

## SHORT COMMUNICATION

# Flying insect abundance declines with increasing road traffic

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**Abstract.** 1. One potentially important but underappreciated threat to insects is road mortality. Road kill studies clearly show that insects are killed on roads, leading to the hypothesis that road mortality causes declines in local insect population sizes.

2. In this study we used custom-made sticky traps attached to a vehicle to target diurnal flying insects that interact with roads, sampling along 10 high-traffic and 10 low-traffic rural roads in southeastern Ontario, Canada. We used a paired sampling design to control for potentially confounding differences in the road characteristics (e.g. road width) and surrounding land covers (e.g. housing density) between high-traffic and low-traffic roads. We then used these data to test the prediction that fewer flying insects collide with vehicles, per vehicle (i.e. insect abundance is lower), on high-traffic than low-traffic roads.

3. We found significantly fewer insects at the high-traffic roads than at the low-traffic roads as predicted. There was a 23.5% decline in the number of insects/km/vehicle on high-traffic relative to low-traffic roads.

4. Given the high rates of insect mortality observed in previous studies, it is likely that road mortality contributes to these observed negative effects of traffic intensity. Thus the growing global road network is a concern for conservationists and land managers, not only because insect population declines contribute to the ongoing global losses of biodiversity but also because insects play a vital role in food webs and provide important ecosystem services.

**Key words.** Biodiversity, insect, invertebrate, population decline, road ecology, road kill, road mortality, traffic intensity, traffic volume.

## Introduction

There is alarming evidence of global declines in insects (Dirzo *et al.*, 2014). This is concerning not only because it contributes to the ongoing global losses of biodiversity, but also because insects play a vital role in food webs. For example reduced availability of some insects (e.g. Coleoptera) has been implicated in insectivore bird declines (Hallmann *et al.*, 2014; Pomfret *et al.*, 2014). Insects also provide vital ecosystem services such as crop

pollination. Approximately 65% of crop types consumed by humans are moderately to highly dependent on pollination services (Klein *et al.*, 2007), with global pollination services valued at approximately \$153 billion euros/year (in 2005; Gallai *et al.*, 2009).

One potentially important but underappreciated threat to insects is road mortality. Road kill studies clearly show that insects are killed on roads (Riffell, 1999; Mckenna *et al.*, 2001; Rao & Girish, 2007; Yamada *et al.*, 2010; Baxter-Gilbert *et al.*, 2015), with estimates as high as 250 individuals killed/km/day on a high-traffic highway (Baxter-Gilbert *et al.*, 2015). Given the extent of the global road network, with more than 40 million km of road lanes worldwide (Dulac, 2013), billions of insects are likely killed on roads each year.

Nevertheless, it is not clear whether this mortality is an important driver of insect population declines. A few

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studies have found that local insect abundance or richness decline with increasing traffic intensity (Boháč *et al.*, 2004; Dunn & Danoff-Burg, 2007; Flick *et al.*, 2012; but see also Melis *et al.*, 2010). This is consistent with the hypothesis that road mortality reduces local insect population sizes because road kill is generally higher when there is more traffic (Yamada *et al.*, 2010; Soluk *et al.*, 2011). But, it is also possible that these road effects are driven by other factors that are correlated with traffic intensity, rather than road mortality. For example comparison of high-traffic urban roads to low-traffic rural roads is likely confounded by the effects of housing density on insects (Gagné & Fahrig, 2011). Comparisons of high-traffic multi-lane highways to low-traffic two-lane roads may also be confounded if wider highways are more likely to act as barriers to movement (as observed in some vertebrates; e.g. Rondinini & Doncaster, 2002) and thus negatively affect insect populations via habitat fragmentation. Thus an important step towards determining whether road mortality drives insect population declines is to test whether insect abundance is lower at high-traffic versus low-traffic roads when controlling for other factors (e.g. surrounding land cover, road width) that could also affect insect population sizes.

In this study we tested our prediction by sampling diurnal flying insects along 10 high-traffic and 10 low-traffic rural roads in southeastern Ontario, Canada, using a paired sampling design to control for potentially confounding differences in the road characteristics and surrounding land covers between the high-traffic and low-traffic roads. We targeted the insects that interact with roads, and are thus at risk of road mortality, using custom-made sticky traps attached to a vehicle (Fig. 1a) to collect insects flying directly over the roads at vehicle height. Although the total road kill across all vehicles might be higher when there is more traffic (even if the insect abundance is lower), the number of insects colliding with a given vehicle should be lower when there are fewer insects in the local population. Thus we predict that fewer flying insects will collide with vehicles, per vehicle (i.e. insect abundance is lower), on high-traffic than low-traffic roads.

## Methods

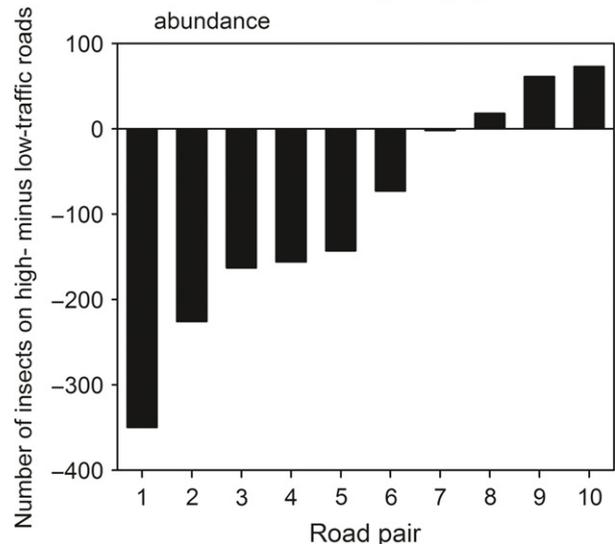
### Site selection

We selected 10 high-traffic and 10 low-traffic road segments in southeastern Ontario, Canada, where a road segment was defined as a stretch of road between two cross-streets. We used a paired sampling design to control for potentially confounding differences between high-traffic and low-traffic roads (i.e. differences other than traffic intensity). To select our paired roads, we first selected a set of 10 high-traffic road segments, limited to rural locations where the segment was 1–3 km long, 2-lane, and paved. A location was considered ‘rural’ if it had

(a) Vehicle-mounted sticky traps



(b) Effect of traffic intensity on flying insect abundance



**Fig. 1.** (a) Vehicle-mounted sticky traps, used to estimate flying insect abundance on the roads. We attached one sticky trap ( $0.5 \times 1.2$  m area) to the front grill of the vehicle and a second trap ( $0.3 \times 1.2$  m) to the roof. Sticky traps consisted of a plywood frame into which we inserted a thin board covered in a piece of heavy plastic wrap. Traps were secured to the vehicle frame using ratcheting straps. A coating of a sticky medium (Pestick; Phytotronics, Inc.) was applied to the plastic wrap. Inset shows one of the insects trapped in the Pestick. (b) Effect of traffic intensity on flying insect abundance, measured as the difference in the number of insects collected by vehicle-mounted sticky traps between the high-traffic and low-traffic road within each of the 10 pairs. Negative values indicate that there were fewer insects at high-traffic roads than low-traffic roads ( $t_9 = 2.23$ ,  $P = 0.03$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

<15 houses km<sup>-2</sup> within a 250 m radius around the road segment. Then we selected a rural low-traffic road segment to pair with each high-traffic segment. Differences other than traffic intensity between the high-traffic and low-traffic roads within a pair were controlled by ensuring that the low-traffic road segment (i) was 1–3 km long, 2-lane, and paved; (ii) had similar land cover within a 250 m radius around the road segment to the landscape around its high-traffic pair, i.e. a  $\leq 0.1$  difference in the proportion of water cover (wetland, ponds, streams, and rivers),  $\leq 0.1$  difference in the proportion of forest, and  $\leq 5$  house km<sup>-2</sup> difference in the housing density; and (iii) was <10 km from its high-traffic pair. Water cover, forest cover, housing density, and road segment lengths were derived from Ontario Ministry of Natural Resources and Forestry (Peterborough, ON, Canada) land cover data sets.

#### Data collection

Each road segment was sampled twice in 2014: once between June and mid-July and again between mid-July and September. All sampling occurred between 12:00 and 17:00, on rain-free days when the temperature was >15 °C and the wind was <20 km h<sup>-1</sup>. Paired roads were sampled within 1 week of each other, and the order in which pairs were sampled was random. Insect abundances may vary from early to late afternoon; for example Herrera (1990) found that some species of Lepidoptera, Hymenoptera, and Diptera were more active in early afternoon than late afternoon. To avoid potential biases in our abundance estimates between high-traffic and low-traffic roads due to variation in insect abundance over the sampling period, we randomised the order in which road segments were sampled on a given day.

We used custom-made sticky traps attached to a pickup truck to assess abundance of flying insects on high-traffic and low-traffic roads (Fig. 1a). As different insect species may fly (and encounter vehicles) at different heights (e.g. Wermelinger *et al.*, 2007), we attached two sticky traps to the truck: (i) at the front grill of the vehicle and (ii) at the roof. The front grill sticky trap covered a 0.5 × 1.2 m area and the roof sticky trap 0.3 × 1.2 m. These traps consisted of a plywood frame into which we inserted a thin board covered in a piece of heavy plastic wrap. A coating of a sticky medium (Pestick; Phytotronics, Inc., Earth City, MO, USA) was applied to the plastic wrap. Traps were secured to the vehicle frame using ratcheting straps.

Insects were sampled by driving the road segment twice per survey, keeping speed constant at ~80 km h<sup>-1</sup>. Because road segment lengths varied among sampling sites (1–3 km), we standardised sampling effort by sampling less of the surface area of the sticky traps on longer roads. To subsample the sticky trap area, we divided the surface area of both the grill and roof sticky traps into six equal-area portions. For each 0.5 km increase in the road

segment length, we randomly removed one portion of the grill sticky trap and one portion of the roof sticky trap from sampling. Thus the full surface area was sampled when the road segment was 1 km long, and 1/3 of the area was sampled when the road segment was 3 km long. We note that there was no systematic difference in road segment lengths between high-traffic and low-traffic roads ( $t_9 = -0.14$ ,  $P = 0.89$ ); thus this subsampling procedure is unlikely to bias our estimates of relative abundance on high-traffic versus low-traffic roads. We classified body length into 5-mm size classes (<5, 5–10, 10–15, etc.) and recorded the order of each collected insect.

To evaluate our *a priori* selection of high-traffic and low-traffic roads, we collected traffic volume data during the second sampling period (mid-July – September 2014), recording the number of vehicles heard passing a random location along the road during a 15-minute period, using an audio recorder (Zoom Corporation, Tokyo, Japan).

#### Data analysis

We tested our prediction of fewer insects on high-traffic than low-traffic roads using a paired, one-tailed *t*-test assuming unequal variances to compare insect counts between high-traffic and low-traffic roads. Insect counts were the summed counts for the roof and grill sticky traps for the two surveys at each of the 20 road segments. We repeated the above analysis for different insect orders and different size classes. To ensure adequate sample sizes in our analyses, we included only the orders/size classes found on more than 25% of road segments.

We also tested for greater traffic volume on high-traffic than low-traffic roads using a paired, one-tailed *t*-test assuming unequal variances. Traffic volume for each road was the number of vehicles heard in the audio recording per minute.

All statistical analyses were done in R (version 3.4.0; R Core Team, 2017).

## Results and Discussion

In total, we collected 7225 insects with the vehicle-mounted sticky traps, and 7213 of these were identified to order. Diptera, Hymenoptera, Hemiptera, Thysanoptera, and Coleoptera were each collected on more than 25% of road segments. More than 99% of collected individuals (7202/7225 individuals) belonged to one of these five orders (5568 Diptera, 679 Hymenoptera, 435 Hemiptera, 383 Thysanoptera, and 137 Coleoptera). We collected Lepidoptera on five roads, Odonata on two roads, and the remaining orders (Neuroptera, Orthoptera, and Trichoptera) on one road each. Samples were dominated by smaller-bodied insects, with 6996 insects <5 mm long, 200 insects 5–10 mm, 24 insects 10–15 mm, and four insects 15–20 mm. The first three size classes were each found on more than 25% of road segments.

Fewer insects were captured on high-traffic roads than on low-traffic roads, as predicted ( $t_9 = 2.23$ ,  $P = 0.03$ ; Fig. 1b). There were fewer insects on the high-traffic than low-traffic road for 7/10 road pairs and there was a 23.5% decline in the number of insects/km/vehicle on high-traffic relative to low-traffic roads. This negative effect of traffic intensity on overall insect abundance was driven by the significant, negative effect of traffic intensity on Diptera (30.7% decline;  $t_9 = 2.52$ ,  $P = 0.02$ ) and a marginally significant, negative effect on Coleoptera (38.8% decline;  $t_9 = 1.53$ ,  $P = 0.08$ ). Traffic intensity effects were similar for the three body size classes. There were 23.2%, 33.3%, and 40.0% declines in the number of insects/km/vehicle on high-traffic relative to low-traffic roads for the <5 mm, 5–10 mm, and 10–15 mm body size classes respectively. Nevertheless, we note that these differences were only statistically significant for the smallest two classes (<5 mm:  $t_9 = 2.08$ ,  $P = 0.03$ ; 5–10 mm:  $t_9 = 2.51$ ,  $P = 0.02$ ; 10–15 mm:  $t_9 = 1.03$ ,  $P = 0.16$ ).

We attribute the lower overall abundance of flying insects, and lower abundances of Diptera and Coleoptera, at high-traffic than low-traffic roads in our study to the traffic itself. High-traffic roads had an order of magnitude higher traffic volumes (mean = 6.11 vehicles/minute, SD = 3.73) than their low-traffic counterparts (mean = 0.68, SD = 0.31;  $t_9 = -4.66$ ,  $P < 0.001$ ). In addition, we specifically controlled for potential confounding variables that can be correlated with traffic intensity, including characteristics of the road (e.g. its substrate or width) and the surrounding landscape (e.g. water cover, forest cover, or housing density) that could be related to insect abundance. Therefore our study suggests that traffic itself has a negative effect on insect abundance.

Given the high rates of insect mortality observed in previous studies (Riffell, 1999; Mckenna *et al.*, 2001; Rao & Girish, 2007; Yamada *et al.*, 2010; Baxter-Gilbert *et al.*, 2015), it is likely that road mortality causes, or at least contributes to, our observed negative effect of traffic intensity on the abundance of insects that interact with roads. Nevertheless, there are other correlates of traffic that could contribute to the effect by increasing insect mortality or reducing reproduction. For example local air and ground pollutants can increase with traffic intensity (Wheeler & Rolfe, 1979; Aldrin & Haff, 2005), and this might cause greater mortality or sub-lethal effects for insect populations along higher traffic roads (e.g. Lob & Silver, 2012). In addition, roads with higher traffic may have higher abundances of non-native plant species than roads with lower traffic (Joly *et al.*, 2011). This could reduce insect abundances along higher traffic roads if non-native plants are unsuitable insect habitat or food.

It is also possible that there are fewer insects interacting with high-traffic than low-traffic roads because insects are less likely to attempt to fly over high-traffic than low-traffic roads. To our knowledge this has not been studied. Some mark-recapture studies have reported that few marked insects successfully cross high traffic roads (see Muñoz *et al.*, 2015 and references therein). But, it is not

clear whether this is due to road avoidance or because the insects that tried to cross the road died and were thus not recaptured.

Although our results are suggestive, future study is needed to determine if road mortality is driving the negative effect of traffic intensity on insect abundance. Thus an appropriate next step would be to test the prediction that insect species with the highest per capita probability of road mortality are most negatively affected by traffic intensity. If supported, this would suggest that road mortality was negatively affecting insects, rather than other factors associated with traffic intensity such as changes in local pollution or numbers of invasive species. If pollution levels or invasive species were driving the negative effect of traffic intensity on insect abundance we should see no cross-species relationship between the per capita probability of road mortality and the strength of traffic intensity effects. Such a study will be complicated by the fact that a species that is frequently killed on roads may be observed less frequently on high-traffic than low-traffic roads because its population size along the high-traffic road has been depressed as a result of higher rates of road mortality (Zimmermann-Teixeira *et al.*, 2017). Thus estimates of per capita probability of road mortality must account for each species' relative abundance along the sampled roads, for example by simultaneously sampling the numbers of road-killed and live individuals along road transects (e.g. Skórka *et al.*, 2013).

Our results suggest that such future studies should focus on the effects of road mortality on Diptera and Coleoptera, which were most strongly affected by traffic intensity in our study. Moreover, both taxonomic groups play vital roles in food webs and provide important ecosystem services. For example Diptera provide important agricultural ecosystem services, by pollinating crops (Orford *et al.*, 2015) and through biocontrol of crop pests (Satar *et al.*, 2015). Coleoptera are an important prey species for insectivore birds (Razeng & Watson, 2012, 2015), many of which are in decline (North American Bird Conservation Initiative Canada, 2012).

Future studies are also needed to determine whether the effects of traffic intensity we observed in diurnal flying insects are also found in nocturnal flying insects. We speculate that traffic intensity may have a larger impact on nocturnal insects than diurnal ones. This is because many nocturnal insect species are attracted to artificial night lighting associated with roads (e.g. street lights; Wakefield *et al.*, 2017), which may increase the per capita probability of road mortality.

If road mortality does indeed contribute to insect declines, this should be concerning for conservationists and land managers. The already extensive global road network—with more than 40 million km of road lanes worldwide (Dulac, 2013)—likely kills billions of insects each year, and this network is projected to increase by approximately 60% (25 million km of road lanes) between 2010 and 2050 (Dulac, 2013). Increasing road mortality associated with this growing global network of roads may

accelerate the global declines in insects (Dirzo *et al.*, 2014), which is of concern not only because it contributes to the ongoing global losses of biodiversity, but also because insects play a vital role in food webs and provide important ecosystem services.

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