Mortality Census of the Road-Killed Butterflies in Mahendra Highway, Nepal

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ABSTRACT. Increasing roads become the serious conservation threats to the animal populations. The most direct effect of roads on them is deadly collision with vehicles, leading to high levels of injury or death. Estimates of detectability of road-killed higher vertebrates have been determined but not for the invertebrates like butterflies, although they are frequently killed insects group on roads. This is the first comprehensive mortality census of road-killed butterflies in Nepal. The main aim of this study was to estimate the detectability of road-killed butterflies in Mahendra Highway, the busiest highway in Nepal. We established eight transects, each of 500 m long within the randomly selected forest and human settlement landscapes. Pearson’s correlation was calculated to test the link between the number of road-killed and living butterflies. All together 1000 butterfly individuals were counted throughout the study periods including road-killed and living butterflies. Among them, 364 butterflies were counted roadkilled whereas 636 butterflies were living. Forest landscape contributed higher number in both road-killed and living butterflies than human settlement landscape. Also, the number of butterflies killed on the roads were significantly correlated with number of living butterflies on the road. Our results indicate that road has the significant impact on loss of butterfly population. Also, higher the number of living butterflies more will be road mortality. The public awareness and maintaining the habitats with high forbs cover, gardening, avoid depositing asphalts, etc. in the vicinity of road can denigrate the rate of road mortality of butterflies.

Key words: Devdaha Municipality, Nepal, Roads, Butterfly, Conservation

Introduction

Roads constitute substantial parts of our environment (Husby, 2016). Increasing the human population increases road encroachment that leads to the rapid development of the automotive industry across the globe (Selva et al., 2011). Roads are known to be a cause of...
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disturbance for some population (Skórka et al., 2015). In addition, roads also act as a barrier that isolates the population, increase habitat fragmentation (Keller et al., 2004; Riley et al., 2006) and impeding the movement of individuals and gene flow (Jackson & Fahrig 2011). Consequently, roads become the major conservation threats in natural population composition (Laurance et al., 2009; Selva et al., 2011).

The road mortality of animal population are poorly documented throughout the world (Ries et al., 2001; Mckenna et al., 2001). However, previous studies have shown the effect of roadway traffic in different faunal populations to exemplify amphibians (Fahrig et al., 1995), snakes (Bernardino & Dalrymple, 1992), koala (Canfield, 1991), wolves (Mech, 1989), turkeys (Holbrook & Vaughan, 1985), badgers (Davies et al., 1987), birds (Husby, 2016) and other vertebrates (Lalo, 1987; Putman, 1997). Additionally, insects are also largely killed invertebrate groups by traffic (Rao & Girish, 2007; Soluk et al., 2011). Despite this fact, they have been receiving relatively very little attention in studies (Rao & Girish, 2007), and butterflies are not exceptional (Sony & Arun, 2015). Though butterflies are one of the most common group of insects that adversely affected by roads (Mckenna et al., 2001; Rao & Girish, 2007; Yamada et al., 2010; Skórka et al., 2013). Some studies had been carried out to discern the impact of roads in butterfly population in different parts of the world (Dennis, 1986; Selva et al., 2011; Vidivalagan et al., 2012; Skórka et al., 2013; Kalarus & Bakowski, 2015; Skorka et al., 2015; Sony & Arun, 2015).

This is the first comprehensive study on the mortality census of butterflies on road in Nepal. They have been listed in the non-priority taxa in conservation by the general public. This study brings attention to the conservation of the butterfly population in the place where traffic pressure is maximum. However, if road mortality is high then the conservation value of the respective road verges would be diminished (Skórka et al., 2013). Although, the main aim of this study is to make an extensive survey of road-killed butterflies and to quantify the data to compare on species richness and abundance of road-killed butterflies with the living butterfly individuals encountered. We also aim at investigating the factors that leading the butterflies toward road-killed.

Material and methods

Study area

Having identified the high traffic volumes and number of biodiversity collision we selected the Mahendra Highway (192 km) as a study road. The highway is one of the busiest highway which is extended east to west of Nepal. The study was conducted in two landscapes (Forest and Human settlement) of Devdaha Municipality (Latitude; 27°39’37.88” N and Longitude; 83°34’12.95” E, altitude: 154 m asl.), Rupandehi district (Fig. 1). Lowland Nepal which touches 10 km of the highway. The study area was mostly occupied by deciduous, mixed deciduous and flowering forest (65%) and scattered human settlement (30%) at 5-10 m away from either side of the main highway. The river site, agricultural land, and open grassland cover a small part of the study area near human settlement (2-5%).

Transects Selection and Sample Collection

For monitoring species richness (N), road-killed (n) and living (nL) butterflies fixed-route transects were established as suggested by Pollard (1977, 1982) in the randomly selected forest and human settlement landscapes. Counts along transects are the standard methods to study the butterflies population (Pollard & Yates, 1993) and also allow detection of butterflies
Figure 1. Map of Rupandehi district (Blue) (Above) and location of transects in the highway of Devdaha Municipality (Below).

through distance sampling approaches (Nowicki et al., 2008). All together eight transects (five transects in the forest landscape and three in the human settlement landscape), each of 500 m long were established. The distance gap between every two consecutive transects was made 500 m in order to avoid overlapping of the living butterflies. The road extends maximum with forest landscape, hence transects number were established more than the human settlement landscape. Two parallel lines were laid at each transect one on either side of the road as suggested by Skórka et al., (2013). Two teams with two persons in each team were formed. The teams were walking at a constant pace in parallel on each side of the transects. We counted the butterflies that were killed colliding with vehicles and living individuals following the recommendations of Skórka et al., (2013). All the killed individuals were eliminated from each transect in order to restrict double counting (Sony & Arun, 2015; Skórka et al., 2013). In the case of confusing living butterflies, we used a butterfly net to capture and identified with the help of standard literature grids (Smith, 1997, 2011a, 2011b) and released (Khanal et al., 2012). The dead
butterflies were collected and preserved in the triangular transparent envelope for further identification and study. The survey was conducted six times from September 10 to November 4, 2017, during a sunny day. The interval between consecutive observations on particular sites was made one week. Each survey was completed for four days. The time of sampling was made between 08:00 am to 03:00 pm, the time of high activeness of butterfly species. Each transect was visited for 1.5 hours. Traffic volumes with vehicles sizes of two-wheelers and multi-wheelers plying both the directions during survey periods were counted (Sony & Arun, 2015). Vehicles sizes were categorized in to small (i.e. two-wheelers) and large sizes (i.e. multi-wheelers).

**Data Analysis**

Pearson’s correlation was tested to seek a link between the number of road-killed and living butterfly individuals (Skórka et al., 2018). The result was declared significant if $P<0.05$. The analysis is performed in R-studio 3.5.0 software.

**Results**

Altogether, 1000 individuals of butterfly species were recorded throughout the study periods in which we counted 364 road-killed butterfly individuals (36.4%) of 29 species with 23 genera belonging to four families (Nymphalidae, Pieridae, Hesperiidae and Papilionidae) and 636 living butterfly individuals (63.6%) of 33 species with 27 genera under five families (Nymphalidae, Pieridae, Papilionidae and Lycaenidae) (Table 1). The butterflies belong to family Nymphalidae contributed highest road-killed number (n=195; 53.6%) followed by Pieridae (n= 100; 27.5%), Hesperiidae (n=52; 14.3%) and Papilionidae (n=17; 4.7%), whereas none of the lycaenid butterflies were found road-killed (Table 1; Fig. 2). The living butterfly individuals were registered highest belonging to family Nymphalidae (nL=329; 51.73%) followed by Pieridae (nL=170; 26.73%), Hesperiidae (nL=76; 11.95%), Lycaenidae (nL=32; 5.03%) and Papilionidae (nL=29; 4.56%) (Fig. 2). We counted 464 living, and 270 road-killed butterfly individuals in the forest landscape and 172 living, and 94 road-killed butterfly individuals in human settlement landscape (Table 1). In both the landscapes, Nymphalidae butterflies were counted highest road-killed individuals i.e. in forest (57%); human settlement (43%) followed by Pieridae i.e. forest (24%); human settlement (37%), Hesperiidae i.e. forest (15%); human settlement (13%) and Papilionidae i.e. forest (4%); human settlement (7%) (Fig. 3). During the survey periods, the highest number of road-killed and living butterfly species recorded were *Euploea core* (C.) (Lepidoptera: Nymphalidae) (n=38; nL=51) *Precis almana* (L.) (Lepidoptera: Nymphalidae) (n=37; nL=46), *Terias blanda* (C.) (Lepidoptera: Pieridae) (n=37; nL= 46), *Danaus chrysippus* (Lepidoptera: Nymphalidae) (n=29; nL=31), *Parnara guttata* (M.) (Lepidoptera: Hesperiidae) (n=25; nL=38), *Catopsilia pomona* (F.) (Lepidoptera: Pieridae) (n=21; nL=43), *Melanitis leda* (C.) (Lepidoptera: Nymphalidae) (n=19; nL=37) and *Precis iphita* (C.) (Lepidoptera: Nymphalidae) (n=19; nL=35) (Table 1). There was only one individual of two butterfly species namely; *Danaus genutia* (C.) (Lepidoptera: Nymphalidae) and *Atrophaneura lateillei* (D.) (Lepidoptera: Papilionidae), found road-killed during study periods. Both were observed in forest verge transects (i.e. T2 and T4 respectively) (Table 1). The detail lists of the number of road-killed and living butterfly individuals are given in Table 1. Although, highly damaged butterflies that difficult to identify were excluded from the list. We only counted three butterfly individuals in such condition. Statistically, there was a highly significant positive correlation ($r = 0.942, P = 0.000; P<0.05$) between the number of roadkilled and the number of living butterflies.
Figure 2. Family wise number of road-killed, living and species richness of butterfly counted.

Figure 3. Family wise composition of road-killed butterfly individuals in forest and human settlement landscapes.
### Table 1. Species wise observed road-killed and living butterfly individuals along all transects; RK=Road-killed Butterfly Individuals (n) and L=Living Butterfly Individual (n).

<table>
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<th>SN</th>
<th>Family/Scientific Name</th>
<th>Family: Nymphalidae</th>
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<td>3</td>
<td>6</td>
<td>9</td>
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<p>|    |                            | Family: Pieridae    |                     |                            |       |
|    |                            |                     |                     |                            |       |
| 16 | Catopsilia pomona          | 3                   | 7  | 0  | 2  | 2  | 5  | 1  | 3  | 6  | 10 | 1  | 4  | 4  | 7  | 4  | 5  | 21 | 43 |
| 17 | Catopsilia pyranthe        | 2                   | 4  | 0  | 2  | 0  | 2  | 0  | 2  | 3  | 0  | 1  | 0  | 0  | 3  | 4  | 7  | 16 |</p>
<table>
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<tr>
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<th>Human Settlement Transects</th>
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</tr>
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</table>

**Family: Hesperiidae**

|    | Parnara guttata         | 4 5 3 5 4 6 4 6 6 6 0 3 2 4 2 3 | 25 38 |       |
| 23 | Borbo bevani            | 0 0 4 4 0 0 2 4 1 3 3 3 3 0 1 2 | 14 16 |       |
| 24 | Sarangesa dasahara      | 4 6 5 9 1 3 0 0 0 0 1 2 0 0 0 0 11 20 |       |
| 25 | Notocrypta curvifascia  | 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 2 2 |       |

**Family: Papilionidae**

|    | Atrophaneura lateillei | 0 0 0 0 0 0 1 2 0 0 0 0 0 0 0 0 0 0 0 0 1 2 |       |
| 27 | Papilio polytes        | 2 3 2 5 1 3 1 3 1 1 1 2 3 3 2 3 | 13 23 |       |

**Family: Lycaenidae**

|    | Lampides boeticus      | 0 3 0 0 0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 6 |       |
| 30 | Zizina otis            | 0 2 0 1 0 0 0 2 0 1 0 1 0 0 0 0 0 0 0 0 0 7 |       |
| 31 | Pseudozizeeria maha    | 0 4 0 3 0 2 0 0 0 0 0 3 0 3 0 1 0 16 |       |
| 32 | Zeltus amasa           | 0 0 0 2 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 3 |       |
| 33 | Total                  | 61 103 81 122 48 89 34 74 46 76 19 48 39 60 36 64 |       |

Grand Total  
RK = 270; L = 464  
RK = 90; L = 172  
364 636
Discussion

We confirmed that the roadways acted the significant loss of butterfly individuals and strong behavioral barriers to adult butterflies. Coinciding with other studies (Dennis, 1986; Skórka et al., 2013, 2015; Sony & Arjun, 2015; Skórka et al., 2018) also showed strong negative effect on butterfly communities due to road. Indeed, this type of study provided significant data on the diversity of butterflies from the sites (Vadivalagan et al., 2012). We found that heavy large multi-wheelers vehicles have high mortality impact on butterfly individuals rather than two-wheeler motorbikes as butterflies had frequently collision with such vehicles (Authors’ observation). This suggests that vehicle size has a greater impact on road mortality. However, such is not the case. Instead, traffic volume has a greater effect on the population composition and cause of exceeding roadkill in the butterfly individuals (Authors’ observation). However, the study still needs completion to disseminate the relationship between traffic volumes and butterflies in the surveyed highway. Similarly, our observation is in agreement with Skórka et al. (2013, 2015), suggesting that the high traffic volume had an important, and obvious, effect on increasing number in roadkill butterflies. In contrast, Mckenna et al. (2001) found a decline in road mortality of butterflies at the highest traffic volume.

Statistical result showed an abundance of butterfly mortality on road were directly associated with the increased number of living butterfly individuals. We found that the number of road-killed butterflies increased with an increased abundance of living butterflies on the road. The result is consistent with Skórka et al. (2013). Our result also showed the butterfly species like Euploea core, Precis almana, Terias blanda, Danaus chrysippus, Parnara guttata, Catopsilia pomona, Melanitis leda, Precis iphiita and Gandaca harina (H.) (Lepidoptera; Pieridae) that occurred with high abundance were road-killed in more numbers (Table 1). These butterfly species are very common in low-land Nepal (Smith 1997; Khanal, 2008). Hence this indicates that the commonly occurring butterfly species kill in more number with deadly collision to the vehicles on the road. Unlike, Sony & Arun (2015) had obtained a less number of butterfly mortality on the road which was very common. These butterflies were frequently observed puddling on cattle dung, asphalt and moist parts at the road verge and prone to more casualties with the vehicle’s presence.

We counted maximum road-killed and living butterfly individuals in the forest landscape than human settlement (Table 1; Fig. 3). This finding corresponds with the previous conclusion by Skórka et al. (2013) where they also argue that forest cover landscape near road verge increased butterfly road mortality and abundance. This is probably, the butterfly species are strongly related to the availability of heterogeneous composition of nectar plants species in the forest verges (Saarinen et al., 2005; Kalaus & Bakowski, 2015; Shrestha et al., 2018) and presence of warmer microhabitats than surrounding landscape (Ockinger et al., 2012). Thus forest landscape in the vicinity of roads increases the butterfly number and concurrently influx the butterfly individuals into the roads and result the collision with vehicles and lead to dead (Skórka et al., 2013). Therefore, forest coverage sizes in the vicinity of roads may be regarded as low conservation values for butterflies (Skórka et al., 2013). However, Saarinen et al. (2005) and Shrestha et al. (2018) had contradict conclusion arguing the forest coverage land is a suitable habitat for butterflies and carried high conservation concern. In addition, we observed the speed limits of the vehicles in the forest landscape is relatively higher. Indeed, butterfly individuals were seen to be caught not being able to escape from high-speed vehicles and collided into it. Thus, increased the road mortality of butterflies to a certain extent (Mckenna et al., 2001). In human settlement landscape, we counted relatively very less butterfly individuals
in terms of both road-killed and living butterflies. A similar finding was obtained by Skórka et al. (2013) concluded that settlements have no role to increase the number of roadkills. Human disturbance/or encroachment always favor the least butterfly diversity and abundance (Shrestha et al., 2018). However, the effect of human settlements on butterfly kills is more difficult to understand (Skórka et al., 2013). In fact, artificial gardens with numerous flowers, farmland, vegetables and crops land etc. near the human settlements provide supplementary food for butterflies (Rosin et al., 2011) and have positive effects on butterfly species richness and abundance (Skórka et al., 2013).

During the survey periods, we found the highest number of road-killed, species richness and living butterfly individuals from family Nymphalidae (Fig. 2) as consistent with other surveys, reported highest road-killed butterfly species from family Nymphalidae from Western Ghats, India (Sony & Arun, 2015) and National Highway 50, Kalaburagi district, Karnataka, India (Saraf & Jadesh, 2017). However, spatial and temporal study in such issue might give different results from the study sites. The mass dispersal (random and aimless movement away from the site) of the butterflies when they encounter hostile habitats such as arable farmlands, roads, building etc. (Khyade et al., 2018) highly prone to getting killed on the roads, compared to other families. The butterflies belonging to family Lycaenidae did not find any road-killed, although they were counted 32 living individuals from four species namely; Lampides boeticus (L.) (Lepidoptera: Lycaenidae), Zizina otis (F.) (Lepidoptera: Lycaenidae), Pseudozizeeria maha (K.) (Lepidoptera: Lycaenidae) and Zeltus amasa (H.) (Lepidoptera: Lycaenidae) (Table 1). This pattern may result from the fact that the vulnerability of small patches of grassland distributed on the road verge of both the study landscapes. Most other studies found that the grassy roadsides have a direct effect on small butterflies to exceed the roadkill mortality risk (Ries et al., 2001; Skórka et al., 2013) and living number as well (Skórka et al., 2018). They also fly low to the ground, possess short flight behavior which restricted to cross the road (Fjellstad, 1998; Saraf & Jadesh, 2017) and lower the proportion that cross the road in comparison with other butterflies, resulting less road-killed (Saraf & Jadesh, 2017) and zero mortality as well. In contrast, Skórka et al., (2018) demonstrated the overrepresentation of road-killed small butterflies due to their slow speed and low altitude flying behavior. Butterflies belonging to family Papilionidae contributed the least percentage (i.e. 4.56%) of the total road-killed butterfly individuals. The family constitutes the larger size butterfly group (Smith, 2011a). The larger species often fly over roads with high altitude (Skórka et al., 2013) and hence less susceptible to collide with the vehicles.

This study provides the first estimates of detectability and persistence of dead butterflies on roads in the country. This helps to improve our understanding of the impact of road mortality on the butterfly population (Skórka, 2016). Furthermore, an estimate of detectability also helps to assess how many species individuals killed and thus better known what proportion of individuals in local populations near roads is affected by this type of mortality (Rao & Girish, 2007). In addition several types of vertebrates like dogs, cats, rodents, civet, different bird species, and invertebrates like ants, grasshopper, dragonflies, damselflies, etc. have been found involved in the roadkill cases. This could represent the topic of future study. The evidence of such high mortality suggested the high conservation threat to butterfly species due to roads. However, further extension of studies should overtly quantify the overall impact of traffic on butterfly composition. Thus, could be easy to identify the major conservation level and conservation priority within the countryside. Moreover, such a study provides baseline information that may have a significant role in designing conservation programs and conservation action plan for reducing this mortality as it affects protected butterfly species and
pulling toward vulnerability of very common species. To mitigate the large number of butterfly roadkill, controlling the speed limits of vehicles in high conservation priority sites like forest areas might be an effective tool alleviating road mortality in butterflies (Mckenna et al., 2001). Managing alternative habitats like preparing artificial gardening, regular farming without using pesticides, and increasing diversity and amount of forbs in the vicinity of roads (Skórka et al., 2013) will increase the nectar and host-plant resources (Ries et al., 2001), and hence play pivotal role in protection of butterflies in such landscapes. This is perhaps more significant for ecological beneficial prospect like pollination, ecosystem balance etc. In addition, avoid deposition of asphalts, cattle dung, and reduce moisture in the road verge can be accepted to minimize the road mortality of common butterflies. The most important fact we obtained from this study is local people, drivers were found very less responsive towards the butterfly conservation. Hence, public conservation awareness was realized urgent if for the sustainable conservation of butterfly species to make effective.

**Conclusion**

On the basis of the above result, we concluded that the study area is rich in butterfly species richness. However, the road mortality is the serious conservation issues prevailing in such sites. This study clearly indicates that forest sites in the road verges have greater risk for butterflies to lead higher road-killed. Hence, to mitigate such conservation issue and to establish sustainable conservation practices, a detailed study on the effect of road to the butterfly abundance patterns at different seasons become urgent need. Furthermore, regular conservation awareness programs within the local level, drivers etc. could be an alternative option to minimize the number of road-killed butterflies.

**Acknowledgments**

We are thankful to our volunteers Prabesh Kafle and Gaurab Gaudel for helping us in counting and collecting dead butterfly specimens during survey periods. We also grateful to Arjun Thapa for guidance during manuscript preparation.

**Conflict of Interests**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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آمار مرگ‌ومیر پروانه‌ها در بازگرده ناهندر، نپال

پراکانش گاودل، مانیشا پاودل، پرابین گاودل، بال رام گیری، بیمال راج شرستا

چکیده: نوسه جاده‌ها به‌تنهایی جدی در موضوع حفاظت از جمعیت جانوران تبدیل شده است. برخورد جانوران با وسایل نقلیه اثر مستقیمی این پدیده است که در نهایت منجر به صدمات شدید یا مرگ آنها می‌شود. در مورد مهره‌داران تخمین روشنی از آمار مرگ و میر بیشترین‌ترین این موضوع اطلاعاتی در مورد جانوران یافت شده است. این موضوع مانند پروانه‌ها که به‌تعداد زیاد در جاده‌ها تلف می‌شوند، وجود ندارد. این تحقیق اولین سنجش جامع از مرگ و میر جاده‌ای پروانه‌ها در کشور نپال است. هدف عمده این تحقیق تخمین آمار تلفات پروانه‌ها در بازگرده ناهندر و عدم علایق جاده‌ای پروانه‌ها در بزرگراه ماهندرا با عنوان پرترافیک‌ترین است. این مسیر در نپال واقع است. هشت ناحیه هشت متری در مناطق استیلوت انسانی و جنگلی به طور تصادفی انتخاب شدند. ضریب همبستگی پیرسون برای تعیین رابطه بین تعداد پروانه‌های زنده و تلف شده محاسبه شد. در کل، تعداد هزار نمونه پروانه در طول هفته تلف شده و تعداد هزار نمونه زنده مانده بود. تعداد کل پروانه‌ها در منطقه جنگلی نسبت به مناطق استیلوت انسانی بیشتر بود. همبستگی تعداد پروانه‌های تلف شده چه در جاده‌های همبستگی در جاده‌های همبستگی زیادی دارد. تعداد پروانه‌های زنده را به‌طور کلی از جاده بهتر می‌کردند. بنابراین این تحقیق نشان می‌دهد که جاده‌ها اثر معنی‌داری بر جمعیت پروانه‌ها دارند. به‌علاوه، هر چه جمعیت پروانه‌ها بیشتر شود، تلفات جاده‌ای نیز بیشتر خواهد بود. ارتقای گامهای عمومی و حفاظت از زیستگاه‌ها با نصب حصارهای مرتفع، احداث پذیره و اجرای برنامه‌های تهیه‌سازی جاده‌ها، منجر به کاهش میزان تلفات پروانه‌ها در این نواحی خواهد شد.