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1. Introduction - Background information

1.1. Introduction

The present Report regards the elaboration of the "Assessment and mitigation of impacts on reptiles and amphibians in Kresna Gorge for the Struma motorway (Lot 3.2) alternatives". The elaboration of the study has been assigned to the Greek Environmental Consulting Company "ENVECO SA" by the Bulgarian Ministry of Regional Development and Public Works/ Road Infrastructures Agency, according to the contract signed on 15-05-2017.

Lot 3.2 is situated in the Blagoevgrad Province in the Municipalities of Kresna and Simitli.

Kresna Gorge is an ecologically sensitive area included in the European Ecological Network Natura 2000. In particular, the following Natura 2000 areas are found in the broad area of the gorge:

- BG0000366, "Kresna - Ilindentsi" protected under the habitat Directive 92/43/EC as amended
- BG0002003, "Kresna" protected under the birds Directive 79/409/EC as amended.

1.2. Study team

The team that elaborated the present report was compiled of the following scientist:

- Spyros Papagrigoriou, Civil Engineer
- Yannis Bekiaris, Environmental Scientist
- Yannis Ioannidis, Biologist
- Yorgos Papamihail, Environmental Scientist
- Manolia Kalfaki, Environmental Engineer
- Alexandra Kavvadia, Biologist
- Vaggelis Kaniastas, Agriculturalist - Ornithologist
- Vaggelis Pantelias, Infrastructure Works Engineer (technological education)

1.3. Studies taken into account

The following studies were taken into account during the elaboration of the present Report:

- Final Reports on "*Surveillance, analysis and evaluation of the mortality of the animal species in the section of road E-79 (I-1) passing through Kresna protected areas Kresna - Ilindentsi*" (for the years 2013, 2014, 2015 and 2016).
- "*Preliminary Study for the comparative environmental impact assessment on alternative alignments of the Struma Motorway (Lot 3.2) in Bulgaria - Final Report*" (ENVECO S.A., 2015)
- Proposed mitigation measures, sent digitally by the Bulgarian Ministry of Regional Development and Public Works/ Road Infrastructures Agency.

1.4. Scope of the Report

The scope of the present Report includes the following:

a) Forecast of the impact of road traffic during the:
   - operation of the "East alternative G10.50" (right carriageway, direction "Sofia-Kulata" on the route of the existing E79 road in the gorge) and Forecast of the impact of the remaining traffic in the gorge during the exploitation of the "East alternative G20" (left and right carriageways outside the gorge);
   - the construction of the Long Tunnel;

   on animal species in the Kresna gorge, especially amphibians and reptiles, subjects to conservation in SCI BG0000366 "Kresna – Ilindentsi", in respect of:
   - Mortality - of individuals on the road and the reflection on their populations;
   - Barrier effect - creating physical barriers for migration of species on the carriageway;
   - Fragmentation – of the habitats and populations of species;

b) Assessment of the mitigation measures proposed in the AA Report during the operation of the "East alternative G10.50" - right carriageway to limit the negative impact on the animal species and their habitats in the Kresna Gorge.
   Specific additional effective mitigation measures at the "East alternative G10.50" - right carriageway are also presented.

2. Estimation of impacts on reptiles and amphibians according to the motorway alternatives in Kresna Gorge

2.1. Synopsis of existing situation

The road E79 passes through Kresna gorge from north to south moving parallel to Struma river and it bysects the Natura 2000 area BG0000366 "Kresna-Ilindentsi". This road has a significant and increasing traffic load at it is the main connection rout between Sofia and the Greek borders. The position of the road and the increased traffic have as a consequence an increased mortality rate for most species found in the area. The most commonly found casualties are the reptiles. They represent more than 50% of the total animal casualties. Regular mortality monitoring has been carried out on this road during the period 2013-2016. The daily mortality rate for reptiles was 7.2-10.8 animals per day and for the amphibians 0.6-1.4 animals per day during 2014-2016 and lower in 2013. These are the observed mortality rates as a number of dead on road (DOR) animals are removed from scavengers before the researcher has a chance to count them. The inefficiency of road mortality counts can be high especially in areas with many scavengers present and this is more prominent in amphibians. The true mortality numbers are always higher than the observed.

Table 2-1 Mortality monitoring results on E79 for the period 2013-2016

Year	2013	2014	2015	2016
Days of monitoring	38	48	36	36
Total number of DOR	213	875	652	462
Reptiles DOR/day	2.00	10.77	10.50	7.19
Amphibians DOR/day	0.37	1.42	1.00	0.56

Almost all species of reptiles present in the SCI BG0000366 have been found as road casualties. Most road casualties belong to the species *Natrix tessellata* (dice snake). This can be attributed to its daily movements from the foraging area at the river to the basking areas and shelters in the surrounding land. It also uses often the road surface for thermoregulation and has the highest population densities from all snake species present because Struma River is an optimal habitat for dice snake. The most common lizard found dead was *Lacerta viridis*, a very common species in areas with bushes and trees, it uses extensively the openings near the road and utilizes the road surface for thermoregulation.. Among them the two tortoise species (*Testudo graeca* and *Testudo hermanni*) are regular casualties. Those two species seem to be relatively common in the gorge area and are included in Annex II of the 92/43 EC directive. The two snake species of Annex II (*Elaphe situla* and *Elaphe quatuorlineata*) are considered rare in the area and possibly more specimens have been found DOR than alive. The same seems to be true for the turtle *Emys orbicularis,* a species that probably utilizes those parts of Struma where the riverbed is wider and the water speed diminishes.

The mortality rates for amphibians are much lower but the composition of casualties also represents most of the species present in the SCI. *Bombina variegata* and *Triturus karelinii*, the only two species of Annex II, are very rare casualties. For both species Struma river is not an optimal habitat, *Bombina variegata* prefers smaller tributaries of Stuma and temporary pools on higher elevations while *Triturus karelinii* prefers standing water basins and seems to be more common east of the Kresna gorge. The most commonly found amphibian road casualties on E79 are the two species of toad, *Bufo bufo* and *Bufo viridis*. Those are the most vagile amphibians making regular movements from and to their reproduction areas and this makes them prone to road mortality especially in spring and autumn. Their copses also stay longer on the road in relation to other amphibians.

Table 2-2 Example of one years results. Reptile and amphibian casualties on E79 during the 2015 mortality monitoring

Species	casualties found (in 36 days)	% of class casualties
Amphibians		
Pelophylax ridibundus	5	7,35
Rana dalmatina	4	5,88
Salamandra salamandra	1	1,47
Bombina variegata	1	1,47
Bufo viridis	5	7,35
Bufo bufo	24	35,29
Bufo sp.	1	1,47
Anura sp.	27	39,71
Amphibian totals	**68**	**100.00%**

Reptiles		
Emys orbicularis	4	0,77
Testudo greca	4	0,77
Eurotestudo hermanni	4	0,77
Testudo greca/ Eurotestudo hermanni	16	3,09
Lacerta trilineata	1	0,19
Lacerta viridis	86	16,63
Anguis fragilis	5	0,97
Lacerta sp.	33	6,38
Malpolon monspessulanus	1	0,19
Dolichophis caspius	4	0,77
Platyceps najadum	14	2,71
Typhlops vermicularis	17	3,29
Zamenis situla	10	1,93
Vipera ammodytes	7	1,35
Tellescopus fallax	2	0,39
Natrix natrix	7	1,35
Natrix tessellata	142	27,47
Natrix sp.	122	23,60
Serpentes sp.	37	7,16
Reptilia	1	0,19
Reptile totals	**517**	**100.00%**

2.2. Main characteristics of motorway alternatives

The scenarios that where evaluated are the proposed design variant solutions for route of Lot 3.2 of Struma Motorway. The three scenarios include passage through the gorge and bypass of Kresna gorge.

“East alternative G 10.50”

The design solution of east alternative G 10.50 is designed as two separate lanes. The right lane (direction Sofia-Kulata) at the end of Lot 3.1 follows the existing road through the Kresna Gorge, and after exiting the gorge it leaves the existing road and the town of Kresna is bypassed from the east on a new terrain, ending at the beginning of the Lot 3.3. The left lane (eastern bypass of the gorge) passes on a new terrain alongside the Gradevska River between the neighborhoods of the town of Simitli - Oranovo and Dalga mahala, as developing eastwards around the villages Brezhani, Stara Kresna and Oshtava by consistently built tunnels, viaducts, supporting walls and reinforced embankment, ending again at the beginning of the Lot 3.3. Under this scenario there will be no significant changes on the existing E79 road, and the technical works will be mainly focused on mitigation measures for the elimination of impacts on fauna species. The traffic on the right lane during the operation will compose mainly of the traffic from the right carriageway and some local traffic. During operation, the forecast of mean daily traffic load for the year 2040 is 6303 vehicles.

“East alternative G 20”

In this design solution all the traffic of the “Sofia-Kulata” will pass outside of the Kresna gorge through a new larger road east of the gorge area. This motorway will largely follow the direction of the left lane of East alternative G 10.50. During operation the traffic on the existing Road E79 inside Kresna gorge will compose mainly from the local traffic. The forecast of mean daily traffic load on E79 inside Kresna gorge for the year 2040 is 2011 vehicles.

“Long Tunnel”

The developed conceptual design for a long two-pipe tunnel with a length of 15.4 km is located on the west side of E79 and below the Kresna Gorge. The passing of the route through Kresna tunnel is from km 379 + 267 to km 394+605 (left pipe) and from km 379 + 255 to km 394 +600 (right tube). After the exit of the Kresna tunnel, the motorway intersects an existing third class road and Struma River as passes east of it and southwest of the residential area of the town of Kresna. During the construction two side tunnels will be constructed and used by the trucks that carry excavation and other material through E79. This load will be added to the existing traffic on E79 and the mean expected daily traffic load will be near 9000 vehicles. During operation, the forecast of mean daily traffic load for the year 2040 is at similar levels to the “East alternative G 20”. However all trucks transporting dangerous (hazardous) loads will not use the long tunnel (there is a ban on movement in the tunnels according the local legislation), but the gorge. Also all dangerous loads (including all kind of chemicals) will be transported via E 79.

2.3. Forecast of the impact of road traffic under three traffic scenarios

2.3.1 Mortality

Logistic regression was used to reveal the important parameters affecting probability of reptile and amphibian road mortality along 16km part of E79 (Simitli – Kresna). The analysis was based on field mortality data collected from 3/2013 until 1/2015. Logistic regression models were used to predict road mortality for the year 2014. Probability curves from the literature (Hels and Buchwald 2001) and 2014 results were used to predict road mortality of reptiles and amphibians under three scenarios (G10.50, G20 remaining traffic and Tunnel construction).

2.3.2 Methodology

Preparation of data and coordinates:

Mortality data were extracted from monitoring reports of 2013, 2014 and 2015 coordinates, were tested on the map and obvious mistakes were corrected using the available spatial information (e.g. value km of the road). A maximum of 1063 recordings was reached with reliable coordinate data along the road for the monitoring period from March 2013 until January of 2015.

Parameters estimates:

A Kernel Density Estimator (a decreasing density function known as KDE) was used to visualize the accumulation of mortality records along the road. Four separate KDEs were calculated from the point data layer (in QGIS) for amphibians, reptiles, mammals and birds respectively.

Figure 2-1 Kernel density estimation for the point data of animal road mortality.

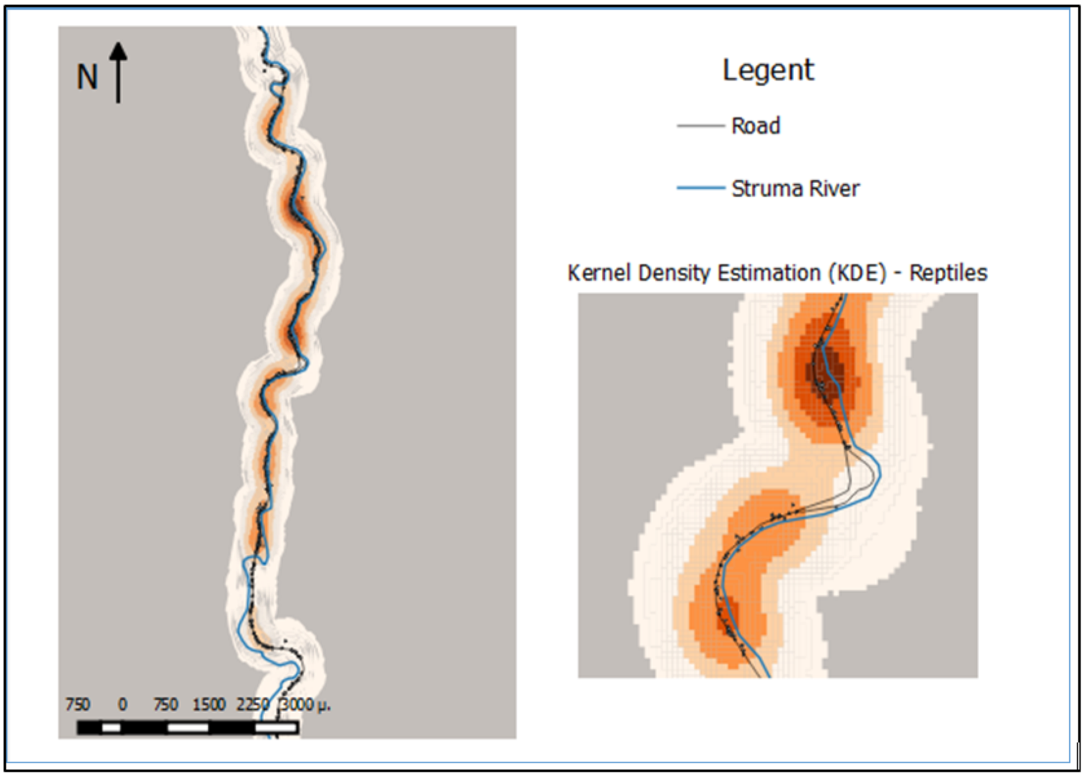
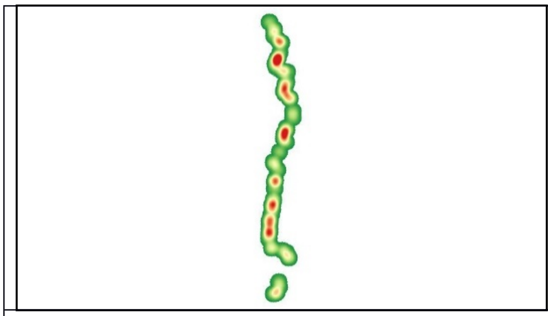
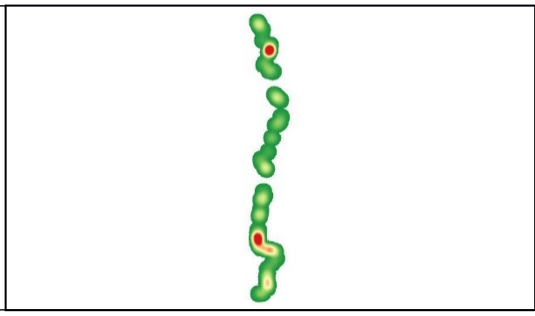
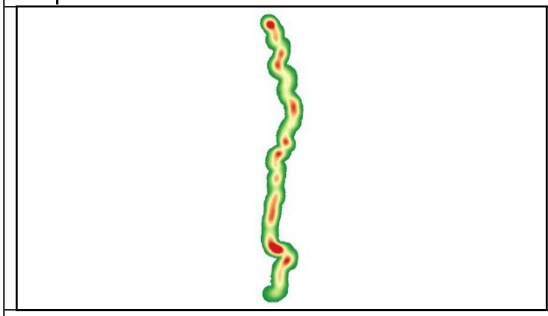
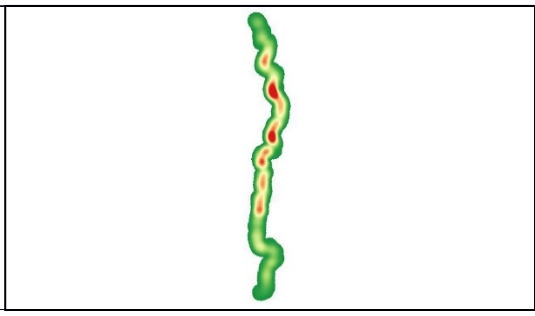


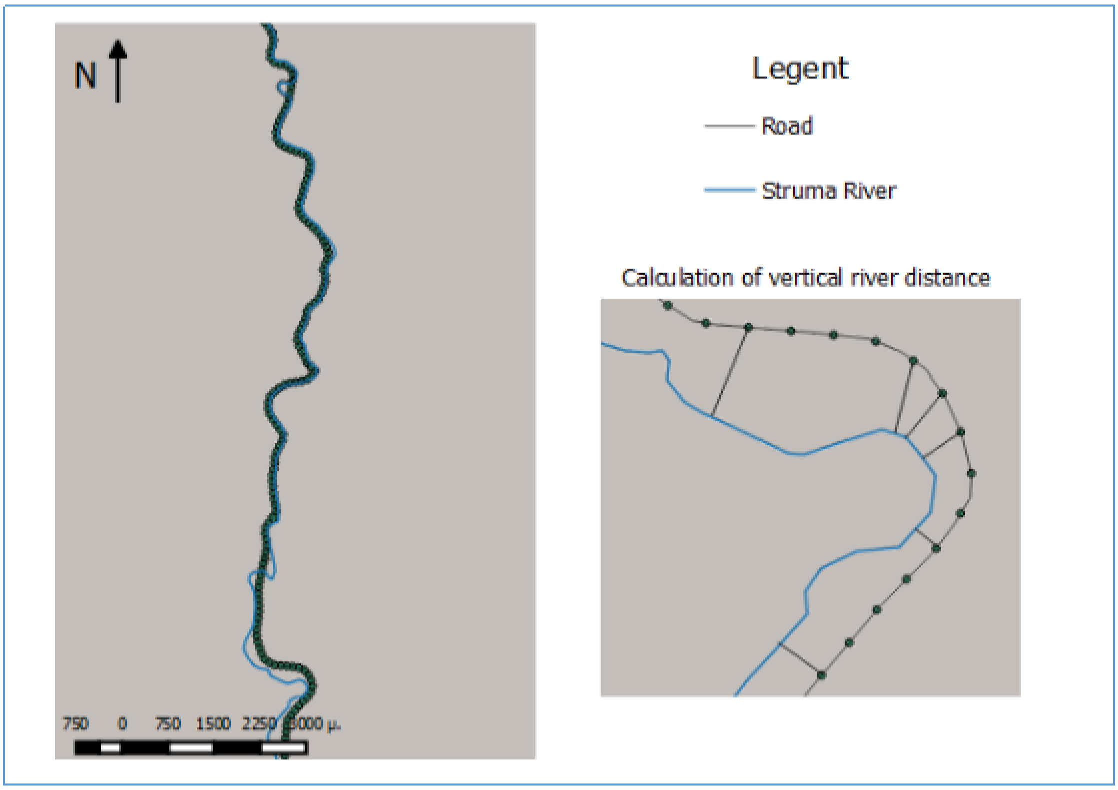
Figure 2-2 Kernel density estimator for Amphibians, Birds, Mammals and reptiles found dead during the monitoring years 2013 – 2014. Colors represent the gradient of probability (from green to red, 0 to 1 respectively) of finding a dead animal.

	
Amphibians	Birds
	
Mammals	Reptiles

River distance parameter:

calculation of the nearest (vertical distance) of each point from Struma River line feature (digitized on google earth) as a measure of water proximity.

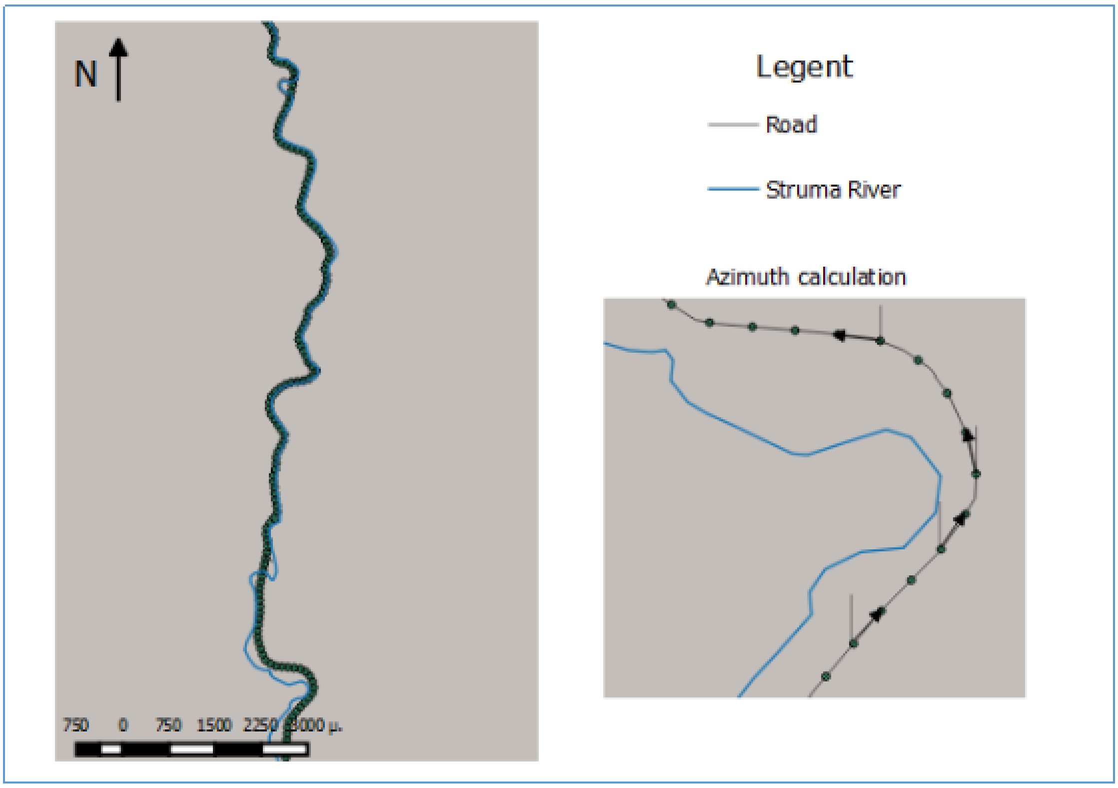
Figure 2-3 Calculation of vertical river distance



Direction change parameter:

In order to test the effect of road shape in animal road mortality a point layer with equal distance points for 100m along the road was created (QGIS 8.1). Azimuths between those subsequent points were calculated (using a python script in QGIS 8.1) and then the positive difference between those subsequent values was calculated as a measure of road direction change.

Figure 2-4 Azimuth calculation for the preparation of direction change parameter



Land cover parameters:

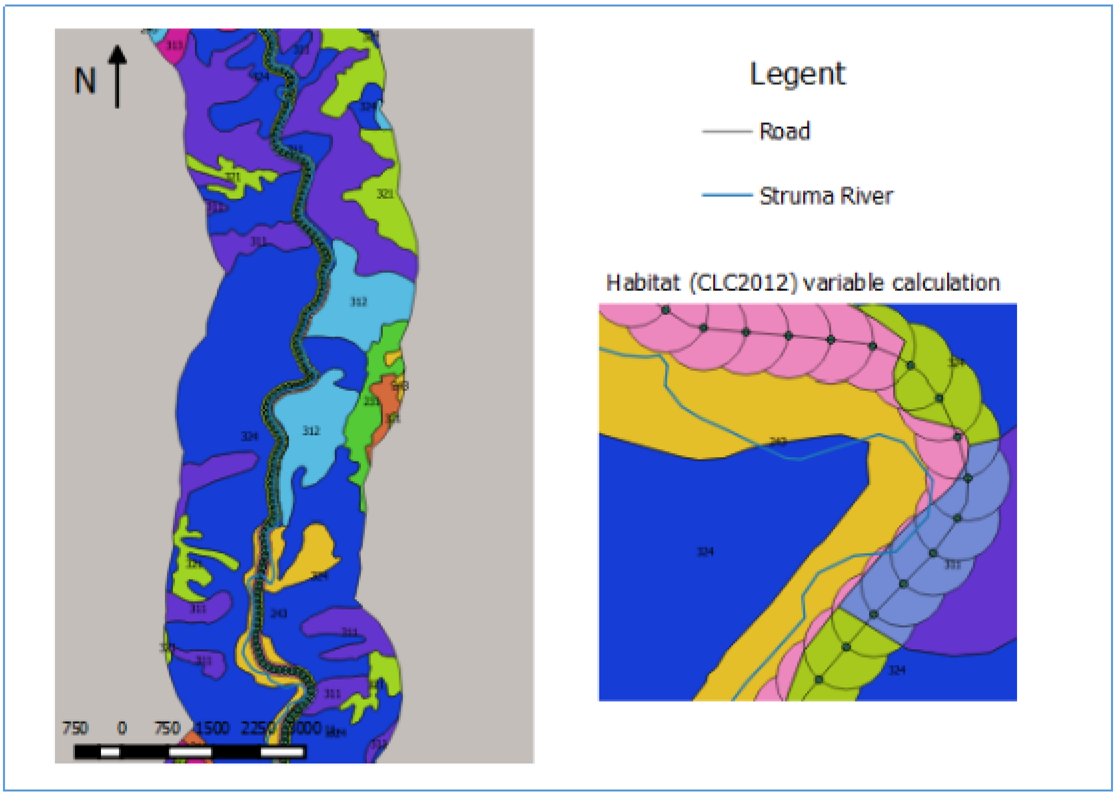
Calculation percentages (using buffer→intersect in Qgis 8.1) of cover of the CLC_2012 (http://land.copernicus.eu/pan-european/corine-land-cover/clc-2012) categories in four spatial scales (buffers 100m, 500m, 1000m, 1500m) around each sample point. All land cover categories appearing average percentages less than 5% were rejected from the analysis. We finally concluded in to five land cover categories (243, 311, 312, 324, 321) generating 4 variables in 100m scale and 5 for each other scale respectively (500m, 1000m, 1500m) a total 19 variables.

Table 2-3 Land cover classes found in a buffer 1500m around data E79 along (Simitli – Kresna)

243	Land principally occupied by agriculture, with significant areas of natural vegetation
311	Broad-leaved forest
312	Coniferous forest
324	Transitional woodland-shrub
321	Natural grasslands

After comparison of the scales (100, 500, 1000, 1500) in terms of full model performance (total variance of the dependent variable explained) it was concluded that 100m scale performed best.

Figure 2-5 Calculation of habitat (CLC 2012) variable for data points.

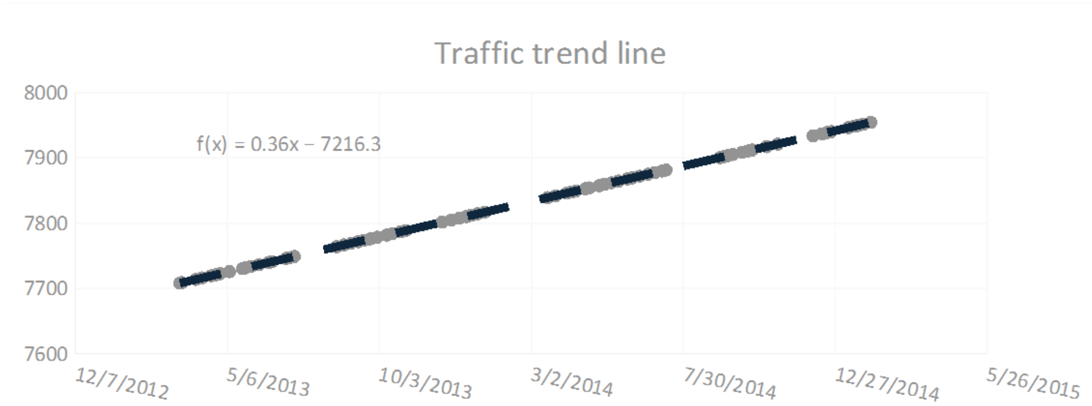


Traffic variable:

Due to the absence of daily traffic data for the available monitoring days (3/2013 – 1/2015) the traffic variable was created as follows:

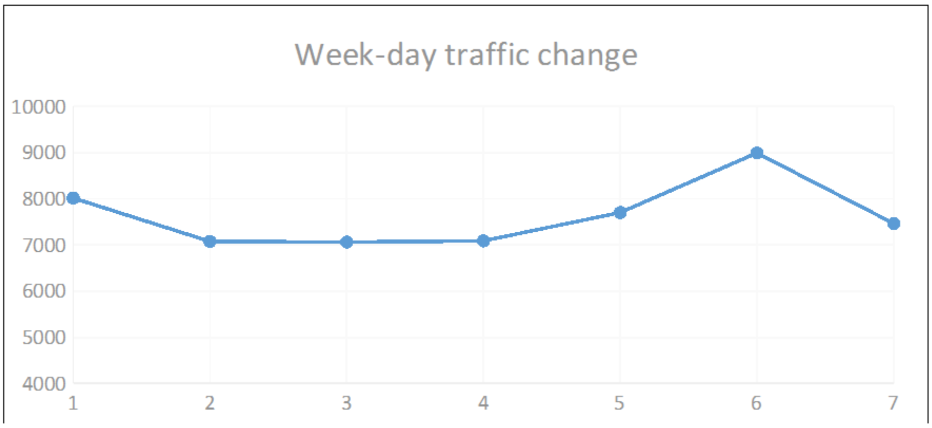
1. Expected Increasing trend of traffic load (linear regression line calculated from given predictions from EIA corrected according to 10/2013 daily data)

Figure 2-6 Traffic intensity trend line used on the calculation of traffic variable.



2. Weekly variation of traffic loads (in terms of percentage of traffic change according to 10/2013 daily data)

Figure 2-7 Weekly variation of traffic intensity used on the calculation of traffic variable (the percentage of change between consequent days was used on the preparation of traffic variable).



The combination of the above patterns resulted the traffic variable under the scenarios tested (G10.50 2040, G20 2040 and Tunnel construction 2020). Furthermore the variable was tested in the logistic regression models also with a delay (-1 day) of one day in order to represent dead animals from the previous day from recording of those specimens.

Weather variables:

The weather history for the sampling period was extracted from www.wunderground.com. A total of 4 variables were extracted (High daily temperature, Humidity (%), sum of daily precipitation (mm) and Visibility (km).

Table 2-4 Variables entered to the analysis.

Variable	Description	Type of variable
	Dependent variables	
Rc	Reptiles found dead / not found (D/N)	Binary – Dependent variable
Ac	Amphibians found dead / not found (D/N)	Binary – Dependent variable
	Independent variables	
season	Winter, spring, summer, autumn	Categorical
km_cat	Km of the road, 16 categories (0-1km, 1-2km, ...15-16km)	Categorical variable
weekday	Day of the week (7) (1 = Sunday, 2 = Monday ... , 7 = Saturday)	Categorical variable
Traffic	Traffic of the sample day	scale
Traffic-1lag	Traffic of previous day	scale
Direction change	Direction change of the road (positive, azimuth difference between equally spaced [100m] subsequent points along the road)	scale
RivDis	Vertical distance from Struma River	scale
100m - CLC: 243, 311, 312, 324	Percentage of CLC 324 on a 100m buffer around a data point	4 scale variables
500m - CLC: 243, 311, 312, 324, 321	Percentage of CLC categories using 500m buffer for each data point	5 scale variables
1000m - CLC: 243, 311,	Percentage of CLC categories using 1000m buffer for each data point	5 scale variables

Variable	Description	Type of variable
312, 324, 321		
1500m – CLC: 243, 311, 312, 324, 321	Percentage of CLC categories on a 1500m buffer around a data point	5 scale variables
Hu_high	Maximum daily humidity (%)	Scale
T_high	Maximum daily temperature (°C)	Scale
Vkm_low	Minimum daily visibility (km)	Scale
Prec_sum	Sum of daily precipitation (mm)	Scale

2.3.3 Results

Logistic regression for reptile species

A logistic regression was performed (e.g., Langen et al. 2009) to ascertain the effects of traffic intensity, distance from Struma River, direction change of road, kilometer of the road (categorical km intervals 0-16km from north to south) and four CLC2012 land cover categories (243, 311, 312, 324) on the likelihood of reptiles get killed on the road. Furthermore random variables were tested such as max daily temperature, humidity, precipitation daily sums and visibility km.

Result: The logistic regression model was statistically significant, $\chi2 = 28.064$, $p<0.0005$. The model explained 31.6 % (Nagelkerke *R2*) of the variance in reptile road mortality and correctly classified 70.7 % of cases.

Table 2-5 Statistically significant variables (p<0.05) entered to the final model for reptile mortality

Variable	Description	Type of variable
Rc	Reptiles found dead / not found (D/N)	Binary – Dependent variable
Season	Winter, spring, summer, autumn	Categorical
Weekday	Day of the week (7) (1 = Sunday, 2 = Monday … , 7 = Saturday)	Categorical variable
km_cat	Km of the road, 16 categories (0-1km, 1-2km, …15-16km)	Categorical variable
Traffic	Traffic of the sample day	scale
100m CLC 243	Percentage of CLC 243 on a 100m buffer around a data point	Scale
Avg Of T_high	Maximum daily temperature (°C)	Scale

Logistic regression for amphibians

A logistic regression (binary logistic) was performed to ascertain the effects of traffic load, distance from Struma River, direction change of road, kilometer of the road (categorical km intervals 0-16km from north to south), four CLC2012 land cover categories (243, 311, 312, 324) and weather on the likelihood of amphibians get killed on the road. Furthermore random variables were tested such as max daily temperature, humidity, precipitation daily sums and visibility km.

The logistic regression model was statistically significant, $\chi2 = 24.103$, $p < 0.005$. The model explained 29.2% (Nagelkerke *R2*) of the variance in reptiles killed and correctly classified 93.7 % of cases.

Table 2-6 Statistically significant variables (p<0.05) entered to the final model for amphibian mortality

Variable	Description	Type of variable
Ac	Amphibians found dead / not found (D/N)	Binary – Dependent variable
Season	Winter, spring, summer, autumn	Categorical
km_cat	Km of the road, 16 categories (0-1km, 1-2km, …15-16km)	Categorical variable
Weekday	Day of the week (7) (1 = Sunday, 2 = Monday … , 7 = Saturday)	Categorical variable

Variable	Description	Type of variable
Traffic	Traffic of the sample day	scale
Traffic-1lag	Traffic of previous sample day	scale
RivDis	Vertical distance from Struma River	scale
Hu_high	Maximum daily humidity (%)	Scale
T_high	Maximum daily temperature (oC)	Scale
Prec_sum	Sum of daily precipitation (mm)	Scale

Statistical model prediction:

Statistically significant variables entered to the following model:

$$\log\left(\frac{p}{1-p}\right) = \hat{\alpha} + \hat{\beta}_1 X_1 + \hat{\beta}_2 X_2 + \cdots + \hat{\beta}_p X_p \,.$$

The predicted probability computed as follows:

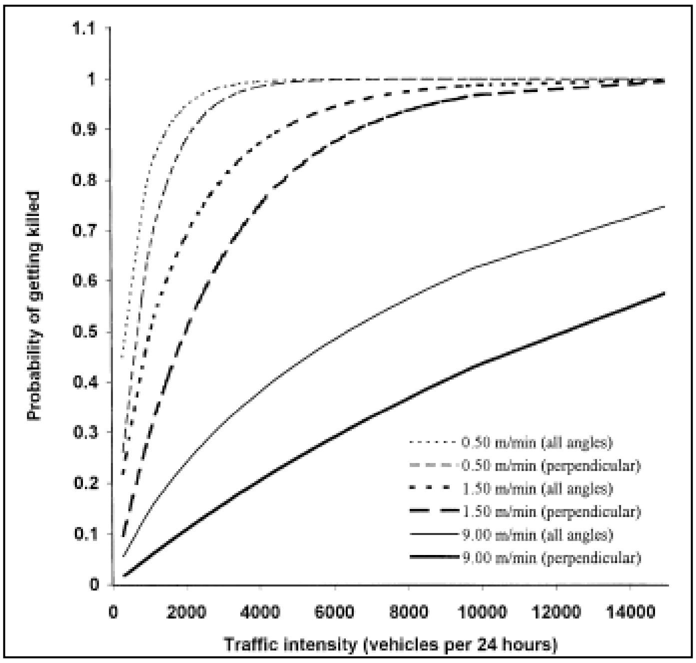
$$p = \frac{e^{\alpha + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p}}{1 + e^{\alpha + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p}}$$

Predicted numbers of animals getting killed on the road were then selected based on 0.5 cut value.

The problem with the above analysis is that we assume that the probability of a reptile or amphibian getting killed is linearly related to the traffic load per day. Nevertheless the relation between traffic loads per day Vs probability of getting killed is better described by a nonlinear curve (Hels and Buchwald 2001).

We tried to use the above statistical models to predict the mortality probability under the scenarios tested using the parameters selected by the analysis calculated for the scenario road (e.g. river distance parameter for the new road planed). Unfortunately but not unexpectedly, when traffic intensities out of the data range are used, the mortality probability is maximized to 1 for large values (traffic intensities) and minimized to 0 for small values.

Figure 2-8 The effect of traffic intensity on probability of getting killed by crossing the road (randomly - all angles and perpendicularly) is given for three representative velocities of amphibians (0.5m/min, 1.5m/min, 9m/min) by Hels and Buchwald (2001).



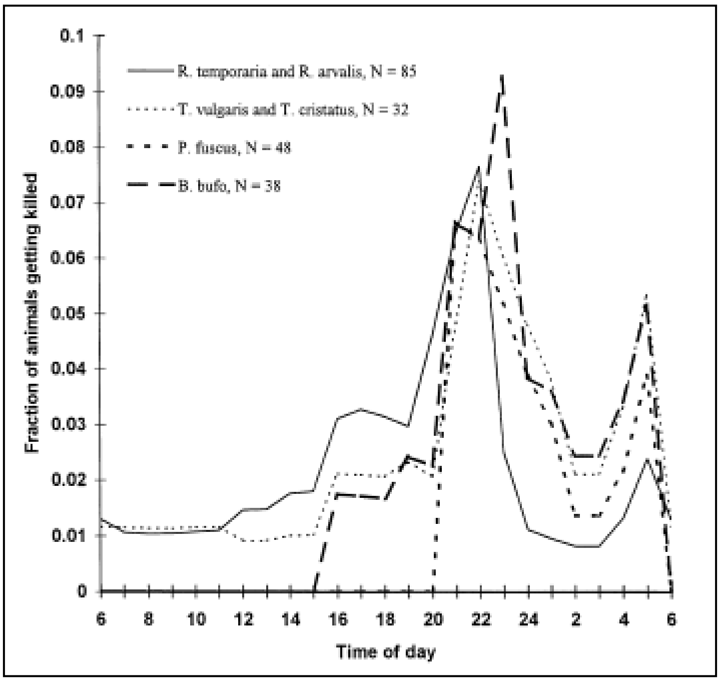
The probability predicted form the curves of figure. 2.8(Hels and Buchwald 2001) for 0.5m/min and 9m/min velocities (for random crossing) was used to evaluate amphibian and reptile mortality respectively under the three scenarios of traffic intensity [per day] (E79).

The probability predicted by curves for the given scenarios of traffic intensity (G10.50, G20 and Tunnel construction) was used to improve the prediction given by logistic regression of the data for year 2014. The calculated probabilities under the scenarios of traffic load tested were multiplied with the respective results of logistic regression for 2014 to produce predictions for reptiles and amphibians.

Logistic regression shows a relation between amphibian mortality and traffic intensities of the current day and the previous day, this result indicates that probably the most amphibian casualties were killed late in the afternoon of the previous day and early in the morning of the monitoring day, thus the logistic regression will tend to overestimate the amphibian mortality.

To overcome this overestimation the probability of getting killed within a time range 06:00 until 20:00 was used, an average value of 0.18333 in order produce a realistic prediction of amphibian road mortality.

Figure 2-9 Diurnal variation in probability of getting killed for the amphibians investigated by Hels and Buchwald (2001).



The predictions produced are shown at the following tables

Table 2-7 Predicted numbers of reptiles and amphibians killed per year for the scenarios tested.

Prediction\Taxon	Reptiles killed per year	Amphibians killed per year	mean traffic load/day
2014	2087.5	287.2	7925.0
2040 -G10.50 without mitigation	1758.1	286.0	6302.9
2040 -G10.5 with mitigation	175.8-703.2	28.6-114.4	6302.9
2040 -G20 or Long Tunnel	876.4	214.4	2010.7
2020 - Tunnel construction	2219.4	296.1	8977.5

Nevertheless the exact number predictions are not so important in this case, the most meaningful calculation is the expected percentage of change in road mortality (in terms of % increase or decrease) which are shown in the following table.

Table 2-8 Percentage of change in road mortality of reptiles and amphibians under the scenarios tested.

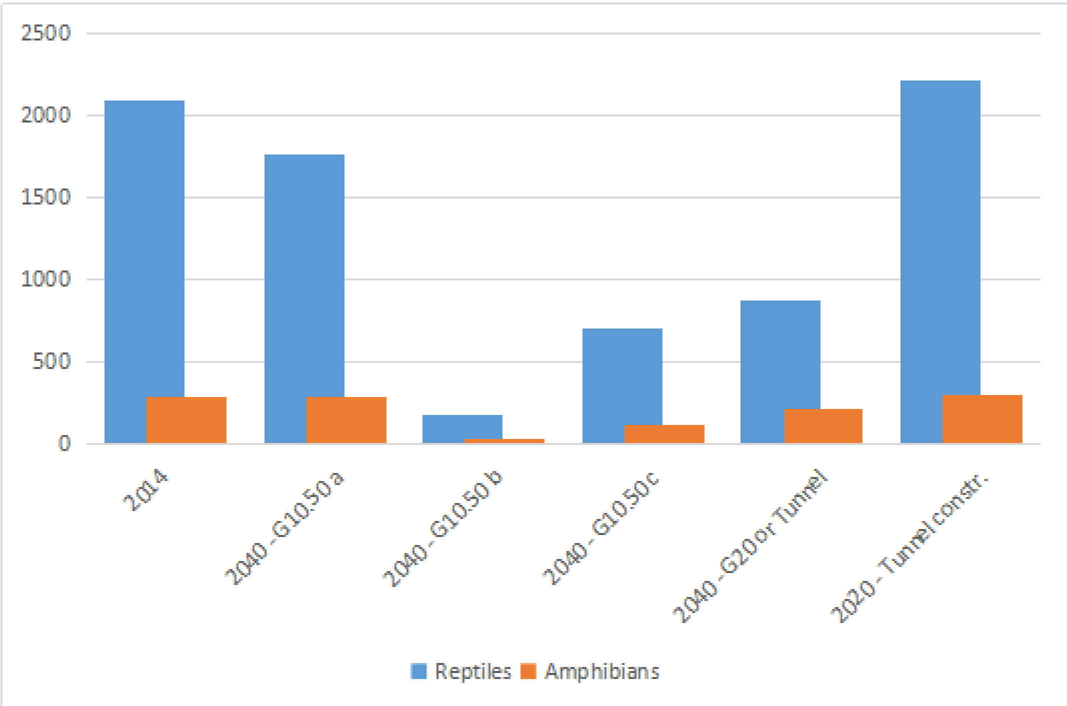
Prediction\Taxon	% change on reptile mortality	% change of amphibian mortality	mean traffic load/day
2040 -G10.50 without mitigation	-15.8% ↓	-0.4% ↓	6302.9
2040 -G10.50 with mitigation	-66.3% to -91.6% ↓	-60% to -90% ↓	6302.9
2040 -G20 or Long Tunnel	-58% ↓	-25.4% ↓	2010.7

2020 - Tunnel construction	6.3% ↑	3.1% ↑	8977.5

Increase ↑, Decrease ↓

The prediction for the G10.50 doesn't take into account the mitigation measures. A proper implementation of exclusion and connectivity structures as discussed in the following chapter is expected to significantly decrease road mortality. However it is not realistic to expect all fencing and defragmentation facilities to be perfectly constructed and function absolutely efficiently. The proposed monitoring methodologies will identify where the weak spots are in the fence/defragmentation system and to define spots with significant mortality rates or sections with damaged passes and approaches. These locations should be identified and the situation should be analyzed for the purpose of reporting to the contractor to take appropriate measures to improve the functionality of the mitigation measures. This approach will increase the efficiency of measures and will contribute to reduction of traffic-related mortality to very low impact levels.

Figure 2-10 Annual predicted mortality for reptiles and amphibians under the scenarios tested. G10.50a: Without mitigation measures, G10.50b: Good implementation of mitigation measures, G10.50c: Worst scenario with implementation of measures.



2.3.4 Habitat fragmentation

"Habitat fragmentation is a landscape-level process in which a specific habitat is progressively sub-divided into smaller, geometrically altered, and more isolated fragments as a result of both natural and human activities, and this process involves changes in landscape composition, structure, and function at many scales and occurs on a backdrop of a natural patch mosaic created by changing landforms and natural disturbances (McGarigal and McComb 1999)."

This is one of the most comprehensive definitions of habitat fragmentation which is a difficult process to define on the total of its aspects due to the complexity of the changes that take place during the fragmentation, the scale and components of the fragmentation process, as well as the causes and consequences of this process. (McGarigal et al., 2005).

A road is a linear element that induces a fragmentation effect related to the dissection processes which increases the number of landscape patches, decreases interior habitat area and increases the extent of edges. However there is not enough knowledge and data to predict with any precision the consequences of habitat fragmentation to many species including reptiles and amphibians. Theoretically the first step is a population decline that is linearly correlated to the amount of *habitat loss*. At some point (fragmentation degree) their is a threshold beyond which the decline becomes sharp. This is the point when the edge effect, the barrier effect (isolation) and other fragmentation effects accelerate the loss of the population. A way to approach this is to try to evaluate the current and future condition regarding habitat fragmentation based on empirical data.

Edge effects are changes in population or community structures that occur at the boundary of two habitats. Increased habitat fragmentation leads to pronounced edge effects. Regarding the response or amphibians and reptiles to the edges we would expect that amphibians would have a negative correlation because of their preference for moisture environments while the reptiles should favor them as they prefer warmer and drier conditions. Studies in Mediterranean ecosystems have shown an increased preference of most reptile species for mosaics of habitats while some diurnal heliothermic species have shown increased population densities in the ecotones. Although some degree of caution is necessary due to the increased detectability of the animals in those areas, it is a fact that many edges provide better thermoregulation and feeding opportunities for reptiles due to the abundance of herbs and insects. Generally it is expected that negative edge effects will start to appear in reptiles only at very high fragmentation levels. On the other hand there is strong evidence of negative edge effects on some species of amphibians (e.g. Rothermel and Semlitsch 2002) especially in salamanders. Contrary to that Rosenberg and Raphael (1986) reported greater amphibian richness in landscapes with greater availability of edges but species differed in their preference for ecotones. Although a significant increase of for high contrast edges can have adverse effects on amphibians the current habitat fragmentation at Kresna gorge is not at a level that edge effect is expected to be a significant reason for population declines.

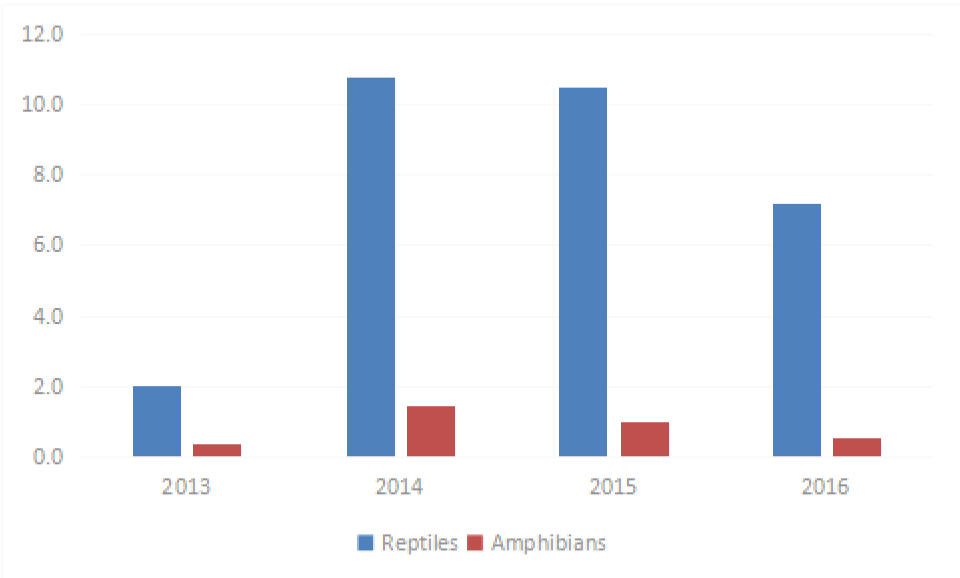
Barrier effect

Roads can act as barriers to animal movement. The physical surface of a 7 meter width road such as E79 does not represent an obstacle to crossing for the reptiles. For the amphibians which are more sensitive to the dry open surfaces it can be more of a problem but they easily cross it during wet/rainy days or during the night. It is the traffic of vehicles that is responsible for the barrier effect of the road through mortality during crossing attempts or behavioral road avoidance. Amphibians appear particularly vulnerable to road mortality regardless of their relatively low vagilities. The probability of getting killed can reach 0.34 to 0.61 when crossing a road with a traffic load of 3207 vehicles/day, and from 0.89 to 0.98 when crossing a motorway (Hels & Buchwald, 2001). In the later case the road is a very strong barrier to their movement but in either case it depresses their population growth rates and has negative demographic and genetic consequences that can result in local or regional extinction. In the case of E79 the road passes near Struma river which has some important reproduction areas and this amplifies the negative effects. For the reptiles high mortality rates lead in the long term in significant declines around the road and it can also lead to extinction in isolated patches on a fragmented habitat. High mortality during road crossing often results on a selection for road avoidance. Studies have shown that many reptile species with homeranges that overlap a road, cross it a lot less often than predicted. Road avoidance can reduce the road mortality over time but it also results in diminished gene flow. The negative genetic effects of that may take many decades to appear making the problem undetectable and thus placing populations at high risk.

The barrier effect is with regard to patch isolation effects which inhibit the movement of organisms for focal ecological processes occurring between patches (Richardson et al., 1997). The consequences of barrier effect and fragmentation are not easily detected at the metapopulation level. A metapopulation can consist of the populations existing in the different habitat patches of a fragmented habitat. Theoretically the population of each patch has a probability of extinction, but it has also a colonization rate from the other occupied patches in the metapopulation. Populations in certain patches can go extinct, but the metapopulation as a whole persists if colonization rate equals the extinction rate.The main factors influenced the success of recolonization are the vagility of the species, the connectivity of the patches and mortality rates during dispersal. If the balance between extinction and recolonization is broken the metapopulation can go extinct.

One very important question to be addressed is what is the current situation of the populations of reptiles and amphibians with regard to the existing E79 road passing through Kresna gorge. The is not a straightforward answer to this question in the current state of knowledge regarding reptiles and amphibians in the area. The road mortality data from the relevant studies for the period 2013-2016 show a very high mortality rate for reptiles (Fig.1). The models we used to predict future road mortality under the different scenarios make the basic assumption that the populations remain relatively stable and the losses are replaced from reproduction and immigration. While this is a valid approach in order to compare different solutions, this is not what really happens in a true population. Above a critical threshold, mainly related to the increased traffic, the losses can't be replaced and the population declines. At this point the mortality rate decreases and sometimes this trend is also enhanced from an increased road avoidance. However it is clear that this decline is a bad sign because even though the number of casualties decreases the populations along the road, or parts of it at least, are on a negative trend. The numbers of amphibians found DOR during the mortality studies (2013-2016) were lower than expected from a road of such characteristics. This could be an indication of decreased populations but it could also represent an inefficiency of the method. Hels & Buchwald (2001) found monitoring amphibian mortality by foot inefficient with only 7% to 67% of the road victims discovered.

Figure 2-11 Observed daily mortality rates of reptiles (blue) and amphibians (red) in the period 2013-2016 on E79.



There is no way to tell what stage the observed casualties of reptiles and amphibians represent. There is a variation and a negative trend in the number of road casualties from 2014 to 2016 (Fig.1) but we can't clearly attribute that to decreasing populations or other coincidental factors. The low rates of 2013 are very hard to explain especially because they are also similar for birds and mammals. It could possibly be related to a difference in methodology but if this is not the case it requires closer attention. Although it is hard to tell from the available data if the populations are on a declining stage, it is easier to predict that the increasing traffic and mortality rates are not sustainable and are contradicting the management priorities of the SCI BG0000366. There is an urgent need to rectify this situation.

It is clear that the fragmentation of the landscape in the broader area of Kresna gorge hasn't reach a level that could potentially threaten the survivor of any species of reptile or amphibian. Nevertheless there is an area of special concern. The area between E79 and the river Struma could be isolated from two barriers. Various ecological studies on habitat suitability and movement patterns indicate features such as rivers to act as barriers that may impede gene flow in certain animal groups. This is obviously not the case for the amphibians, the water snakes (Natrix sp.) and the turtle *Emys orbicularis.* All these species often use the waterbodies as dispersal corridors. However for most other reptile species the river represents a barrier that impedes their movement. Clearly a river is not an impenetrable barrier, most species are physically able to pass the river, in certain parts at least but usually they will prefer a least energy approach and avoid it. In this way the passage of new recruits to the habitat patches available on this belt is expected to be lower both from the river side and from the road side. Eventually the numbers of new recruits won't be enough to offset the mortality leading to decreased population densities and even to the extinction of certain species from this belt. Tortoises (Testudo sp.) are especially prone to this threat. Furthermore the lost of this narrow belt of habitat patches, a total size of 1.412 km^2, is not the major consequence on this line of events. The establishment of a bet like that, could significantly decrease the probabilities of gene flow between the east and west part of Struma in the Kresna gorge area. Obviously this bad scenario is not unavoidable and it depends on various factors, most significantly from the connectivity at the road side. There is an empirical approach to verify if fragmentation has decreased the value of this area and it requires comparative measurements of presence/absence and density of reptile populations on patches of similar habitat in both sides of Struma river. If the fragmentation effects are significant then the differences between measurements in the road/river part and the opposite part of the river are expected also to be significant.

2.3.5 Conclusions

- On the E79 road that passes through Kresna gorge the levels of road mortality of reptiles and amphibians are very high and a potentially significant barrier effect at the current traffic loads. This situation contradicts the management priorities of the SCI BG0000366 Natura 2000 area and should be improved.

- All of the studied scenarios are an improvement in relation to the current situation.

- During the operation of both the "East alternative G20" scenario and the "Long Tunnel" scenario the predicted mortality for the year 2040 will be lower by 58% for the reptiles and 25.1% for the amphibians in relation to 2014. The "Long Tunnel" scenario has higher risk levels as all trucks, from both directions, transporting dangerous (hazardous) loads will use the E 79 road.

- For the construction of the long tunnel there will be a period of around 6 years with increased level of road mortality - 6.3% for the reptiles and 3.1% for the amphibians- due to the additional load of traffic created by the trucks that will use the side tunnels.

- The most successful scenario is the "East alternative G10.50" with mitigation measures. Under this scenario, the predicted mortality for the year 2040 will be significantly lower for the reptiles and amphibians in relation to 2014 and to the other two alternatives. It must be emphasized that the degree of success largely depends on the proper implementation, monitoring and maintenance of the exclusion and connectivity structures.

- There will be no significant additional structures that will increase the fragmentation of the habitats inside the Kresna gorge area. The side tunnels for the Long Tunnel construction will increase the pressure in the area for a period of a few years but on the long-term the impacts are expected to be low.

- The effects of fragmentation to the populations of reptiles and amphibians and especially of the barrier effect inside the Kresna gorge are expected to be lower in relation to the current situation under all three scenarios. The most improved option will be the "East alternative G10.50" with mitigation measures as long as the connectivity structures are used as corridors. This requires monitoring of their use and a predetermined decision to make improvement if they proved necessary.

  On the long term all of scenarios that were evaluated represent an improvement in comparison with the current condition regarding the conservation objectives for the reptile and amphibian species included in the Annex II species of the 92/43/EEC directive. "East alternative G10.50" with mitigation measures is the best from the three approaches in order to minimize the existing pressures and comply with the requirements of the directive. However this is strongly related to the proper implementation and regular maintenance of the mitigation measures. Therefore a species by species assessment will follow in chapter 3.5 after the assessment of mitigation measures.

- Compliance with Recommendation 98 (2002)

  With the Recommendation 98 (2002) the standing Committee of the Bern Convention makes 9 Recommendations to the Bulgarian Government:

  "1. take account, in the development of this project, of the imperatives of conserving fauna, flora and habitats as well as the concerns of the local communities in the municipalities concerned;"

  All three alternatives take account of the imperatives of conserving fauna, flora and habitats

  "2.ensure that the decision on the routing of the motorway is taken on the basis of an in-depth environmental impact assessment (EIA) supplemented by scientific and mapping data and any other useful source of knowledge on the area concerned by the project, to justify the choice of alternative as recommended in the expert's report;"

  All three alternatives comply with this recommendation.

  "3. consider the possibility of abandoning the option of enlarging the current road since this would substantially increase damage to a unique site, without possible measures of compensation, and continue studying alternative routes located outside the gorge that would respect the natural constraints as far as possible and provide for the integration of engineering works and compensate for environmental impact;"

  All three alternatives are based on the abandonment of enlarging the current road. The "Long Tunnel" and "East alternative G20" are alternative routes while the "East alternative G10.50" is using an alternative route to diminish the traffic load and mitigation measures to improve the current situation.

  "4.ensure that the choice of alternative is based not only on technical, legal and economic criteria but also on social and ecological criteria;"

  Regarding the ecological criteria, all three alternatives comply with this recommendation. The "East alternative G10.50" has the best positive effects on the improvement of the current situation.

  "5. institutionalise dialogue and seek consensus solutions with the different partners concerned; active partnership could be forged with non-governmental organisations with sound knowledge of the location of habitats and the presence of protected species, and the setting up of advisory groups could be envisaged;"

  Not relevant to the design of three alternatives.

”6. provide for the downscaling and rehabilitation of the existing road, restoring its initial status of a local road used by the farming community and tourists and thus ease current pressure on the site, with suitable planning to revitalise damaged areas and provide user information services;”

It must be noticed that the three motorway alternatives (G10.50, East alternative G20, and the Long Tunnel) do not comply with this recommendation. In all cases the E-79 road through the gorge will not be closed. It is a first-class international road and can never be closed or become a walking or bicycle corridor, no matter what alternative will be selected. Concerning the East alternative G20, road E-79 will be used by the residents of the nearby settlements and tourists. In case of the long tunnel will also be used for the transfer of environmentally hazardous or flammable substances. However the three alternatives can contribute to revitalizing damaged areas and provide user information services.

”7. establish periodic site assessments (Kresna Gorge and motorway route), providing, as soon as the EIA is produced, the mapping and biological inventories necessary for long-term bio-monitoring;”

The compliance with this recommendation is independed from the alternative selection.

“8.select the zone concerned for the Emerald Network, by extending the central site to cover the gorge entrance and exit areas, to take stronger account of the biological functioning of the natural habitats and the connecting areas between the sites (ecological network of core area plus complementary areas);”

All three alternatives do not contradict this recommendation.

“9. ensure that adequate legal protection is given to the whole of the gorge site and its development areas.”

All three alternatives do not contradict this recommendation.

2.3.6 References

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3. Proposals on the mitigation measures for reptiles and amphibians

3.1. Main characteristics of the proposed mitigation measures

The assessment of mitigation measures for reptiles and amphibians in the Kresna gorge during the operation of the "East alternative G10.50" is based in the AA report and related studies, the road mortality study conducted during 2013-2016 and on fieldwork carried out during May 2017.

The main form of exclusion structure proposed as a mitigation measure on the E79 road in the Kresna gorge area are metal poles that support a wire mesh fence with a smaller mesh (5 mm) covering the lower part. There are also some short concrete retaining walls proposed in both sides of some underpasses. The related technical drawings are presented in Annex II. The proposed network of connectivity structures between Km 375+560.00 and Km 396+100.00 of E79 is composed of 223 elements that include 10 existing bridges, 2 existing tunnels (347 m and 76 m long acting as an overpasses), 20 existing box culverts/small bridges and 191 tube culverts most of them new. The details of these structures are presented in Annex I.

3.2. General guidelines of connectivity and exclusion structures

During the last decades the combination of exclusion structures and connectivity structures along motorways is a common practice in order to prevent road mortality (Luell et. al, 2003, BC Minisrty of Water, Land and Air 2004, Clevenger and Waltho 1999, Bank et al. 2002, Arizona Game and Fish Department 2006, Ruediger and DiGiorgio 2006, Jackson 2003, Huijser et al. 2008). These two forms of structures work as an effective mitigation measure only if they are combined in a way that guarantees that both structures are functional. Underpasses, which are the most commonly used connectivity structures, will be useless without an appropriate form of fencing that blocks animals from entering the road. Generally reptiles and amphibians will prefer to use the open sunlit surface of the road instead of a dark passage. Fencing on the other side will prevent animals from entering the road but if it does not directs them to the entrance of a suitable passage, it will be a barrier that cuts the connection of the populations.

3.2.1 Connectivity structures

A wide range of structures has been used and tested over the years as a way to facilitate the reptiles and amphibians crossing the roads. These include box culverts or tube culverts, small or larger bridges, overpasses and larger tunnels. All these structures had been used with varying degrees of success depending on a number of factors.

Size

The general rule is that a big, sunlit underpass is more suitable for most species of reptiles and amphibians. The recommended size or the underpass is also related to its length. A longer tunnel must be significantly larger in order to be attractive as a passage and sometimes breaking the underpass in two with an open surface in between is necessary for 20 meters width roads. The minimum recommended size for an underpass of less than 10 meters length is 75x100 cm. or a diameter of 1 m. in the case of a pipe. However size is not the most significant factor determining the usage of underpasses. Baxter-Gilbert (2015) report a case where turtles preferred to cross the road via a 1 m diameter drainage culvert instead of a 3.4 m x 2.4 m concrete box culvert. The usage of the drainage culvert should not be explained as a preference for smaller structures but rather as a result of its placement in an observed movement corridor for the species. In another example vipers (*Macrovipera schweizeri*) regularly used 60 cm diameter drainage culverts to pass under a 6 m road although they could easily bypass the less than 50 m. long barrier. These examples demonstrate that although an adequate number of larger underpasses should be available along the road, it is also important to have a wider choice of passages connecting areas of suitable habitats.

Location

As mentioned above the location of a passage is a very important factor for its approval and usage by reptiles and amphibians. The passage should connect areas of suitable habitat on both sides of the road or it should be on a known movement corridor. The availability of habitat does not represent a problem in the Kresna gorge area with small exceptions such as in the areas of the railway station and the restaurants. This assumption is also supported by the distribution of DOR animals found during the 2013-2016 Mortality studies. If underpasses were constructed for only a very limited number of species, then specific habitats should be targeted. In this case the goal is to mitigate for a wide variety of species, so diversifying passage locations assists in maximizing the likelihood of their use.

Entrance

Reptiles and amphibians should be able to reach the entrance of an underpass from both sides of the road. In those cases where the entrance is on a steep bare slope, usually formed from loose material, additional works are necessary following three steps.

- Smoothening of the slope in the area that leads to the entrance as much as it is technically feasible.
- Covering of the approach with a layer of fertile soil.
- Planting the area (slope) of approach with local bushes and other plans (but not trees) in order to stabilize it and also to provide a cover for reptiles and amphibians

Generally the vegetation should reach near the entrance of a passage in order to provide a covered approach for the animals but it should not cover it. An entrance surface free from vegetation is recommended so the vegetation around the entrance needs to be well maintained.

Spacing

It is important to have a wide choice of passages placed on diverse locations. In order to achieve that, the network of passages should be relatively dense. According to the literature (Iuell et al. 2003) a distance up to 150 m. between two underpasses is adequate for most reptiles and amphibians. However, as it has been mention a choice of different underpasses should be available so it is also suggested that a larger passage, eg. a box culvert 1mx2m or larger should be available every 600 meters.

Form

Underpasses for small animals such us amphibians and reptiles consist of pipes or rectangular tunnels. Rectangular tunnels are considered preferable for amphibians, and possibly other species, because the vertical walls provide better guidance. Pipes on the other hand are often cheaper than rectangular tunnels and easier to build under existing roads. Both types have been used successfully as underpasses. Prefabricated concrete elements are appropriate for rectangular tunnels. The connection between elements has to be smooth. Design solutions should be adopted that will prevent the tunnel from becoming waterlogged. To allow for a free-draining tunnel, the minimum gradient is 1%. Large gradients (30% or more) should be avoided. The lower point of the underpasses should at all times be above the level of the ground water.

Surface

The floor of the passage should be as natural as possible: soil, sand or small rocks form a suitable surface mainly for the amphibians as some reptiles species will tolerate even the cement. In the case of tube culverts the bottom surface should be filled to provide a leveled movement surface. It must be mentioned that any type of underpasses is expected to develop a soil layer overtime imported from the water and the wind. It is advisable to speedup this natural process during construction in order to optimize the use of connectivity structures. This will have no effects on the functionality of a well designed and constructed culvert. Maintenance works will ensure the adequate operation of the culvert.

Figure 3-12 An existing tube culvert in Kresna gorge area demonstrating a surface of soil, rocks and branches brought in from the water. In this case the middle section is partly blocked and needs maintenance.

3.2.2 Exclusion structures

An exclusion structure is a form of barrier that prevent animals from entering the road. The most common form of exclusion structure on highways is the wire mesh fence erected on metal poles. This form of barrier is primarily intended to prevent larger mammals such as deers, canids etc. from entering the road in order to avoid animal mortality but also for the safety of the drivers. In order to exclude also reptiles and amphibians, modification on these structures have been made. The most common form is the addition of a layer of material in the lower part of the fence that would prevent smaller animals to pass. The material that has been used in a number of cases is fine mesh (5mm), net, corrugated iron, heavy-gauge geotextile, plastic etc. The height of this layer is usually up to one meter and the lower 20 or 30 cm are often buried in the ground in order to avoid having animals pass underneath.

Some studies (eg.Baxter-Gilbert et al. 2015, Wilson & Topham 2009) have shown that this approach can be problematic. There are case where fencing has even increased road mortality in the sections of the road where it was applied. The main issues mentioned are rips and tears and other forms of degradation of the fencing, poor installation and also problems associated with fencing becoming washed-out, unburied etc. The deterioration of fencing can be very fast and it creates gaps. Reptiles and amphibians are experts in using tiny gaps in the fencing to gain access on the road. However the animals spend more time on the road trying to pass the fence on the other side, practically trapped on the road, and this can explain their increased mortality. On the other side the literature has also positive reports of drift fences that have significantly reduced road mortality of reptiles (eg. Aresco 2005) but the conclusion is that fencing need constant monitoring and repairs.

The most successful form of exclusion structure for reptiles and amphibians are the concrete retaining walls (Barichivich & Dodd 2002; Dodd et al. 2004) placed bellow the road surface into the sloped gravel between the shoulder and ditch. This form of barrier has been proven very successful with a 93% reduction in reptile and amphibian abundance on roads when hylid frogs were removed from the analysis (Dodd et al. 2004). The main problem is a much higher initial cost for the installation of this more durable, permanent exclusion structure. However a cost benefit analysis should take into account the high annual cost for monitoring, repairing and replacing parts of the fencing system. Over the long-term the concrete wall option may be more cost effective. Up to now it has been proven the most biologically effective option.

Figure 3-13 Fig. Construction phase of a concrete wall build as an exclusion structure for reptiles on an existing road.

Height

The minimum height of the barriers should be at least 60 cm. A vertical barrier of this height will prevent all the amphibians and most of the reptile species present in Kresna gorge area to reach the road. In the case of concrete retaining walls this height is a compromise that will stop the vast majority of species, while allowing animals that have managed to reach the road to pass/drop to the other side with no injury. From the species present in Kresna gorge only the largest individuals of three species of snakes (*Malpolon insignitus, Dolichophis caspius and Elaphe quatuorlineata*) are expected to be able to pass over a 60 cm. obstacle. These represent only a small portion of the population and and even smaller part (<1%) of the animals found DOR.

In the case of fencing, the barrier for the reptiles should have a height of 80 cm in order to stop most of animals, because if they reach the road they would be trapped and eventually killed with no escape option. In this case an additional 20 cm of barrier should be dug inside the soil in order to avoid having tortoises and other species dig their way under the fence. It is also advisable to have the top part of the barrier for the reptiles bend in a 90° angle. This will prevent the animals to exploit weak spots and climb over the fence.

Material

In the case of concrete retaining wall, it is the cement the forms the barrier. In this case the vertical element should reach the horizontal in a 90° angle or less (negative slope). The surface of the concrete wall should be as smooth as possible because many species are able to use knobs and recesses as steps in order to climb over. Any irregularities during the construction must be smoothed.

Concrete is thermic instable and may don not “work” properly under severe cold and extreme hot weather. In such conditions may present breaks or deformations which can constitute entrances for small animals to the motorway. In order to avoid such cases dilatation silts should be foreseen in the retaining walls. These silts should be covered by sheet metals which are anchored to the ground and not on the walls.

In the case of fencing the use of durable material in the design of exclusion structures is necessary. Flexible fencing is prone to rips and tears and very often presents weak points from the installation. It also quickly degrades in a short period of time and requires regular maintenance. Plastic and metal mesh fences are easily climbed by many reptile species and nets are totally unsuitable for the same reason. More durable materials such as steel box culverts can be used but in this case drainage should be taken into consideration. Washouts and flooding must be minimized, as exposure to water will degrade the structures.

Over or under the road

Concrete retaining wall runs under the road level while fencing is erected alongside the transport infrastructure near or in some distance from it but is always a barrier above ground level from both sides. There are two main advantages of the “under the road level” placement of retaining walls. Technically, they do not have the drainage problems associated with fencing and even in the worst cases they act as drainage ditches. Biologically, they permit animals that manage to reach the road surface to leave the road by jumping/ falling from a small height. Animals can be trapped on road in the case of fences if they pass either from weak points of the exclusion structures or from areas that can not be adequately fenced as in the case of side roads meeting the motorway.

Position

Exclusion structures must run alongside the transport infrastructure to prevent amphibians and reptiles from entering on the motorway. They are always placed outside of the crash barriers to avoids having vehicles getting caught or destroy the fence or fall from the structure. Fencing can be placed close to the road if there is no other option but it is preferable to be erected in some distance from it. The main purpose of exclusion structures is to stop the animals from entering the road but there is a secondary equally important function, guiding the animals to the underpasses. Guiding is important in order to give to as many animals as possible the choice to pass to the other side of the road through the connectivity structures and to avoid the fragmentation of the populations. In order to have the exclusion structures serve their guiding function and eventually lead the animals to an underpasses they have to be fitted very carefully.

The interaction between the exclusion and connectivity structures is critical. The exclusion structures must lead the animals directly to the entrance of the underpass and not just pass over it, because the animals usually will just follow the fence without been able to find the entrance.

Where the guiding structure joins the entrance to the tunnel, corners and edges that could be used for climbing should be avoided.

The join between the vertical part of the exclusion structure and the movement surface with a 90° angle is important as rounded surfaces don't provide adequate guiding.

The ends of the fences, in case of a side road or other obstacle, should be U shaped to block the animals from leaving the fence.

There is an alternative approach with a continuous fencing and differentiated guiding structures that lead the animals from the fence to the underpasses, but it is usually more complicated and expensive without any significant advantage.

Drainage

Generally fencing do not interfere with motorway drainage, since water can pass through the fence. The position of the concrete retaining walls should be designed in order not to change or stop run off water. As for the motorway drainage, the upper level of the retaining walls should not be higher than the motorway level, as it is presented in figure 3-13. Consequently the wall will not act as an obstacle to motorway runoff water. In cases where walls are constructed in the side of the motorway where runoff water is coming from the surrounding area (in case of cut), a ditch should be foreseen (as shown in figure 3-13). The ditch needs to be regularly cleaned, during the operation of the motorway, as is the case with all the hydraulic works of the motorway. In case of constructing the wall on embankment, the motorway designer should decide if a ditch is necessary.

Road safety

Generally fencing does not affect road safety. Concrete retaining walls do not affect road safety given that they are under the road level in order not to interfere with the car in case of an accident. The minimum height of the walls should be at least 60cm and the upper level of them shall have to be at the level of the motorway or lower.

Accessibility

Whether fencing or walls are used, the possibility should be foreseen, for personnel access from the motorway, for the implementation of maintenance works. This can be achieved through the installation of maintenance doors.

Comparison between Concrete retaining walls and Fencing

Based on the above mentioned concrete walls, even they have more difficulties in construction and technical adaptation to the motorway design are considered more effective than fencing on reducing amphibians and reptiles road mortality.

However, where the construction of retaining walls is technically very difficult (e.g. in case where there is no enough space between the motorway edge and the cut) proper fencing can be foreseen.

In any case, monitoring and maintenance works are essential to keep the very low road mortality rates.

3.3. Assessment of mitigation measures proposed - Additional proposals

The assessment of mitigation measures for reptiles and amphibians in the Kresna gorge during the operation of the "East alternative G10.50" is based in the AA report and related studies, the road mortality study conducted during 2013-2016 and an on spot appraisal during May 2017.

3.3.1 Assessment of connectivity structures

In the area of Kresna gorge, between Km 375+560.00 and Km 396+100.00 the proposed network of connectivity structures is composed of 223 elements that include 10 existing bridges, 2 existing tunnels (347 m and 76 m long acting as an overpasses), 20 existing box culverts/small bridges and 191 tube culverts most of them new. The details of these structures are presented in App I.

The number of planned underpasses is considered adequate as the mean distance between them is less than 90 m.

The largest distance between two underpasses is between Km 391+990.00 and Km 392+530.00. This is a distance of 540 meters with no connectivity structures, due to the existence of high cuts close to the road. It is also the area of the restaurants in the side of the road which is obviously unsuitable for most reptiles and amphibians. The unsuitability of this part is also supported from the lack of casualties according to the road mortality data.

The area between Km 376+280.00 and Km 377+220.00 is covered by only 4 underpasses. However this is acceptable as this area, which is north of the gorge and just outside of the village Simitli, includes a large road junction, private fenced properties and sub optimal habitat for most reptile species.

If we exclude the restaurants area and the unsuitable northern part of the road, the average density of connectivity structures is nearly one every 80 meters. This density is very good and it is expected to allow an adequate number of animals to pass in order to avoid fragmentation of the populations.

In order to assess the proper location of underpasses, the localities of DOR animals found during the road mortality studies were superimposed over the localities of the connectivity structures. The correlation was very good and the vast majority of the reptiles and amphibians was found in a distance of less than 40 m. from a proposed underpass.

Regarding the size of connectivity structures we can separate them in two categories. The existing bridges, tunnels (overpasses) and box culverts represent the larger structures while the tube culverts are considered as smaller structures.

The larger connectivity structures cover well the largest part of the road. However the following three parts of the road have no large underpasses while there are important habitats (Annex II species of 92/43)

From Km 383+685.00 to Km 385+570.00

From Km 386+070.00 to Km 388+230.00

From Km 392+760.00 to Km 394+900.00

This could be improved by replacing some of the proposed 1 meter diameter pipes with box culverts 1m x 2m or larger.

The smaller connectivity structures are tube culverts with the following diameters

111 tubes Φ 100 cm

27 tubes Φ 80 cm

34 tubes Φ 50 cm

19 tubes Φ 30 cm

The larger Φ 100 tubes are more appropriate to be used as underpasses. However the smaller tubes are used in between larger ones and the distance between two consecutive larger tubes is less than 150 m. This creates an adequate system of underpasses for reptiles and amphibians.

Overall, the proposed connectivity structures are considered appropriate in order to avoid the fragmentation of the populations of reptiles and amphibians in Kresna gorge after the installation of exclusion structures.

3.3.2 Suggestions for improvements of connectivity structures

Replacement of proposed 1 meter pipe (88) at Km 384+975.00 with box culvert 1m x 2m or larger.

Replacement of proposed 1 meter pipe (117) at Km 386+670.00 with box culvert 1m x 2m or larger.

Replacement of proposed 1 meter pipe (130) at Km 387+460.00 with box culvert 1m x 2m or larger. This area is important for *Eurotestudo hermanni*.

Replacement of proposed 1 meter pipe (186) at Km 393+675.00 with box culvert 1m x 2m or larger. This area is important for *Emys orbicularis*.

Wherever it is technically possible replace the smaller Φ 50 cm and Φ 30 cm tubes with larger ones.

Cover the bottom surface of the new pipes with soil and stones during the installation to provide a leveled movement surface.

Note. According to the initial analysis of the proposed connectivity structures the following suggestion was also added. “*Replacement of proposed 80 cm pipe (74) at Km 384+130.00 with box culvert 1m x 2m or larger. This area is important for Emys orbicularis.*” However during the on spot appraisal it was noted that underpass 74 was already a box culvert. This needs confirmation.

3.3.3 Assessment of exclusion structures

The main form of exclusion structure proposed as a mitigation measure on the E79 road in the Kresna gorge area are metal poles that support a wire mesh fence with a smaller mesh (5 mm) covering the lower part. There are also some short concrete retaining walls proposed in both sides of some underpasses.

The main type of fencing proposed has many disadvantages for the reptiles and amphibians, because many species can climb over the fine mesh and also it has a very short life. Weak spots that will enable animals to pass are expected from the first year.

3.3.4 Suggestions for improvements of exclusion structures

Examine the possibility to totally replace the fencing with concrete retaining walls or at least replace it in the more important parts of the road taking into consideration the 2013-2016 road mortality studies. In the cost benefit analysis, the high annual maintenance costs that are necessary to maintain the fencing in working order on the long term should also be included.

In the case of fencing, the replacement of the fine mesh wire with a more durable material is recommended. It should also be more suitable as a barrier for reptiles and amphibians. One suggestion for such a material is galvanised steel panel 1.5 mm, with an expected life of nearly 20 years, but there are other options also depending on local availability.

Take into consideration the general guidelines for the connectivity structures and mainly the following points.

- In the case of fencing and panels the fencing element for reptiles and amphibians must be dug (20) cm in the ground
- Fences or retaining walls must guide the animals to the entrances of the connectivity structures.
- There should be no openings or other forms of "weak points" (eg. points that will allow animals to climb over) in the connection between two fencing panels or between fencing and underpass entrance.)

3.4. Assessment of impacts on the conservation priority species of the reptiles and amphibians from the implementation of East alternative 10.50 with mitigation measures

According to the Standard Data Form for the SCI BG0000366 "Kresna-Ilinntetsi", 2 species of amphibians and 5 species of reptiles are present in the Kresna gorge area. According to the 92/43/EEC Directive core areas of their habitat are the designated as sites of Community importance (SCIs) and included in the Natura 2000 network. These sites must be managed in accordance with the ecological needs of the species. The assessment of impacts on those species from the implementation of East alternative G.10.50 with mitigation measures is focused in the Kresna gorge.

Bombina variegata

For the "Kresna-Ilinntetsi" area, according to the SDF, the species is characterized as Common with 23 known localities and the conservation degree of its habitats as excellent. Finding casualties of this species on E79 is a very rare event therefore the species is not at significant pressure from mortality. The reason for that seems to be its preference for temporary pools on higher elevations and smaller water bodies such as some of the smaller tributaries of Stuma, while avoiding larger water bodies such as Struma. During operation phase the implementation of East alternative G.10.50 with mitigation measures will have no significant (at a population level) effect on its mortality and will possibly result in a small improvement in the connectivity of its population. The same impacts are expected from the "East alternative G20" and the "long Tunnel".

Triturus karelinii

For the "Kresna-Ilinntetsi" area, according to the SDF, the species is characterized as Rare with only 5 known localities and the conservation degree of its habitats as excellent. Finding casualties of this species on E79 is a very rare event therefore the species is not at significant pressure from mortality. The reason for that seems to be its avoidance of large bodies of moving water such as Struma river. It prefers standing water basins and seems to be more common at the foothills of the Pirin slopes, east of the Kresna gorge. During operation phase the implementation of East alternative G.10.50 with mitigation measures will have no significant (at a population level) effect on its mortality and will possibly result in a small improvement in the connectivity of its population. The same impacts are expected from the "East alternative G20" and the "Long Tunnel".

Testudo hermanni (=Eurotestudo hermanni)

For the "Kresna-Ilinntetsi" area, according to the SDF, the species is characterized as Common with 25 known localities and the conservation degree of its habitats as excellent. This species is one of the more common road casualties on E79. It is a rather common species in the habitats and their ecotones near the road. During operation phase the implementation of East alternative 10.50 with mitigation measures will have very significant positive impact on the mortality and a significant positive impact in the connectivity of its populations. It is therefore a recommended solution to meet the 92/43 conservation objectives. It must be noted that retaining walls are a much more successful form of exclusion for this species than any form of fencing and it is strongly suggested to be implemented at least at the important areas for the species. The "East alternative G20" and the "Long Tunnel" will have positive medium impacts both on mortality and population connectivity.

Testudo graeca

For the "Kresna-Ilinntetsi" area, according to the SDF, the species is characterized as Common with 23 known localities and the conservation degree of its habitats as excellent. This species is one of the more common road casualties on E79. It is a rather common species in the habitats and their ecotones near the road. During operation phase the implementation of East alternative 10.50 with mitigation measures will have very significant positive impact on the mortality and a significant positive impact in the connectivity of its populations. It is therefore a recommended solution to meet the 92/43 conservation objectives. It must be noted that retaining walls are a much more successful form of exclusion for this species than any form of fencing and it is strongly suggested to be implemented at least at the important areas for the species. The "East alternative G20" and the "Long Tunnel" will have positive medium impacts both on mortality and population connectivity.

Emys orbicularis

For the "Kresna-Ilinntetsi" area, according to the SDF, the species is characterized as Present with no information about its population and the conservation degree of its habitats as excellent. This species is often found as road casualty on E79 but usually in very specific areas. This can be explained by its habitat preferences as it probably utilizes mainly those parts of Struma river where the riverbed is wider and the water speed diminishes. During operation phase the implementation of East alternative 10.50 with mitigation measures will have significant positive impact on the mortality and a significant positive impact in the connectivity of its populations. It is therefore a recommended solution to meet the 92/43 conservation objectives. It must be noted that retaining walls are a much more successful form of exclusion for this species than any form of fencing and it is strongly suggested to be implemented at least at the important areas for the species. The "East alternative G20" and the "Long Tunnel" will have low positive impacts both on mortality and population connectivity.

Elaphe situla

For the "Kresna-Ilinntetsi" area, according to the SDF, the species is characterized as Very Rare with only 1 known locality and the conservation degree of its habitats as excellent. However this species is one of the more common road casualties on E79 as large parts of the habitat near the road are optimal for the species. During operation phase the implementation of East alternative 10.50 with mitigation measures will have very significant positive impact on the mortality and a significant positive impact in the connectivity of its populations. It is therefore a recommended solution to meet the 92/43 conservation objectives. The "East alternative G20" and the "Long Tunnel" will have positive impacts both on mortality and population connectivity.

Elaphe quatuorlineata

For the "Kresna-Ilinntetsi" area, according to the SDF, the species is characterized as Rare with only 9 known localities and the conservation degree of its habitats as excellent. This species is a rather uncommon road casualty on E79 verifying its status as rare. During operation phase the implementation of East alternative 10.50 with mitigation measures will have small positive impact on the mortality and a significant positive impact in the connectivity of its populations mainly by facilitating the dispersal of young individuals. The

"East alternative G20" and the "Long Tunnel" will have small positive impacts both on mortality and population connectivity.

3.5. Proposal for monitoring

A monitoring program must be implemented during the operation of "East alternative G10.50" in Kresna gorge for the evaluation of the results of mitigation measures as a basis for subsequent repairs and other decisions. The program should focus at least on the following subjects.

Road mortality.

Any form of road mortality of reptiles and amphibians is a sign of a weak point in the exclusion structures that should be repaired or upgraded as soon as possible.

Use of connectivity structures

A significant number of underpasses (more than 10% at any given time) of different form and size should be monitored during the active period for reptiles and amphibians. Emphasis must be given in the underpasses of the more important corridors. Monitoring can be done with movement detection cameras, tracks on the sand in underpass entrances and other methods. Although great differences in the use of underpasses is expected, the absence of regular movement of reptiles in at least a part of the connectivity structures would be an early sign of an increased risk of population fragmentation that should be addressed.

Maintenance of the exclusion and connectivity structures.

At least once annually and before the beginning of the main active season for reptiles and amphibians (March) all the elements of the mitigation measures should be closely checked. The main points that should be checked are the following.

- Integrity of the fence. Check for openings in the fencing system or spots where it has collapsed, washed out etc.
- Condition of connectivity structures. Check for underpasses that have been blocked from materials carried from the water flow and other problems.
- Vegetation along the exclusion structures. The development of the vegetation near the fences or walls provides "bridges" for the reptiles and amphibians that helps them climb over the barrier. In this case cutting back the vegetation is necessary.

Any problems reported should be addressed as soon as possible.

Some other, more specific studies could also be included in the monitoring program depending on the preliminary findings eg. Radio tracking the movements or tortoises and other priority species near the road etc.

A detailed monitoring approach in order to maximize the effectiveness of the fieldwork during the first years of operation could be as following.

Research team

The research team should be composed from the field specialists and the analysis specialists. A researcher could participate in both teams but generally separating these two categories generates more reliable results.

The field team should compose from at least three researchers. The two of them must have experience with reptiles and amphibians and the skills to identify the animals to species level without necessarily capturing them. Walking on difficult terrain carrying a load will be necessary.

The analysis team should be composed with researchers specialists in reptiles and amphibians and population modeling.

Fieldwork intensity

The fieldwork for reptiles and amphibians should cover all the main activity season, generally from March to October. One field visit per week is the minimum for the first years. Especially during the first year it is advisable to increase it to two field visits per week during spring as this is the period with high levels of activity and mortality. Intensifying the fieldwork during the first year will maximize the likelihood to identify weak spots in the exclusion structures. The following years monitoring intensity will be adjusted according to the previous year findings.

Description of fieldwork

A three member team is the minimum because during the monitoring, inspections will be necessary at the road and in the areas left and right of the road. After the installation of fencing it will not be easy to move from the one side of the road to the other. Safety protocols must be developed and followed and the whole distance of the gorge will be covered on foot. The estimated time necessary for every field visit is around 10 hours but this must be checked in situ.

The team member responsible with mortality on road will record every dead animal, not only reptiles and amphibians, using the same protocols and procedures that were used in the mortality monitoring during the period 2013-16.

The other two members of the field team will be concentrated on the monitoring of the populations in the sides of the road and on the use of underpasses. Moving parallel to the road they will record all the reptile and amphibian individuals they will spot. This is a line transect method which can give estimates of abundance. A sample protocol for that is included.

For the monitoring of underpasses it is advisable to use two different techniques, movement detection cameras and tracks on a suitable surface. Cameras can be placed in at least 20 underpasses and they usually give good results for the species that use them. However they tend to loose small or slow moving individuals and underestimate the use of underpasses. The cameras will be placed inside and on the top of the underpass near one of the two entrances. In every visit, the researcher must check and replace if necessary the memory card and the batteries. He also must check if the camera is working properly and if the date and time are correct. For the track method, both entrances of the underpass must be covered with a thin layer of material suitable for track imprinting such as marble dust or sand. The researcher must note all his findings in a special protocol and then level the soil and prepare it for the next period. This method gives good information about the numbers of reptiles and amphibians that use an underpass and also if they use it to pass (tracks on both sides) or if they only enter end exit from the same entrance. However it does not give good information about the species that use them as it is generally very difficult or impossible to recognize species of reptiles from tracks but only the main categories they belong (lizards, snakes, tortoises). The combination of cameras and tracks gives enough information to evaluate the degree of usage of underpasses and this means that the track method must be implemented in the same underpasses with the cameras. However it is advisable to use the track method in a larger sample (eg. 60 underpasses) as it is easier and cheaper to implement. This will also allow to move cameras to underpasses that are used more often.

For the selection of the underpasses that will be monitored emphasis must be given in the areas of the more important corridors, based on the available data on the areas with higher mortality during the 2013-16 period. The sample must also be representative of the different types and sizes of underpasses used. A random selection can be used after these two main criteria have been applied. The underpasses that will be monitored will change from year to year but it is advisable to keep some of them eg. one third constant in order to check long-term trends. Each underpass must have a code (number) painted in order to avoid mistakes.

If a dead reptile of amphibian is found on road, the team must check for weak points in the fencing in order to explain how the animal was found on the road. It the problem can be easily fixed eg. branches of a rock that bridged the barrier, they must solved it. If it is something more complicated, then it should be reported as soon as possible, to the body responsible for the road. A standard procedure for that must be developed.

Protocols

For the development of databases and the data analysis standard data recording protocols are important. These can be used by the field team either in paper form or electronic form. The electronic form (eg. with a tablet) is more effective and less prone to errors because a number of fields, such as date, time and coordinate, can be filled automatically. Generally the protocols must have all the fields necessary in order not to miss important information but they must also avoid the mistake to ask for a lot of information that will not be used making their use a time consuming and difficult task. For the road mortality the existing protocol can be used.

For the population monitoring the following fields can be included.

- Date
- Time
- Researcher code
- Species
- Distance from main line of movements
- Additional info (if possible juvenile, adult, male, female)
- Coordinates
- Weather (Sun, Clouds, Rain)
- Temperature
- Notes

This is a minimized protocol that does not include a number of variables such as habitat, altitude etc. These variables can be extracted from the location (coordinates) and will be included in the analysis.

For the track method on the underpasses the protocol can include the following fields.

- Date
- Researcher code
- Underpass code
- Side (E or W. East or west considering only the general direction of the road and not the specific spot)
- Species (or group)
- Size (if possible small or large)
- Direction (in or out. If the individual moved in the underpass or came out of it)
- Notes (free text)

Those fields must be filled for every single individual or line of tracks.

Additional protocols must be developed for the analysis of the camera images, placement of cameras and analysis of tracks.

It is noticed that the monitoring results will verify the estimation of impacts that are presented in this report. Activities related to the further minimization of impacts on reptiles and amphibians should be determined and specified, according to these results. These activities will include the following:

- Further improvement or adaptation of the monitoring methodology.
- Type and frequency of maintenance works especially on the connectivity and exclusion structures.
- Possible additional rehabilitation works in order to improve biotope conditions affected by the motorway construction and operation phases.

3.6. References

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ANNEXES