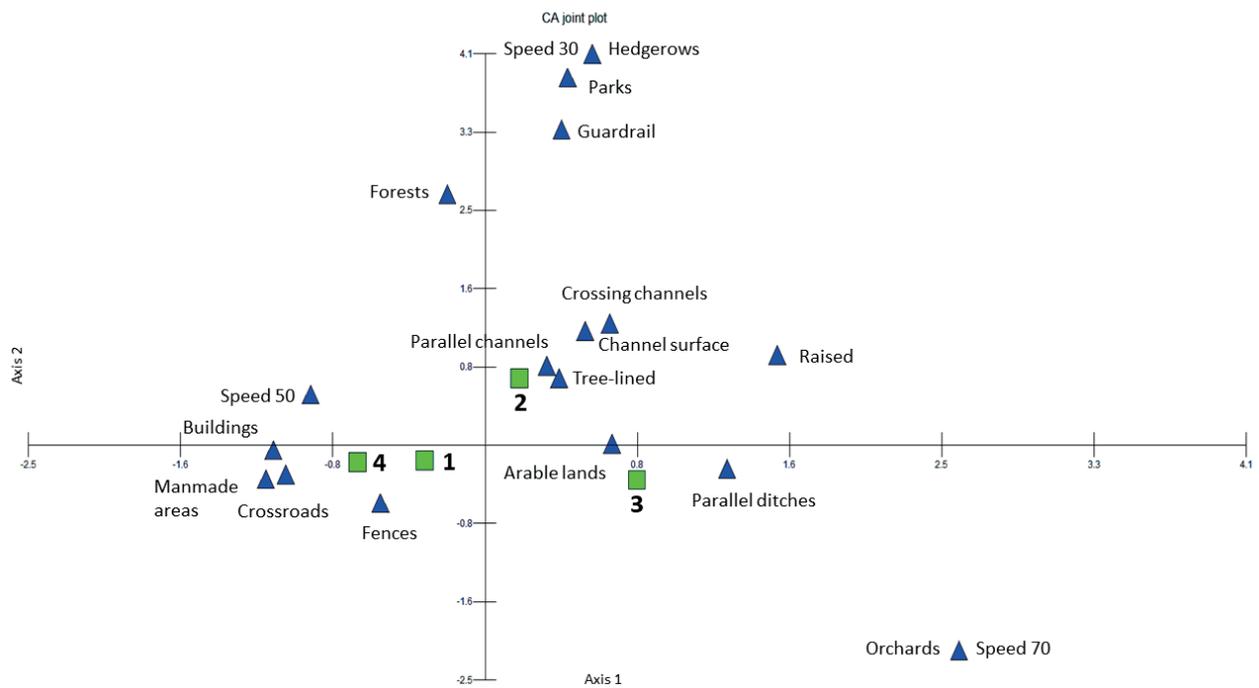


Fig. 7 - Correspondence analysis performed to characterize the four road sectors. X and y axes account for 98% of eigenvalues.

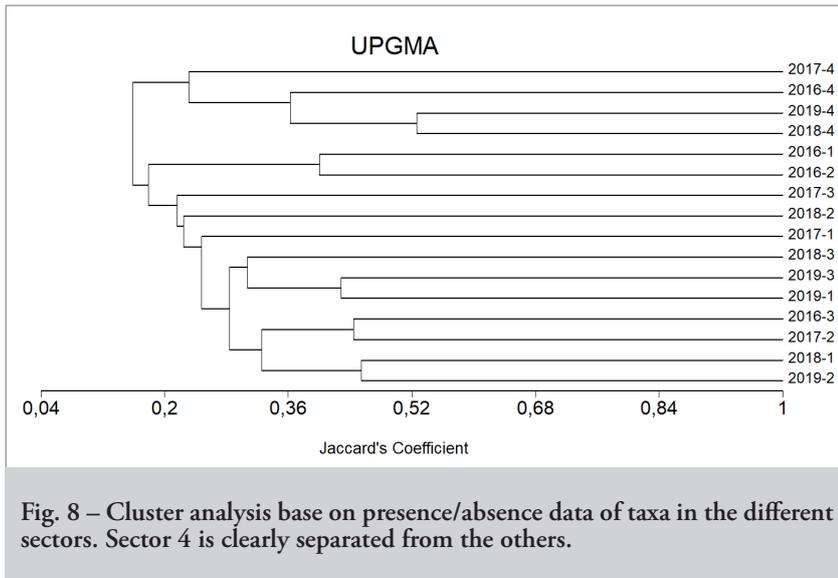


Fig. 8 – Cluster analysis base on presence/absence data of taxa in the different sectors. Sector 4 is clearly separated from the others.

Hedgehog mortality was very high, close to results found in Ukraine on national roads for *E. roumanicus* (MOORE *et al.*, 2020). *E. europaeus* roadkill was the same in all road sectors and among the years but not along the months. Its mortality peaked in July and secondarily in early autumn: we have some evidence of casualties involving juveniles for autumnal roadkills but few insights for what concerns the age of summer victims. There is no estimate of the abundance of *E. europaeus* populations in Italy (RONDININI & CAPASSO, 2013) but it is considered abundant and stable and a least concerned IUCN species; on the contrary, the ecological requirements of *E. europaeus* have been studied in detail in North-Western European countries, where its population is declining. The temporal trend of hedgehog roadkill we observed (minimum occurrence in February and maximum occurrence in July) is consistent with data and models proposed in Great Britain by WRIGHT *et al.* (2020) and with the results of Irish studies on

its mobility (HAIGH *et al.*, 2013). Hedgehogs are traditionally thought of as being a rural dwelling species, but some studies have highlighted that in some countries hedgehogs are more likely to be found in towns and cities, even with densities 9 times higher than those in agricultural ecosystems (HUBERT *et al.*, 2011; VAN DE POEL *et al.*, 2015): urban habitats may provide more refuges for hibernation, larger availability of invertebrate preys than intensively cultivated fields, and the food left available to pets. Avoiding predation by badgers or foxes may be another drive, forcing hedgehogs in urban habitats. It has been recognized long ago that *E. europaeus* has a wandering attitude (REEVE, 1986) due to the high polygamy of males, and no evidence of exclusive territoriality. They often change nesting sites during summer and hibernation sites during winter

(REEVE & MORRIS, 1985; RASMUSSEN *et al.*, 2019). The villages along Via dei Calzolari proved to be interesting shelters for the hedgehogs. Neither the presence of fences, often interrupted by the gates of the townhouses, nor the upraised embankments of some parts of Via dei Calzolari stopped its movements. The quantitative aspects of hedgerows (density, width, height, length) as well as their management are important to favor hedgehog populations (YARNELL & PETTETT, 2020). Reversely, high numbers of hedgehogs may indicate the existence of a network of adequately preserved hedges: that points to a good quality standard of the semi-rural habitats flanking Via dei Calzolari.

Even if some studies suggest that road mortality can cause significant depletions in population sizes (MOORE *et al.*, 2020), we did not find significant relationships among hedgehog yearly mortality: our results suggest that *E. europaeus* population was not affected by roadkill.

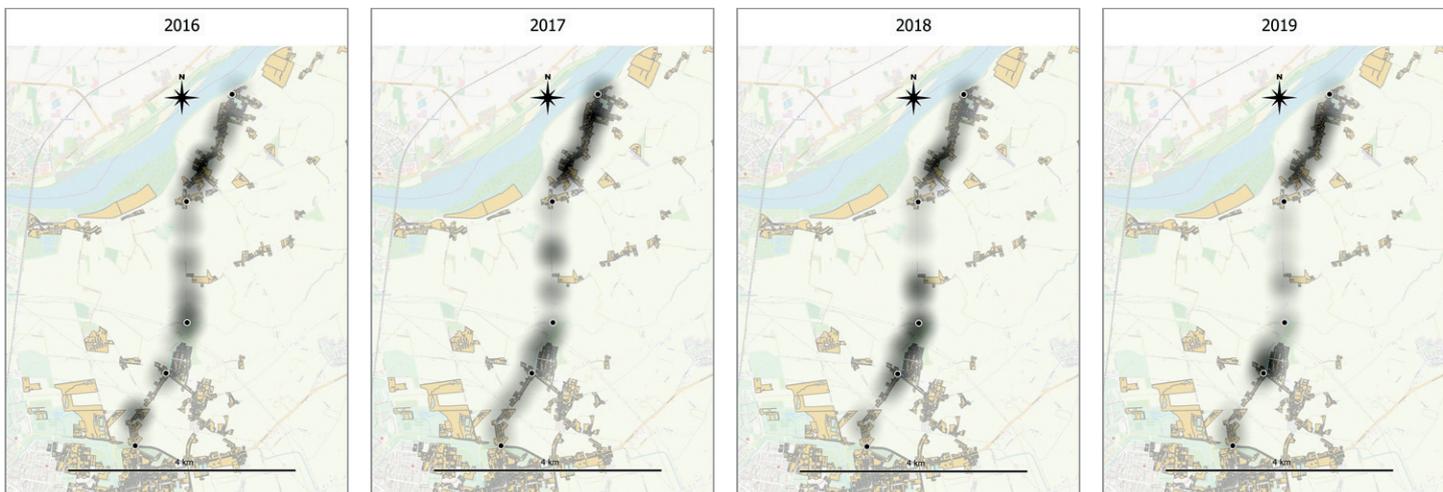


Fig. 9a. Heatmaps of the roadkill points. Black dots are the milestones that separate the different monitoring sectors.

The large extension of channels flanking sector 2 are at origin of the high number of rats found dead there, a useful suggestion for control actions against the pest.

Other alien or domestic species detected were bullfrog (*Lithobates catesbeianus*), red-eared slider (*Trachemys scripta*) and coypu (*Myocastor coypus*), pheasant and cat, but their records seemed rather occasional and not related with their abundances in the landscape.

Vertebrate mortality gave clear insights in the structure of the zoological community near the Natura 2000 site: in sector 4 we found the strongest mortality of toads with the exclusive mortality of the vulnerable species *Bufo bufo* and the almost exclusive mortality of Passeridae birds, including the vulnerable, or near threatened, exclusive species *Passer italiae*. Other species we detected only in sector 4 are *Hierophis viridiflavus*, *Podarcis muralis*, *Natrix helvetica* among reptiles and *Erithacus rubecula*, *Sturnus vulgaris* among birds.

Roadkill of toads showed three phases: late winter, mid-summer (probably related with wet days) and early autumn.

The farm fields between the road and the Natura 2000 site are rich in small ditches used by toads for laying eggs (e.g. CORAZZA, obs. 10545236 at www.inaturalist.org). The arrival of the toads on the road is probably related to the abundance of perpendicular infrastructures (gates, little paths flanked by small grass verges and ditches) connecting the rural landscape to the road itself: the migrating toads found it easier to move along those paths, so they were conveyed towards the road.

We observed that some reproductive ditches use by the toads are also used for the spawning of *Rana dalmatina*, a species that we never found roadkilled. Other amphibian species that are present in the landscape but not among roadkills are *Hyla intermedia* and *Lissotriton vulgaris*.

The number of toads killed in sector 4 is comparable with that found by HEIGHL *et al.* (2017) in Austria on agricultural and municipal roads. Thus, we must suppose that the toad populations close to the Natura 2000 site is still well preserved as in the Austrian landscape.

Modern agriculture tends to eliminate small ditches replacing them with subsurface pipe drainage systems (NIJLAND *et al.*, 2015), thus eliminating very important microhabitats for many animal and plant species of wetlands: on the contrary, it is very important to preserve those reproductive ditches that dry completely in summer, limiting the presence of predators, especially in the areas surrounding Natura 2000 network.

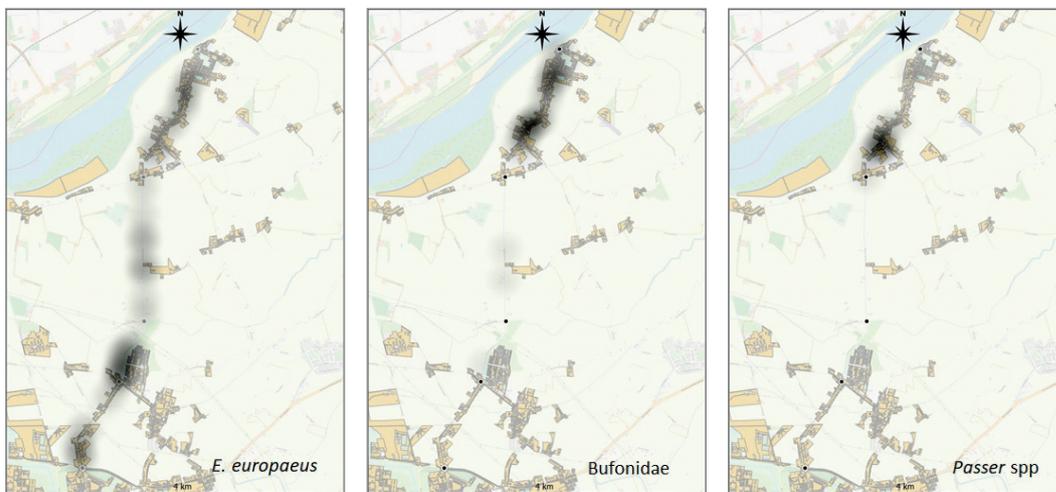
Other species particularly roadkilled in sector 4 were sparrows, *Passer sp.* and *Passer italiae*. The pattern of sparrow mortality can be the result of shelters availability and behavior in response to different traffic intensities: the quieter situation of sector 4 combined with the richness in gardens with shrubs allow them to come closer to the road. Social behavior and the attitude of sparrows to chase each other can increase the risk of roadkill.

For all the other birds the structural aspects of the road were not relevant, but the seasonality was: the most part of birds and of bird taxa died above all in July, probably because of the dispersal of young and inexperienced individuals.

Weather conditions (temperature, relative humidity, wind) may increase the risk for some animals, inducing migrations or making difficult to control flight: toads can die because of a combination of temperature and humidity influencing the timing of migration, that may coincide with the returning home of people after day work. Some of the roadkilled sparrows were found in windy days.

Climate warming can change the phenology of species, exposing them to new dangers, included increased roadkill probability.

Careful studies on the relationships between roadkill accidents and meteorological conditions can help in forecast movements of the animals and to assess the roadkill risk, in some cases making possible to prevent accidents, for instance involving volunteers to help toads to cross, at least in the reproductive period: volunteers may have important roles in both monitoring and rescuing population of endangered species (BONARDI *et al.*, 2011).



Traffic tended to diminish in summer, after school closures, in sector 2 and 3, but school closure had irrelevant consequences on traffic inside the villages (sectors 1 and 4). That testifies that the central segments of the road are used by vehicles as a ring road to bypass the main city, while at the extremes of the road the villages are mainly interested by local, residential traffic: resident people should mainly be targeted with information campaigns.

CONCLUSIONS

Via dei Calzolari is a road that caused an average of 10.5 roadkill among vertebrates per year per every km of its length, a value in line with other results for similar roads in the same landscape (ALDROVANDI *et al.*, 2018) and one of the highest values found in Europe (MOORE *et al.*, 2020).

Many of the involved species are protected under European, national and regional laws, even if Italian sparrows are without legal protection in spite of their vulnerable conservation status.

In Via dei Calzolari neither vertebrates of large size such as deer or badgers nor large birds such as Ardeidae and large raptors were involved in accidents. Moreover, alive large mammals were not observed by citizens even in a buffer of 2 km from the road itself: Via dei Calzolari is an ancient road built more than 200 years ago and we can conclude that the anthropic transformations of the landscape that accompanied its construction have largely expressed their ecological impact on mammal populations, even inside the Natura 2000 site, so the species most conspicuous in size were excluded from the pool of potential victims.

The Natura 2000 site of the Po River is an ecological corridor: urban settlements close to the protected area disrupt its connectivity role because of the multiple activities carried on by human population.

The study was long enough to detect not only some roadkill hotspot points, but also to highlight the main crossing stretches and crossing periods for vulnerable or protected species. Different strategies can be adopted to help animals to cross. Toads, that cross the road in a ground level stretch lined by houses, could be helped by the intervention of volunteers and by the use of temporary barriers to prevent uncontrolled crossings (PEDRINI & FIN, 2014), while underpasses (BENNET, 2033) coupled with appositely designed fencing (RYTWINSKI *et al.*, 2016) can be a good solution for the hedgehogs, that consistently cross the roads in an upraised portion of it. Unfortunately, it is rather difficult to imagine solutions to protect sparrows or little owls. Education campaigns targeting resident people should be adopted.

Moreover, the present work suggests it is necessary to preserve the small ditches among agricultural field for the reproduction of the amphibians and to respect the hedgerows network around part of the road, since the large number of the hedgehogs suggests a good ecosystem functionality of the network itself.

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