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The detectability and persistence of road-killed butterflies: An experimental study

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ABSTRACT

Estimates of detectability and removal of carcasses have been determined for vertebrates but not for invertebrates, although the latter are more frequently killed on roads. The aim of this study was to estimate the detectability and persistence of road-killed butterflies and to assess the relative contributions of the location on the road, volume of traffic, and presence of scavengers to the removal of dead butterflies. During three independent experiments, dead butterflies from 12 species were placed at randomly chosen locations along selected road sections (both on asphalt and on a verge) with varying vehicle traffic volume (including sections without vehicles) and scavenger abundance. Dead butterflies were counted, scavengers' behaviour was observed, and the traffic volume was measured. The detectability was higher for butterflies placed on asphalt (0.979) than on a verge (0.767) and increased with body size. The removal rate of dead butterflies from asphalt on roads with vehicles was four times faster (persistence probability of 12 h: 0.139) than on roads where vehicles were absent (persistence probability: 0.508). Overall, only 5% of butterflies persisted for a 48-h period. Vehicles and bird scavengers removed 14.9% and 9.6% of dead butterflies, respectively. The probability of dead butterfly removal by birds was positively correlated with traffic, indicating that the number of road-killed butterflies might be substantially underestimated on roads with high traffic. This shows that imperfect detection should be taken into account when estimating the number of road-killed butterflies, especially for butterflies on road verges.
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1. Introduction

Roads play an important role in the functioning of animal and plant populations (Forman and Alexander, 1998; Rytwinski and Fahrig, 2007; New, 2015). Roads can represent dispersal corridors (Wynhoff et al., 2011), and road verges are surrogate habitats for several species, mostly insects (Ries et al., 2001; Saarinen et al., 2005; Valtonen et al., 2007). However, roads that have a high traffic volume might also be a source of pollution (Munoz et al., 2015), act as dispersal barriers (Bhattacharya et al., 2003), divide local populations (Fahrig and Rytwinski, 2009), or deteriorate habitat quality in the neighbouring landscape (Port and Thompson, 1980). The most direct effect of roads on animal populations is deadly collisions with vehicles (Malo et al., 2004; Seiler, 2005; Rytwinski and Fahrig, 2007; D'Amico et al., 2015). A high rate of animal road mortality might negatively affect the demographics of several endangered species (Mumme et al., 2000; Fajardo, 2001; Jackson and Fahrig, 2011; Soluk et al., 2011). Driving safety is also affected by collisions with animals, and it is estimated that 1 to 2 million collisions occur between vehicles and large animals each year in the United States and that 5.5% of all serious on-road casualties result from a direct impact with an animal in Australia (Huijser et al., 2008; Rowden et al., 2008).

To assess the impact of road mortality, it is necessary to make robust and unbiased estimates of the number of road-killed animals on a given road section. Road mortality estimates are affected by two factors: carcass detectability and removal. Carcass detectability is related to body size and colour, as well as the location of the carcass on a road (Guinard et al., 2012; Teixeira et al., 2013). Low detectability is especially relevant for small animals, such as butterflies, where observers might easily omit some individuals by chance. Carcass persistence is mostly influenced by vehicle flow (Teixeira et al., 2013), scavenger activity, and weather conditions (Slater, 2002; Munoz et al., 2015). Accounting for these limitations, it is essential to evaluate the effects of road-kill impacts on wildlife populations as well as the efficient allocation of resources needed to mitigate road impacts (Teixeira et al., 2013).

Studies on vertebrates have previously included methodologies to account for the imperfect detection and removal of carcasses (Guinard et al., 2012; Teixeira et al., 2013). Few studies on road mortality have been performed on insects, even though insects (e.g., butterflies) are among the most commonly recorded road-killed animals (Mckenna et al., 2001; Rao and Girish, 2007; Soluk et al., 2011; Baxter-Gilbert et al., 2015; Munoz et al., 2015). No study has addressed the issue of imperfect detection and the removal of butterfly carcasses. It is usually assumed

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that the number of road-killed butterflies found on roads represents the daily estimate of the butterfly-vehicle collision rate, and the removal of dead butterflies by scavengers is normally ignored.

Using a series of three field experiments, I tested three hypotheses:

(1) Detectability is higher for larger and brighter butterfly species than for smaller and darker ones and for butterflies that are present on asphalt rather than on a road verge.
(2) Dead butterflies are more rapidly removed from asphalt than from a road verge because butterflies on asphalt can be removed by both vehicle wheels and scavengers, while butterflies lying on a road verge are usually only removed by scavengers.
(3) Dead butterflies located on asphalt are more likely to be removed by vehicle wheels than bird scavengers, and larger and brighter butterflies are more likely to be scavenged by birds than smaller and darker ones.

Testing these hypotheses allows for the following questions to be addressed: (1) what is the detectability of road killed butterflies and how does their body size and colour affect detectability? (2) How long do road-killed butterflies persist on a road? (3) What is the contribution of the location on a road, volume of traffic and presence of scavengers to the removal of road-killed butterflies?

2. Methods

2.1. General study design

The construction of numerous new roads in Poland due to current road development enabled the research hypotheses to be empirically tested. During the road building process, there is a period in which asphalt is on the road, but vehicles are not yet allowed to use the road (Fig. S1 in Supporting information). This provides an excellent opportunity to obtain unbiased (due to the absence of traffic) estimates of detectability via experimental placement and subsequent counting of dead butterflies on the road. Similarly, the presence of both new roads that have no traffic and nearby used roads creates the opportunity to estimate how quickly experimental dead butterflies are removed and what proportion of the removal can be assigned to traffic. Additionally, fate of dead butterflies may be determined through direct observations at road with varying traffic volumes and bird densities.

The study consisted of three experiments. The first experiment was established to study the detection bias of road-killed butterflies depending on their location on a road as well as on their body size and colour. Dead butterflies were placed at randomly chosen locations along selected roads where vehicle traffic was excluded. In the second experiment, the persistence of dead butterflies was studied on two road types: roads without vehicle traffic and with vehicle traffic. Thus, the effect of vehicle traffic on the removal rates of dead butterflies could be distinguished from the effect of biotic factors (scavenger activity) on the removal rate. In the third experiment, which was complimentary to the second experiment, dead butterflies were placed at different sites on roads with varying traffic volumes and bird densities, and their fate was observed from a hidden location (e.g., whether they were removed by a vehicle, scavenger, or wind).

2.2. Study area

The study was conducted in the Krakow and Tarnow vicinity in southern Poland between 2011 and 2014.

2.3. Experiment 1. Estimation of detectability bias

The estimation of detectability may be obscured by other factors, such as vehicle traffic, wind activity, and scavenger activity. Therefore, selected sections of the road without vehicle traffic were used (Fig. S1 in Supporting information). These consisted of 1-km sections of recently built sections of ring roads in Tarnow (centre of the road section: 50°02′31″N, 21°00′40″E), Skawina (49°59′25″N, 19°50′24″E), Szczurowa (50°07′49″N, 20°36′44″E), and Wojnicz (49°56′29″N, 20°50′43″E). Experiments were conducted in 2011 (Szczurowa and Skawina), 2012 (Tarnów), and 2013 (Wojnicz) (see: file1.kmz in Supporting information).

Dead butterflies were distributed at random locations within a 1-km road section, both on the asphalt and on verge, with a 1-m belt boundary between the asphalt and verge. Road verges were covered by sparse, spontaneously developing ruderal vegetation that was dominated by *Artemisia vulgaris*, *Chenopodium album*, *Elymus repens*, *Lolium perenne*, *Polygonum aviculare*, and *Tanacetum vulgare*. The bare ground cover was approximately 20%. In each road section, an equal number of individuals were placed on asphalt and the verge.

Twelve butterfly species that differed in body size and colour were used for the experiments (Table 1, Fig. S2 in Supporting information). In the case of *Polyommatus icarus* I, males and females were assigned to distinct groups due to their large sexual differences with regards to wing colour and because other less common species were not used.

Twenty-four to twenty-eight individuals of each species were placed on each road section. In total, each species was represented by 100–106 specimens. These specimens were previously acquired from a minimum of fifteen (*Pieris rapae* and *Coenonympha pamphilus*) different meadows to minimise the impact on their populations (well below 1% of individuals present at a given time at each site were captured– up to five individuals per site per year). All of the studied species are common in Poland and have numerous populations, and none of these species are protected by law. No legal permission is required for capturing invertebrates in Poland. The procedure was approved by independent referees during the study proposal evaluation by the funding body. For each specimen, morphological measurements were taken for the purpose of a different study. These butterflies were killed by a gradual decrease in temperature to −20 °C and were subsequently kept at this temperature.

Each dead butterfly placed on the road was marked with an individual number, and only one butterfly species was placed at a time during each experiment. Immediately following the placement of dead butterflies at the appropriate locations, three independent and naive observers counted the dead butterflies that they observed. Each observer walked along the delimitated road section and noted the dead butterflies and their identification number (without removing them). The second observer started their observations once the first had finished counting. The third observer followed the same procedure. Observers did not have contact with each other and could not see one another during the experiment. The observers had no research experience; thus, detectability was not influenced by previous experience. This method of calculation was used for each species. Each observer was followed by the experimenter who had previously placed the dead butterflies who

Table 1
Butterfly species used during the study.

Species	Body size	Body colour	wing span [mm]
Maniola jurtina	Large	Dark	46
Aphantopus hypernatus	Large	Dark	40
Inachis io	Large	Dark	60
Pieris brassicae	Large	Bright	55
Pieris rapae	Large	Bright	43
Gonepteryx rhamni	Large	Bright	51.5
Erynnis tages	Small	Dark	26.5
Boloria dia	Small	Dark	34
Polyommatus icarus (females)	Small	Dark	30
Polyommatus icarus (males)	Small	Bright	30
Thymelicus lineola	Small	Bright	27
Leptidea sp.	Small	Bright	37.5
Coenonympha pamphilus	Small	Bright	32

also noted the butterflies present. The role of the researcher was to determine the potential impact of scavengers (in the time between successive observations, dead butterflies might have been removed, for example, by birds). The experimenter knew the exact location of the dead butterflies and determined which had disappeared from the road and those that were not detected by the observers. These observations were performed on windless, sunny days. The order in which the butterfly species were tested was random. After the observations were completed, all of the dead butterflies were removed from the road and used for the second experiment (with the addition of new individuals if necessary). Completion of the entire experiment took three days (four to five species were studied per day) for a given road section.

2.4. Experiment 2. Estimation of the persistence of dead butterflies on roads

This experiment studied how long dead butterflies remained on two types of roads: (1) with vehicle traffic and (2) without vehicle traffic. Using roads with and without traffic allowed the impact of vehicles on dead butterfly removal to be distinguished from that of scavengers. The same 1-km road sections (file1.kmz in Supporting information) from experiment 1 were used, located in Szczurowa and Skawina (in 2011), Tarnów (2012), and Wojnicz (2013). Nearby roads with vehicle traffic located in a similar landscape were selected (centre of each road section with traffic, Szczurowa: 50°07′26″N, 20°33′51″E; Skawina: 50° 00′44″N, 19°53′00″E; Tarnów: 50°03′34″N, 21°04′35″E; Wojnicz: 49° 59′04″N, 20°50′24″E, see: file2.kmz in Supporting information).

As in experiment 1, the road sections were 1-km long. Three butterfly species were placed at each site (*Aphantopus hyperantus*, *Pieris rapae*, *and Coenonympha pamphilus*). Only one species was tested at a time. Thirty individuals of each species were placed on each road section. In total, each species was represented by 240 specimens (120 on roads with vehicle traffic and 120 on roads without vehicles). The butterflies were arranged at random locations on the asphalt and verge, within a 1-m belt boundary between the asphalt and verge (50 cm on asphalt and 50 cm on a verge). In each road section, equal numbers of specimens were placed on the asphalt and verge. Butterflies were placed starting at 5:30 in the morning. Dead butterflies were counted immediately after they were distributed on roads with traffic and those from which vehicles were excluded. Counting stared at 6:00, and subsequent counts were performed at 18:00 on the same day, at 6:00 and 18:00 the following day, and finally at 6:00 on the third day. All butterflies were counted by the same experienced observer who had placed them at the chosen locations. The purpose of this procedure was to reduce detection bias to a minimum. Observations started on days with good weather (no rain or strong wind). Observers moved at a steady pace of 1 km per 20 min. Dead butterflies from the detectability experiment were reused whenever possible. Similar to the first experiment, additional butterflies were captured at several locations. In addition to counting butterflies, the most important bird scavengers were also counted (Hope Jones, 1980; Erritzoe et al., 2003). Bird counting was performed immediately before and immediately after the experiment. All of the birds within a 100-m belt on both sides of the road sections were counted. Observations to assess the abundance of scavengers on both road types started at 5:00 and lasted for 20 min (the removal of dead butterflies might be dependent on the scavenger abundance).

2.5. Experiment 3. Investigation of factors affecting dead butterfly removal from roads

This experiment was complimentary to experiment two and was performed in 2012–2014. In this experiment, dead butterflies were placed at random locations on different roads and were observed using binoculars to assess whether the butterflies were removed as well as the cause of the removal.

The same species from experiment 1 were investigated in this experiment, and any remaining specimens were used. Additional specimens of the *Aphantopus hyperantus*, *Pieris rapae*, and *Coenonympha pamphilus* species were collected from ten different meadows. Each species was represented by 20–26 individuals (half of which were placed on asphalt and half on a verge).

The dead butterfly placement locations were chosen from randomly selected geographical coordinates in the area between the city of Kraków, the town of Tarnów, and the nearest road (see: file3.kmz in Supporting Information). Butterflies were placed from the start of June to the start of September. Date randomisation was attempted, but was constrained by several factors (other experiments being performed at the time, unpredictable weather, car malfunctions etc.). The distance (in centimetres) from the road-verge boundary was randomised within a 1-m belt. For example, a value of 50 cm indicated that a butterfly was placed on the verge 50 cm from the boundary line between the asphalt and road, while a value of 50 cm indicated that a butterfly was placed on asphalt 50 cm from the boundary line. This coding allowed for the inclusion of the butterfly location as a continuous variable in subsequent analyses, which provided statistical advantages.

Although the dead butterfly placement locations and the survey dates were generated before the study started, it appeared unfeasible to move between randomly chosen locations within such an extensive area. Therefore, usually two to six locations that were geographically close were surveyed per day for practical purposes. This issue was subsequently addressed in the analyses by using generalised linear mixed models (GLMM) (see: Data analysis).

When a dead butterfly was placed on a road, whether the dead butterfly was removed by passing vehicle, scavengers or wind was noted from the distance (100 m). When a butterfly was removed, the time from start of the observation was noted. Observations lasted for a maximum of 2 h, and if a butterfly was not removed during this time, it was removed manually and the observation period ended. The scavenger (birds) density was estimated before the start of an observation over a 10 min period by counting all birds present in a 100 m radius surrounding the selected location. Bird-counting was performed immediately prior to the dead butterfly observation. Vehicle traffic was estimated by counting all vehicles in a 60 min period during the dead butterfly observation.

2.6. Data analysis

2.6.1. Experiment 1

To estimate the probability of dead butterfly detection by observers, a GLMM with a binomial error distribution and logit-link function was used. The dependent variable was coded as 1 (the observer found a butterfly) or 0 (the observer did not record a butterfly). The fixed factors were (1) body-mass index (wing span), (2) colour (bright versus dark), and (3) location on the road (verge versus asphalt), as well as all of the second and third order interactions between these factors. The observer, site, and butterfly identities as well as butterfly species were random factors.

2.6.2. Experiment 2

To estimate the persistence probability of a dead butterfly on the road, a GLMM with a binomial error distribution and complementary log-log link function for observations with no termination event ("survival analysis") was used. The dependent variable was coded as 1 (a butterfly was present) and 0 (a butterfly was not present). The fixed factors were (1) road type (roads with or without vehicle traffic), (2) location on the road (verge versus asphalt), and (3) trial (the time since the placement of a dead butterfly [06:00 first day, 18:00 first day, 06:00 second day, 18:00 second day, 06:00 third day]), as well as all of the second and third order interactions between these factors. The random factors were the site identity, butterfly species, and individual butterfly identity (nested within butterfly species).

To compare the density and species richness of potential scavengers (birds) between the two road types, a GLMM with a negative binomial distribution and log-link function was used. This distribution is appropriate for counting data. The explanatory variable was the road type. Site and survey (first versus second) were random factors. As several recorded birds were mostly granivorous species (e.g., *Emberiza citrinella*, *Columba palumbus*) and some hunt insects in flight (e.g., *Hirundo rustica*, *Apus apus*), the analysis was also repeated according to density and richness of species that were classified as insectivorous birds foraging on (or near) the ground.

2.6.3. Experiment 3

The probability of a dead butterfly being removed by a vehicle or bird scavengers was separately estimated during the 2-h observation period using a GLMM with a binomial error variance and logit-link function. The dependent variable was coded as 1 (dead butterfly taken) or 0 (dead butterfly still present on the road). The explanatory variables were (1) butterfly size (wing span), (2) colour (dark versus bright), (3) location on the road (verge versus asphalt), (4) traffic volume (number of vehicles passing per h), (4) density of potential scavengers (birds), and (5) date in the season. The random factors were butterfly species, year, and spatial cluster (points surveyed during one day). Similar to the second experiment analysis, the analysis of these factors was repeated to include the abundance of birds that were insectivorous species foraging on or near the ground.

Hierarchical partitioning (Chevan and Sutherland, 1991) was also used to calculate for each explanatory variable separately, an estimate of the independent contribution to the probability of a dead butterfly being removed (1) by a vehicle or (2) by a bird. Hierarchical partitioning computes the increase in the fit for all models that contain a given variable and compares it to an equivalent model without that variable. The mean improvement in fit (reduction in deviance) across all possible models containing that predictor is then computed. This process results in an estimation of the independent contribution of each explanatory variable (I) and the joint contribution (J) resulting from the correlation with other variables (Mac Nally, 2002). Thus, the relative independent contribution of each predictor (% I) can be determined. Randomisation tests that yield Z-scores were used to determine the statistical significance of the relative independent contributions based on an upper confidence limit of 0.95 (Mac Nally, 2002).

All GLMMs were performed in SPSS 21.0 (IBM corp.). Hierarchical partitioning was conducted using the 'hier.part' package (Walsh and MacNally, 2005), implemented in R version 3.1.2 (R Development Core Team, 2014). The means are presented with the standard error (SE) and 95% confidence interval (CI).

3. Results

3.1. Experiment 1

The mean detectability was 0.925 ± 0.009 (CI: 0.903–0.942), and the detectability of dead butterflies was higher on asphalt (0.979 ± 0.004, CI: 0.969–0.986, Table 2) than on verges (0.767 ± 0.021, CI: 0.716–0.811, Table 2). The detection probability increased with body size (estimate = 0.043 ± 0.019, CI: 0.002–0.085, Fig. 1, Table 2), and the effect of body colour was statistically non-significant (Fig. 1, Table 2). No interaction term was statistically significant (Table 2). Among the random factors, only butterfly identity was statistically significant (estimate = 2.445 ± 0.224, Z = 10.921, P < 0.001). Species identity had no significant effect (estimate = 0.088 ± 0.080, Z = 1.098, P = 0.272), and site and observer identities had variance estimates equal to zero.

3.2. Experiment 2

The mean persistence ("survival") of dead butterflies in a 12-h period was much lower on roads used by vehicles (0.249 ± 0.022, CI: 0.208–0.296) than on roads from which vehicles were excluded (0.548 ± 0.024, CI: 0.492–0.607; Table 3, Fig. 2). The persistence probability decreased with time (Fig. 2) and was generally higher on verges (0.503 ± 0.023, CI: 0.449–0.560) than on asphalt (0.278 ± 0.024. CI: 0.233–0.329; Table 3). However, a statistically significant interaction term was present between the road type and location of the dead butterfly on a road (Table 3). An examination of this interaction showed that the removal of dead butterflies from asphalt on roads with vehicles was almost four times more rapid (persistence probability: 0.139 ± 0.022, CI: 0.101–0.188) than on roads from which vehicles were absent (persistence probability: 0.508 ± 0.031, CI: 0.446–0.573, Fig. 2). When a dead butterfly was on a verge, its removal was also more rapid on roads with vehicles (persistence probability: 0.422 ± 0.027, CI: 0.368–0.482) than on roads without vehicles (persistence probability: 0.589 ± 0.030, CI: 0.527–0.653), but the difference between the road types was significantly smaller than in the case of asphalt (Fig. 2). The persistence probability decreased with time (Fig. 2).

No differences in the mean number of bird species (GLMM $F_{1,13}$ = 2.928, P = 0.111) or bird abundance (GLMM $F_{1,13}$ = 1.752, P = 0.208) were found between roads with vehicles or without (Table S1 in Supporting information). The mean number of species was 20.9 ± 1.6 (CI: 17.7–24.8) and 17 ± 1.5 (CI: 14.3–20.7) for roads with vehicles and roads without vehicles, respectively. The abundance of birds was 41.9 ± 4.9 (CI: 32.1–54.8) for roads with vehicles and 34.2 ± 4.1 (CI: 26.1–44.8) for roads without vehicles. The repeated analysis of bird species classified as insectivorous species did not reveal statistically significant differences in the number of bird species (GLMM $F_{1,14}$ = 1.735, P = 0.209) or their abundance (GLMM $F_{1,14}$ = 1.541, P = 0.235) between roads with vehicles and without.

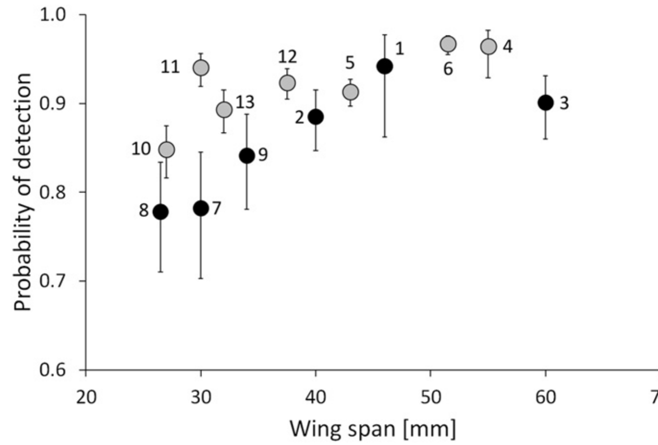

Fig. 1. The effect of body size index (wing span) and body colour (grey circles – bright; black circles – dark) on the detection probability. The effect of body colour was not statistically significant. Estimates (with 95% confidence intervals) from generalised linear mixed models are presented. The numbers represent different species: 1 – *Maniola jurtina*, 2 – *Aphantopus hyperantus*, 3 – *Inachis io*, 4 – *Pieris brassicae*, 5 – *P. rapae*, 6 – *Gonepteryx rhamni*, 7 – females of *Polyommatus icarus*, 8 – *Erynnis tages*, 9 – *Boloria dia*, 10 – *Thymelicus lineola*, 11 – males of *Polyommatus icarus*, 12 – *Leptidea* sp., and 13 – *Coenonympha pamphilus*.

Table 2
Factors affecting the detectability of dead butterflies on roads. Statistically significant effects are shown in bold.

Effect	F	df_1, df_2	P
Location on a road	15.578	1, 3902	**<0.001**
Body size	11.983	1, 17	**0.003**
Body colour	0.967	1, 14	0.372
Location on a road × body size	0.227	1, 3902	0.634
Location on a road × body colour	0.827	1, 3902	0.363
Body size × body colour	0.010	1, 17	0.920
Location on a road × body size × body colour	0.171	2, 37	0.844

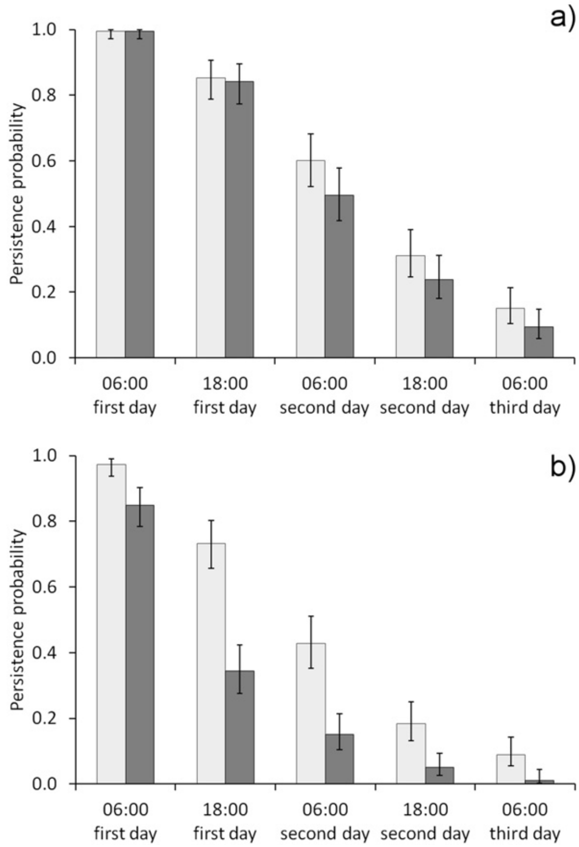

Fig. 2. The persistence probability ("survival") of dead butterflies on roads (a) without vehicles and (b) roads with vehicles. Butterflies were placed both on asphalt (dark bars) and on the grassy verge (bright bars). Estimates (with 95% confidence intervals) from generalised linear mixed models with a log-log link function are presented (see Table 3).

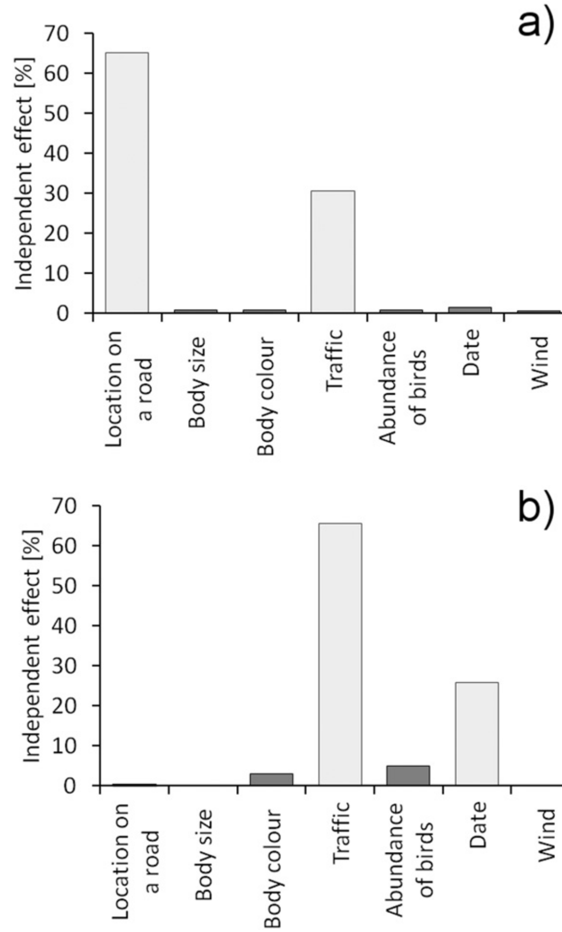

Fig. 3. Decomposition of the total reduction in deviance associated with environmental variables into independent components using the hierarchical partitioning method. The independent contribution of variables to the probability of removal of a dead butterfly by (a) vehicles and (b) bird scavengers. Variables that had the strongest and statistically significant impact on the probability of carcass removal are shown in bright bars.

3.3. Experiment 3

Out of 302 dead butterflies placed on roads, 45 (14.9%) were removed by vehicles, 29 were removed by birds (9.6%), and three disappeared during the 2-h observation periods (Table S2 in Supporting information).

The probability that a dead butterfly was removed by vehicle wheels was positively correlated with traffic (estimate $= 0.013 \pm 0.003$, CI: 0.008–0.019, Table 4) and negatively correlated with the increasing distance from the asphalt centre (estimate $= -0.091 \pm 0.015$, CI: -0.121 to -0.060, Table 4). Other variables, including random factors, were statistically non-significant. Hierarchical partitioning also indicated that among studied variables, traffic and location on a road were the variables that contributed the most to the reduction in deviance (Fig. 3a).

The probability that a dead butterfly was removed by birds was positively correlated with the date (estimate $= 0.018 \pm 0.007$, CI: 0.003–0.032, Table 5, Fig. S3 in Supporting information) and, unexpectedly, with vehicle traffic (estimate $= 0.007 \pm 0.001$, CI: 0.003–0.011, Table 5, Fig. S4a in Supporting information). Vehicle traffic was not correlated with bird abundance (Fig. S4b in Supporting information). Other variables, including random factors, were statistically non-significant. When this analysis was repeated using the abundance of insectivorous birds instead of the abundance of all birds, very similar results (Table S3 and Fig. S4c in Supporting information) were found. Hierarchical partitioning also indicated that among studied variables, the date and traffic contributed the most to the reduction in deviance (Fig. 3b).

Table 3
Factors affecting the persistence of dead butterflies on roads. See: Table 2 for other explanations.

Effect	*F*	df_1, df_2	*P*
Road type	98.646	1, 3580	<**0.001**
Location on a road	55.411	1, 3580	<**0.001**
Trial[a]	206.102	4, 3580	<**0.001**
Road type × location on a road	27.309	1, 3580	<**0.001**
Road type × trial	1.081	4, 3580	0.364
Location on road × trial	2.173	4, 3580	0.069
Road type × location a road × trial	0.526	4, 3580	0.717

[a] "Trial" denotes consecutive counts on a transect.

Table 4
Factors affecting the probability that a dead butterfly will be removed by vehicles. See: Table 2 for other explanations.

Effect	*F*	df_1, df_2	*P*
Location on a road	12.904	1, 294	<**0.001**
Body size	0.615	1, 294	0.434
Body colour	0.186	1, 294	0.667
Traffic	22.549	1, 294	<**0.001**
Abundance of birds	0.288	1, 294	0.592
Date	1.355	1, 294	0.245
Wind	0.040	1, 294	0.245

Table 5
Factors affecting the probability that a dead butterfly will be removed by bird scavengers. See: Table 2 for other explanations.

Effect	F	df_1, df_2	P
Location on a road	0.040	1, 294	0.842
Body size	0.054	1, 294	0.817
Body colour	0.219	1, 294	0.640
Traffic	12.987	1, 294	<**0.001**
Abundance of birds	0.815	1, 294	0.367
Date	4.507	1, 294	**0.035**
Wind	0.007	1, 294	0.932

4. Discussion

This study provides the first estimates of detectability and persistence of dead butterflies on roads, together with a description of the relative impact of vehicle traffic and scavenger activity on the removal of dead butterflies from roads. The estimates of detectability were relatively high, and dead butterflies were quickly removed from roads by vehicles and scavengers. These estimates can improve our understanding of the impact of road mortality on butterfly populations and aids in the assessment of the environmental impact of roads. For example, with known estimates of detectability, it is possible to assess how many individuals die on roads and thus better estimate what proportion of individuals in local populations near roads is affected by this type of mortality (Rao and Girish, 2007; Baxter-Gilbert et al., 2015).

The detectability of dead butterflies was almost perfect when they were placed on asphalt, but it was lower for butterflies placed on a road verge, which was an expected result because clearly, if a dead butterfly is located on a verge, the vegetation, ground structure and its colour can hinder the detectability of the dead butterfly. The diverse locations of dead butterflies should be considered in future studies and monitoring activities. Species-specific estimates of detectability in relation to the location of dead individuals should be implemented and derived in a way that is analogous to the method presented in this paper. For example, previous research (Skórka et al., 2013, 2015) involving the collection of dead butterflies along roads demonstrated that 32% of all butterflies were found on verges (unpublished data). With estimates from this study, this proportion can be corrected to approximately 42%.

The detection probability was determined by the body size of butterflies, but not by their colour. A similar effect of body size was also confirmed in several studies on vertebrates (Santos et al., 2011; Guinard et al., 2012; Teixeira et al., 2013). The studied butterflies were from relatively large species; thus, differential detectability may be a greater problem for smaller insects that are commonly found dead on roads, e.g., bees and hoverflies (Baxter-Gilbert et al., 2015; Munoz et al., 2015). Monitoring projects aimed at assessing road mortality in small butterflies should consider slower observer movement and more thorough inspections of verges to increase the chance of detecting a dead specimen. Ideally, three independent observers should walk along the selected road sections in a short time span, one after another, counting and marking all dead butterflies and noting those marked previously. This allows for effective estimates of the detection probability at the site by using the methods of capture-mark-recapture (Pollock, 1982).

The mean probability of detection for brightly coloured butterflies was slightly higher than for dark-coloured butterflies, but the difference was not significant. Again, body colour might be a more important variable in determining the detectability of much smaller invertebrates when they are present on verges among vegetation.

No variability in the detection ability was found between observers or between study sites. All of the observers had no previous experience in this type of work, indicating that inexperienced observers can detect a comparable and high number of road-killed butterflies. This might be especially important for road-killed animal monitoring programs based on volunteer engagement. As the detection probabilities were high, the involvement of naive observers in such studies does not seem to be a problem.

Butterflies were rapidly removed from roads, even from those without vehicle traffic. On roads with traffic, removal was much faster, indicating the prominent role of vehicles in the removal of dead butterflies. Usually, after 12 h, 83% and 60% of the dead butterflies remained on roads without or with traffic, respectively. After 48 h, these numbers fell to 6.3% and 2.5%. Thus, the removal of dead insects was rapid. This suggests that any collection or count of road-killed butterflies on a particular day represent a sample of specimens that are two days old at most. This has further implications for surveys on road-killed butterflies that are performed to estimate the road-mortality rate. In addition to at least three observers counting and marking dead butterflies repeatedly, surveys should include the counting and marking of all dead butterflies twice during a given day: once at the beginning of the day and once at the end of the day (or in any established time frame). Such surveys should also be repeated on consecutive days. This design is analogous to the “robust design” (Pollock, 1982) aimed to estimate the daily survival of live animals, which would allow to the total number of butterflies killed on roads in a given time period to be estimated and would also separate the rates of detectability from the removal. This strategy enables detection heterogeneity, which in this study means the location of a dead butterfly, on asphalt or a road verge.

Notably, dead butterflies disappeared from the road during the night in this study. The removal rate of dead butterflies was similar to that during the day. Therefore, several types of scavengers might have been involved, such as rodents, snails, and some predatory insects (e.g., ants) (Itzhak, 2008). This represents a topic for future study because little is known about scavenger behaviour on roads at night and no studies exist for dead butterflies. Vehicle traffic is probably the most important factor in the disappearance of dead butterflies, especially when they are present on asphalt, which was an expected result. However, many butterflies end up on a verge after colliding with a car. In these cases, the probability of dead butterfly removal by a vehicle is low, but wind or air flow created by fast-moving vehicles might move them to asphalt. As previously stated, approximately 32% of all dead butterflies were found at road verges in previous studies (Skórka et al., 2013, 2015, unpublished), indicating that collisions with vehicles generally leave dead butterflies on asphalt and, subsequently, these butterflies can be removed by vehicle wheels, which occurs frequently for small butterflies. Occasional observations suggest that when a butterfly is run-over by a wheel, it quickly disintegrates and few remains can be found. Even when the dead butterfly remains on asphalt, it is destroyed by passing vehicles, making it undetectable later.

The observation that the probability of dead butterfly removal by scavenging birds is correlated with traffic suggests a direct positive behavioural response of birds to traffic, especially because the amount of traffic was not correlated with the abundance of birds. The results were the same when only insectivorous bird species were used in analyses. This may result from the fact that in summer, many granivorous birds replenish their diet with insects (Snow and Perrins, 1998). This was confirmed in this study because some scavenging birds were seed-eating birds (e.g. *Emberiza citrinella* and *Passer domesticus*). Therefore, birds might alter their foraging behaviour to rely more on dead butterflies at roads with higher traffic, which is in contrast with studies on vertebrate carcasses where increased traffic diminished the activity of scavengers (Santos et al., 2011). However, dead butterflies are small animals and are thus much easier to capture and carry by birds than some road-killed large vertebrates. Increased activity of scavengers and a high number of vehicles increases the removal of dead butterflies at roads with a high traffic volume, which has an important practical implication for estimating the road-kill rate. At roads with a high amount of traffic, estimates of road-kill rates in butterflies might be disproportionally biased (underestimated) compared to roads with a low amount of traffic (compare: Baxter-Gilbert et al., 2015). Several bird species removed dead butterflies from roads. The most common were *Sturnus vulgaris*, *Motacilla alba*, and *Lanius collurio*, which are typical species in an agricultural landscape. Species such as *M. alba* also use verges and different road structures as nesting sites. Furthermore, several granivorous species (e.g., *Carduelis cannabina*, *Passer domesticus*) removed dead butterflies, and during the breeding season, these birds feed insects to their offspring (Snow and Perrins, 1998). The probability of dead butterflies being eaten by birds was affected by the time of season, which is probably linked to the appearance of young birds that often disperse across the landscape (Erritzoe et al., 2003). Also, in August, the autumn migration of several species starts and they utilise various food resources in the agricultural landscape (Erritzoe et al., 2003). Notably, this corresponds well to the high road mortality rate found in birds during this time. Thus, the increased presence of dead butterflies might possess a risk for bird populations that use roads as foraging sites (Erritzoe et al., 2003).

This study has two possible limitations. First, it did not consider the situation in which butterflies are hit by a vehicle and are removed by the vehicle on a windscreen or radiator. Some studies (Mckenna et al., 2001; Baxter-Gilbert et al., 2015) note this problem, and personal occasional observations indicate that some butterfly carcasses are removed by vehicles. This, of course, leads to an underestimation of detectability and road mortality and underlines the importance of introducing imperfect detection in estimates of road mortality. However, this is perhaps more significant for very small insects, such as small butterflies, mosquitoes, or flies, which often stick to windshields or the vehicle body. Larger butterflies usually drop to the road after vehicle impact (author's unpublished data). Second, the potential role of heavy rain in the disappearance of dead butterflies was not considered in this study because the weather during the experiments was generally good, with no heavy precipitation. However, it is possible that dead butterflies may be quickly flushed away from asphalt and verges, thus becoming undetectable during surveys.

5. Conclusions

The impact of the detectability and removal of dead butterflies by vehicles and scavengers has not been previously addressed in studies of road mortality. Knowledge concerning potential biases in the detection of road-killed butterflies and their removal is essential as verges have become an important surrogate for semi-natural habitats in modified landscapes (Ruiz-Capillas et al., 2013; Skórka et al., 2013). Thus, the estimation of the relative benefits (in terms of surrogate habitats on verges) is dependent on accurate estimates of the road mortality rate. Road mortality for butterflies might be mitigated by widening road verges, sowing flowering plants, mowing less frequently, retaining more grassland in the landscape, and introducing a speed limit at roads crossing butterfly habitats (Munguira and Thomas, 1992; Ries et al., 2001; Skórka et al., 2013). However, all actions aimed at increasing the suitability of road verges for butterflies should be preceded by road mortality studies and monitoring programs that include imperfect detection. These studies and programs should account for detection probability differences between road lanes and verges and the short persistence time of dead butterflies, especially at roads with high traffic.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.biocon.2016.05.026. These data include the Google maps of the most important areas described in this article.

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