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#### How Many Days to Monitor a Wildlife Passage? Species Detection Patterns and the Estimation of the Vertebrate Fauna Using Crossing Structures at a Motorway

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Abstract: The barrier effect imposed by roads and railways on vertebrate populations has aroused both scientific and social concern and has led to the construction of crossing structures for such fauna in new infrastructures. Good practice demands that investment in such mitigation measures should be followed by systematic monitoring of their effectiveness, in order to improve the design of further works. These monitoring schemes need standardized protocols in order to deliver scientifically sound results at an affordable cost. In this context, the present contribution analyzes the suitability of monitoring schemes aimed at determining which vertebrate species use crossing structures in relation to the number of days spent monitoring each crossing structure. The analysis considers data on vertebrates using 22 structures crossing a motorway in northwest Spain, which were monitored for 15-26 consecutive days. Species accumulation curves were fitted by non-linear estimation procedures to the species accumulation pattern detected at each crossing structure in order to estimate the asymptotic number of species using each one of them. Modelling was carried out using 11 functions applied in ecological studies to analyze species accumulation curves in relation to sampling intensity. The results show that species accumulation curves for crossing structures have a rapid increase phase followed by a long tail of slow accumulation. Thus, 25 or more monitoring days may be needed to detect over 80 percent of the species using a crossing structure, but 60 percent of them are detected by day 10, and 70 percent, by day 16. The statistical fit obtained for different function types allows the Clench model to be recommended for evaluating the results obtained in monitoring programs intended to determine the number of species using each crossing structure. This model yielded the highest mean explanatory power (mean r<sup>2</sup>=0.905) using only two parameters; it provided neither a systematic overestimate nor an underestimate of richness, and offered a low degree of uncertainty (2.3% non-significant parameters). In short, 10 to 15 days of monitoring may be enough to provide a basic knowledge of the animal species using crossing structures at a particular time, although the monitoring period could be somewhat shorter or longer according to the requirements of particular cases.

### **Introduction**

Scientific and social concern about the barrier effect imposed by roads and railways on vertebrate populations has led to the construction of crossing structures for fauna in new infrastructures. Economic development is accompanied by increases in the kilometerage of motorways and railway lines, and a corresponding decline in the extent of land patches free from such infrastructures (Forman and Alexander, 1998). This process affects fauna in numerous ways (Robinson et al. 1992), but especially by making it difficult or impossible for animals to move freely (Oxley 1974, Mader 1984, Swihart and Slade 1984, Goosem 2001). Hence, crossing structures, such as overpasses, bridges, and culverts, are being increasingly incorporated into road and railway construction to facilitate faunal movement (Saunders et al. 1991, Clergeau 1993, Rodrígez et al. 1996, Kéller and Pfister 1997, Rosell et al. 1997, McGuire and Morrall 2000). Although the number, complexity, and cost of such structures are rising rapidly, studies of their effectiveness have lagged behind their installation.

Good practice requires that investment in mitigation measures should be followed by a systematic monitoring of their effectiveness. Such reviews will not only assist in their management, but will also optimize any future expenditure on improvements (Forman et al. 2003, luell et al. 2003). At present, information on the use of crossings by fauna is somewhat fragmentary and derives from intensive scientific research carried out in relatively few places, most of them in North America (see review in Forman et al. 2003). A notable gap in knowledge of the effectiveness of crossings would be filled should routine monitoring schemes by transport agencies become generalized. Ideally, monitoring should evaluate different types of crossing structures and should cover the whole vertebrate community, with a view to discovering to what extent such constructions are used by fauna (Yanes et al. 1995, Ng et al. 2004, Mata et al. 2005). Moreover, monitoring schemes need standardized protocols in order to deliver scientifically sound results at an affordable cost.

One of the key aspects of monitoring protocols for faunal crossing structures is the length of the monitoring period at each structure, which reflects directly on the survey costs and on the utility of the resulting data. So far, a range of different monitoring periods have been employed, most often involving monitoring each structure for 10-20 consecutive days (Rodríguez et al. 1996, Brudin 2003, Mata et al. 2005). Nevertheless, the significance of the duration of such surveys has not previously been evaluated.

In theory, monitoring periods should be the minimum required to obtain the most complete picture possible of the process under study. As a first step, it is necessary to discover which species use the crossings over a particular period, e.g., a season or a year. Such data allow the detection of species which do not use the crossings and, hence, whose populations on either side of the route have become separated. It is also possible to see whether any species found as road-kills shy away from the dedicated crossing structures but cross the carriageway routinely instead. This assessment of crossings, based on the entire vertebrate community, clearly differs from those concerned with particular target species(Singer and Doherty 1985, Foster and Humphrey 1995, Gloyne and Clevenger 2001, Cain et al. 2003), but complements studies of the long-term adaptation of fauna to new infrastructures (Clevenger and Waltho 2005).

The problem of whether our monitoring program is representative of the species using the crossings is a recurrent theme in ecological studies, the relationship between the number of species observed as a function of the sampling

effort expended. The problems and usefulness of species accumulation curves have been analyzed repeatedly and have occasionally provoked controversy (Gotelli and Colwell 2001, Gray et al. 2004). The main consideration here is that they may be the only way of estimating a variable that cannot be measured: the species richness that would be detected by an infinitely large sampling. Such an estimate requires a mathematical model, and here another difficulty of species accumulation curves arises: different models tend to produce slightly different results (Flather 1996, Thompson et al. 2003).

The present study has the following objectives: firstly, (1) to analyze the patterns of accumulation of observations of vertebrate species using faunal crossings, to evaluate the representativeness of the data obtained as a function of the sampling period. Also, (2) to establish the capacity for mathematical modelling of the observations obtained by applying functions that permit the modelling of such species accumulation. Finally, (3) to derive recommendations for protocols for monitoring of faunal crossings, relating both to the use of mathematical models and to the duration of the study periods required.

#### **Methods**

#### **Data collection**

The data on use of faunal crossings by animals were collected during summer 2002 on the Benavente-Puebla de Sanabria sector of the A-52 motorway (Zamora Province, NW Spain, fig. 1). This is a fenced dual carriageway, with average traffic levels of 6,000 vehicles/day. A set of 22 crossing structures between kilometer posts 19.5 and 63.5 were selected for monitoring. They comprised six circular culverts, three wildlife-adapted (box) culverts, four open span underpasses, three wildlife underpasses, four overpasses, and two wildlife overpasses (Mata et al. 2005).



Figure 1. Location of the study area (A-52 between Puebla de Sanabria and Benavente) in relation to main roads in Northwest Spain.

The use of crossing structures by vertebrates was analyzed by daily track monitoring. Records were obtained using marble dust, a scentless material which produces imprint tracks of high quality and persistence given its high density (Yanes et al. 1995). A band 1-m wide and 3- to 10-mm deep of the marble dust was installed halfway across each selected crossing structure (Mata et al. 2005). The animal species using each crossing were identified and recorded daily, although only data from days in which the meteorological conditions allowed correct imprinting were used. Monitoring lasted until a minimum of 15 valid recording days were obtained for each crossing structure, but the dataset includes structures monitored for up to 26 days (fig. 2).



Figure 2. Length of monitoring period of crossing structures used in the present study.

Track identification followed Strachan (1995), Sanz (1996), Bang and Dahlström (1995), and Blanco (1998), and was taken to species level whenever possible. However, apart from those species identified specifically, the following groupings were recorded: anurans (all frogs and toads), lacertids, ophidians (including legless lacertids), small mammals (mice, voles, and shrews), water voles (*Arvicola sapidus* and *A. terrestris*), rats (*Rattus rattus* and *R. norvegicus*), lagomorphs (*Oryctolagus cuniculus* and *Lepus granatensis*), small mustelids (*Mustela nivalis* and *M. erminea*), cats (*Felis catus* and *F. silvestris*), and large canids (*Canis familiaris* and *C. lupus*). For simplicity, the text refers to "species" both for species-proper and for groups producing similar tracks.

### Data analysis

The first step of the analysis aimed to test whether the use of each crossing structure by vertebrates was uniform throughout its monitoring period or whether there was any kind of temporal aversion to its use after the marble dust was laid. Hence, an ANCOVA test was carried out with the number of species as a dependent variable, the crossing structure as a fixed factor, and the day as a covariate. Due to the potential non-linear nature of the monitoring day effect, the ANCOVA test was repeated after the log-transformation of the covariate day. A significant covariate with a positive beta parameter value in any of these analyses would mean that fewer animal species used the crossing structure during the initial days following the start of monitoring.

The second level of analysis focused on species accumulation patterns. Thus, the raw data of species using each crossing structure each day were transformed into daily values of accumulated number of species for each structure. These data were then used to fit models of species accumulation curves for each crossing structure. Model fit was carried out through nonlinear estimation methods with Statistica 6.1 (StatSoft, Inc. 2002). The Levenberg-Marquardt estimation method and default settings of the program were set for the analysis. In cases where Statistica was unable to find a solution, a new trial was carried out using the parameters fitted by ModelMaker 3.0 (Walker and Crout 1997) as start values.

In total, 11 species accumulation functions were fitted to the data from each crossing structure. The functions employed represent the spread of those used to a greater or lesser extent in investigations relating ecological problems involving species accumulation to sampling effort (Thompson et al. 2003). Those used include functions with two, three, or four parameters, and there are equations that present one asymptote and others that are infinitely increasing (table 1).

Model name	Mathematical expression	Asymptote			
- Two parameters (a, b)					
Clench	y = (a * t) / (1 + (b*t))	a/b			
Negative exponential	y = a * (1 – exp (-b * t))	а			
Exponential	y = a + (b * log(t))	None			
Power	$y = a * t^b$	None			
B-Logaritmic	y = log(1 + (a * b * t)) / b	None			
- Three parameters (a, b, c)					
Asymptote	$y = a - (b * c^t)$	а			
Chapman-Richards	y = a * ((1 – exp (-b * t)) <sup>c</sup> )	а			
Rational	y = (a + b * t) / (1 + c * t)	b/c			
Hill	$y = (a * b * t^{c}) / (1 + b^{c})$	None			
- Four parameters (a, b, c, d)					
Beta-P	$y = a * (1 - (1 + (t / c)^{d})^{-b})$	а			
Weibull	$y = a * (1 - exp(-(b * (t - c))^d))$	а			

Table 1. Species accumulation functions used in the present study in relation to sampling effort (t =number of monitoring days)

After fitting the functions, the asymptotic value of the number of species present at each crossing point could be deduced according to the different models. The number of species predicted to occur after 100 monitoring days was calculated for those functions that did not generate an asymptote (table 1). The choice of 100 days was arbitrary but represented a period thought long enough (longer than a full astronomical season) to derive the maximum expected species number at a crossing point. It is also clearly longer than would be possible during routine monitoring programs.

A number of complementary criteria were used when evaluating the suitability of the different species accumulation functions, relating to goodness of fit, the predictions made, and the type of function used. With respect to data adjustment consideration was given to (1) the number of cases in which the data could not be fitted because the statistical software was unable to find a correct solution, (2) the variance absorbed ( $r^2$ ) by the adjusted function, (3) the percentage of significant parameters, as a measure of the reliability of the predicted values, and (4) the number of cases in which each function proved to be the most mathematically appropriate. This last analysis was done by comparing the Bayesian Information Criterion values of the different functions (Quinn and Keough 2002).

Two additional criteria were employed in relation to the predictions of the maximum number of species using a crossing. Predictions that were greater than the number of species known to be present in the study area, according to national distribution atlases (Palomo and Gisbert 2002, Pleguezuelos et al. 2002), were rejected as erroneous. The mean number of species predicted by each function was also evaluated with a view to detecting any systematic biases towards over- or under-estimation of species richness (Thompson et al. 2003).

Once the group of functions that best represented species accumulation at the crossings had been identified, the data for each crossing structure were transformed into percentages relating to the predictions of asymptotic (or 100-day) species richness at that crossing. Thus, the observations from the different crossing points were recalibrated to fit a curve showing percentage of observed species as a function of the number of monitoring days. This function is taken as the mean pattern of species accumulation at the wildlife crossing structures, for the purposes of the discussion.

### <u>Results</u>

The study detected 20 species, with a daily mean ( $\pm$ SE) of 1.39 $\pm$ 0.05 species/crossing structure. The number of species using the crossing structures daily varied significantly between crossings (see ANCOVA test in table 2), ranging from 0.52-2.26 species/day. Nevertheless, the number of species using each crossing did not vary significantly during the study period (table 2), despite a slight increase in this number as the study proceeded (*beta*=0.025 $\pm$ 0.017). This same result was revealed by the logarithmic transformation of the day covariate (Covariate In (day), *F*=0.929, p=0.346).

Table 2. Results of the ANCOVA used to control for the effects of the "day of monitoring" covariate and the "crossing structure" factor on the variable number of species detected at a crossing structure.

	MS	d.f.	F	р
Day of monitoring	9.799	1	2.248	0.149
Crossing structure	4.360	21	5.626	<0.001
Error	0.775	413		

By the end of the study period, a mean of  $5.59\pm0.34$  species per crossing had been detected, with a range of 3-8 species. Although most new species at each crossing were detected during the first 10-12 days of study, some new species continued to appear later (figure 3). Indeed a new species was detected on the last day of study at the only crossing point monitored for 26 days.



Figure 3. Raw data of species accumulation in crossing structures along the monitoring period. Note that steep lines represent the detection of new species.

The mathematic modelling was generally feasible, and the data fits obtained may be considered good, although the various models differed in performance (table 3). All functions employed produced high explicative values, these being generally higher among models with more parameters (average  $r^2$  of 0.893, 0.908 and 0.930 for two-, three- and four-parameter models, respectively). The fit obtained was highly significant (p<<0.001) in all cases where the statistical program generated a mathematical solution.

Nevertheless, overparametrisation problems arose with some frequency when fitting models with three and, especially, four parameters (table 3). Thus the statistical program did not find an appropriate solution in 41 percent of cases with the Weibull model and in 27 percent with the Beta-P model (the two models using four parameters). Similarly, the three-parameter Chapman-Richards model could not be fitted in two cases (9%). In contrast, a mathematical solution was possible with all two-parameter functions. Overparametrisation is also reflected in that the highest percentages of significant parameters correspond to two-parameter models (four out of five model families with more than 90 percent significative parameters), this feature declining with more complex models.

Model name	no	r <sup>2</sup>	r <sup>2</sup>	unreal	Best	% significant	expected
	solution	(mean±SD)	(min)	prediction	fit	parameters	richness
- Two parameters)							
Clench	0	0.905±0.054	0.782	2	3	97.7	8.63
Negative exponential	0	0.897±0.065	0.761	2	4	90.9	6.30
Exponential	0	0.860±0.076	0.688	0	0	72.7	8.69
Power	0	0.898±0.054	0.768	0	8	100.0	13.01
B-Logaritmic	0	0.905±0.053	0.799	2	1	90.9	9.57
- Three parameters							
Asymptote	0	0.905±0.058	0.762	3	0	87.9	6.88
Chapman-Richards	2	0.916±0.055	0.768	9	0	56.7	9.50
Rational	0	0.912±0.045	0.829	3	2	59.1	9.55
Hill	0	0.898±0.054	0.768	0	0	77.3	13.01
- Four parameters							
Beta-P	9	0.937±0.042	0.849	4	2	40.4	8.16
Weibull	6	0.924±0.051	0.817	3	2	82.8	7.77

Table 3. Results of fitting species accumulation functions to data from the A-52 motorway

Apart from the problem of a lack of mathematical solutions when fitting certain species accumulation functions, unreal predictions were generated in some instances (table 3). The maximum number of potentially detectable vertebrate species (including taxonomic groups) in the study area was 26, a figure exceeded by eight of the 11 models used. Only the Exponential, Power, and Hill models did not produce this type of error in predictions, although the predictions used for these three models corresponded to the species totals expected at day 100, since they do not have asymptotes (table 1). The models most frequently generating unreal predictions were the Chapman-Richards (9 out of 20 cases) and Beta-P models (4 out of 13 cases).

The models which proved most often appropriate on the basis of mathematical fit (table 3) were the Power (8 cases), the Negative exponential (4), and the Clench (3) models. Considering all the crossing points, two-parameter models were best in 16 cases, three-parameter ones in two cases, and four-parameter models in four cases.

Finally, the different models generate a relatively broad spread of predictions of the numbers of different species using the crossings. The mean numbers predicted (table 3) varied from the 6.30 and 6.88 species generated by the Negative exponential and Asymptote models to the 13.01 species from the Power and Hill models.

On the basis of these results (see Discussion), the fit provided by the Clench function was chosen for further analyses. Once the species accumulation data had been converted to percentages of the asymptotic values, a function was fitted to the data substituting parameter *b* by the expression a/100. This simplification was possible since, by definition, the asymptotic value of the function is 100 percent, and the asymptote of the Clench model is a/b (see table 1).



Figure 4. Average pattern of species accumulation in crossing structures in relation to the asymptotic number of species using each one. Points show mean±SE of percentage species detected, and the line represents the Clench model fitted to the whole dataset.

The resulting function (figure 4) is highly significant (F=141.8; 19 d.f.; p << 0.001) and has a value for parameter a±SE of 15.20±0.73. The figure shows that after 25 monitoring days only 20 percent of the species predicted to use a particular crossing would remain to be detected. According to the model, 10 monitoring days are needed to detect 60 percent of the species using a crossing, and 16 days to detect 70 percent of them.

### **Discussion**

This study shows the pattern of vertebrate species accumulation at the crossing structures of a motorway and the procedure for synthesizing such observations mathematically. Furthermore, the results enable recommendations to be made regarding monitoring protocols for such mitigation measures.

The findings show that detecting all the species which use a faunal crossing is a lengthy and uncertain process. In the present study new species were often detected until the final monitoring day, even after 25 days of observation. Undoubtedly more species would have been detected had the study continued for longer. Most studies on faunal use of a broad range of crossing structures have sampled 10-20 days (Rodríguez et al. 1996, Brudin 2003, Mata et al. 2005), although occasionally fewer (Taylor and Goldingay 2003) and sometimes less than a week (Hunt et al. 1987, Yanes et al. 1995, Ng et al. 2004). Longer studies are unusual and tend to concentrate on a small number of species or on particular crossing structures (Reed et al. 1975, Jackson and Tyning 1989, Foster and Humphrey 1995, Mathiasen and Madsen 2000), or have formed part of extensive investigations into roads and fauna (Clevenger and Waltho 2000, Clevenger et al. 2001).

In any event, the representability of the sampling period must be borne in mind when analyzing and discussing the data obtained. Most studies do not evaluate the potential effects of sampling period duration or sidestep the problem by grouping all the data into one large sample (Clevenger et al. 2001, Ng et al. 2004). In the former case, short sampling periods may underestimate the usage of faunal crossings and may complicate inter-sample comparisons as a result of the random variation between small samples. This problem is more significant if, in addition, the sampling period differs between samples (Rodríguez et al. 1996). Grouping data from different sampling projects avoids these concerns, given that the total sampling period is then quite long, but such a procedure prevents the analysis of the seasonal use of faunal crossings.

Thus, a proper choice of study period permits the optimizing of the amount of work done in relation to the value of the results obtained. The ideal situation is to adjust the study period for each faunal crossing so that the results are directly representative of the animals using the crossing during the season sampled. This permits between-season comparisons (Rodríguez et al. 1996, Mata et al. unpublished data) and the evaluation of long-term changes in the use of the crossings (Clevenger and Whalto 2005).

Mathematical approximations, by means of species accumulation models, may play an outstanding part in deciding how long monitoring periods should be. On the one hand, models extend variables beyond the sample limits (Gotelli and Colwell 2001, Wainwright and Mulligan 2004), in this case to estimate the number of species missed due to the brevity of the sampling period. Although such approximations do not allow the missing species to be known, they do permit the reliability of the data obtained to be evaluated. In addition, the models allow generalizations to be made from sample series which show some degree of variability (Quinn and Keough 2002). Nevertheless, the application of mathematical models to natural processes requires caution to avoid generating mathematically-sound predictions which are unreal from a biological viewpoint (Peters 1991). This applies here in the cases of models which predicted the use of crossing structures by more species than are actually present in the area.

The results support using the Clench species accumulation model to analyze observations of vertebrates using road crossings. The simplicity of the species accumulation pattern detected and the use of short datasets make it unnecessary and even counter-productive to use models based on more than two parameters, which are frequently adequate for large data sets (Flather 1996, Thompson et al. 2003). This aside, two-parameter models enable the use of general purpose statistical software (e.g., Statistica, SPSS) in future applications; whereas, solving more complex models often demands the use of specific modelling programs. Among the two-parameter functions, the Clench model achieved the highest value of  $r^2$  and the second highest percentage of significant parameters. In addition, it has the advantage of having an asymptote, a feature shared only with the Negative exponential model among the two-parameter functions. This characteristic avoids the need to use an arbitrary sampling period (100 days in this study) to estimate the number of species potentially using a faunal crossing. Finally, the Clench model gives intermediate estimates within the range generated by all the models, distinct from the over- and under-predictions of the Power and Negative exponential models, respectively (see also Thompson et al. 2003).

#### **Applied implications**

The results obtained generate recommendations applicable to monitoring programs at faunal crossing structures, although certain considerations must be taken into account. Firstly, the results were obtained from data collected in one area in a single season. Similar studies elsewhere are desirable to confirm the general validity of the conclusions. There could be seasonal differences in the usage patterns of faunal crossings, although these seem to be fairly uniform throughout the year, at least in Mediterranean landscapes (Rodríguez et al. 1996, Mata et al. unpublished data). In addition, differences may arise associated with the faunal richness of the areas surrounding the crossing structures,

in a manner analogous to those encountered in applications of species accumulation curves to other sampling problems (Flather 1996, Gray et al. 2004). Finally, it is necessary to emphasise that the suggestions made are aimed at routine monitoring programs intended to evaluate the use of crossing points by vertebrates. In comparison with these, studies aimed at specific species have radically different characteristics (Singer and Doherty 1985, Foster and Humphrey 1995, Gloyne and Clevenger 2001, Cain et al. 2003).

The present study draws two basic conclusions in relation to the establishment of monitoring protocols for faunal crossing points. Firstly, the species accumulation curves obtained should be examined to evaluate the extent to which the monitoring program has detected the majority of the species using the crossings, to see whether the study period needs extending. The Clench model is indicated for this purpose. Moreover, the use of models may allow comparisons of data derived from sampling periods of different lengths (Flather 1996, Gotelli and Colwell 2001).

Secondly, the results of the present study indicate that study periods at crossing structures should comprise 10-15 days in order to detect most species. Nevertheless, this period may be extended or curtailed depending on various factors. It may be worth shortening the study period to 7-8 days per structure, for example, where there are a large number of crossings to monitor and if the costs of putting on and removing the monitoring systems are small. More than 50 percent of species will have been detected by then, and the rate of appearance of new ones will decline so that it may then be advantageous to switch to another crossing. By this means, at least in theory, the rarest species should eventually be detected at one structure or another, providing a more complete picture of the use of the crossings by fauna. On the other hand, study periods should be longer where there are few crossings structures, such as large ecoducts, to monitor. With these figures as a guide, the suggestion is to employ standardized study periods for all the structures monitored, with only exceptional variation: e.g., extending the period at ecoducts.

In conclusion, our results support the feasibility of establishing compulsory monitoring programs for the measures adopted to remedy the barrier effect of new linear infrastructures. On the one hand, the implementation costs are not very great, given the relative brevity of the monitoring period needed to obtain a basic idea of their use by fauna. Also, the monitoring may produce datasets for large areas which can be of great interest provided that data collection follows certain minimum scientific requirements. Hence, the development of protocols that include an adequate sampling period is essential to operating such monitoring programs and for their results to be of maximum utility. The extension of science-based "low intensity - large area" monitoring schemes would complement in-depth research focused on target species or sites.

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**Biographical Sketch:** The authors are lecturers and researchers of the Terrestrial Ecology Group of the Ecology Department of the UAM, where they conduct diverse studies into applied vertebrate ecology. Their current areas of investigation cover aspects of environmental impact assessment, the design and evaluation of mitigation measures, and the development of automatic systems for tracking fauna.

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