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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.

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