Effect of elevation and plant communities on the flight behaviour of Lepidoptera in Kosciuszko National Park, Australia

Noah Tan*[1]

* Corresponding author: u7541081@anu.edu.au

[1] Research School of Biology, ANU, Canberra, ACT, Australia

Abstract

Lepidoptera play a critical role in ecosystem functioning and are especially vital to the plant-pollinator network in alpine regions. Both their functional traits and broader environmental filters strongly influence their performance in the environment. This study aims to address the lack of research on the functional traits of Australian alpine diurnal Lepidoptera by examining whether their flapping rate differs across elevations and/or plant communities. Field surveys were conducted over four sites in Kosciuszko National Park, comprising heathland and woodland communities at both low and high elevations. Videos of Lepidoptera in flight were recorded and analysed to obtain flapping rates. The results indicate that elevation and plant community interacted to affect flapping rates. Within heathland communities Lepidopterans found at higher elevations exhibited a greater flapping rate than those found at lower elevations. On the other hand, there was no effect of elevation within woodland communities. These results correspond with existing literature, which indicate that elevation alone does not affect flapping rates. We suggest that differences in flapping rates between low and high elevation heathland sites may be driven by variation in food sources within heathland communities at different elevations, with resultant feeding habits imposing constraints on flight behaviour.

Introduction

Lepidoptera, an order that comprises moths and butterflies, are amongst the most diverse and abundant insect groups (Kawahara *et al.* 2019) . They play a critical role in ecosystem functioning, involved in many ecological processes including nutrient cycling and seed dispersal. Moreover, due to their sheer numbers, lepidopterans are a significant food source for other animals such as insectivorous birds and mammals (Sharma S 2020). Despite the frequent heavy documentation of bees as effective pollinators, lepidopterans are also an important part of the pollination network. Mainly feeding on flower nectar for energy, they pollinate a wide range of flowering plant species by transferring pollen that attaches to their bodies while feeding (Ribas-Marquès *et al.* 2022).

In alpine regions particularly, the contributions of lepidopterans to the plant-pollinator network are vital. For instance, higher elevation plant communities in the San Fransisco Peaks have been associated with increased lepidopteran interactions nearly double that of lower elevations (Chesshire *et al.* 2021). Moreover, within the Australian alpine and sub-alpine zones, certain species such as the Bogong Moth (*Agrotis infusa* (Boisduval)) have also been identified as keystone species – likely serving as important pollinators for a large variety of plant taxa in mountain ecosystems (Coates *et al.* 2023).

The functional traits of lepidopterans heavily influence their performance in the environment and are essential in shaping their distribution (Henriques *et al.* 2022). Lepidopterans are dependent on flight to engage in most adult life activities such as foraging, locating mates, escaping from predators and searching for suitable host plants (Gibbs and Van Dyck 2010; Kingsolver and Watt 1983). Over small spatial scales, their functional traits in terms of wing morphology and flight behaviour regulate their interactions with the environment (Blonder *et al.* 2015). For instance, the act of flapping during insect flight contributes greatly to the energetically costly nature of flying, with their thoracic muscles requiring the highest metabolic rates across all locomotor tissues (Mattila 2015). Despite the higher energetic costs of flapping as compared to the other primary flight behaviour of gliding, flapping enables important behaviours such as take-off, climbing, hovering and complex manoeuvring to be performed (Le Roy *et al.* 2019). Consequently, functional traits such as flight behaviour determine lepidopteran's interactions and performance in the environment.

Besides functional traits, broader environmental filters such as elevation and plant community types can also shape lepidopteran flight performance. Environmental filters act on functional traits by favouring the coexistence of species with similar tolerances and responses to surrounding habitat conditions, thereby exerting selection pressures on their diversity and abundance (Mayfield *et al.* 2009). Elevation is one such filter. At higher altitudes, lower air density generates proportionally less lift during flapping (Tsuchiya *et al.*, 2023). This leads lepidopterans to exhibit slower flight at higher elevations and increases their vulnerability to predation, thereby lowering survival (Graça *et al.* 2016). In tropical butterflies it has additionally been shown that these increased selection pressures can in turn lead to reduced butterfly diversity at high elevations (Henriques *et al.* 2022)

Plant communities are another environmental filter that influences lepidopteran's flight behaviour and their distribution. Different plant communities create distinct microhabitats, with spatial variation that alters the evolution of flap-gliding flights (Le Roy *et al.* 2021). For instance, butterfly species that have evolved in cluttered understorey habitats possess more powerful flapping phases relative to species in the open canopy that exhibit more efficient gliding phases; in understories, there is likely a greater need to execute more complex flight manoeuvres to navigate tight spaces, hence placing greater emphasis on flapping compared to open canopies.

Despite being one of the most studied animal groups, there is a stark lack of research on lepidopteran in the Australian alpine habitats; most functional trait research on alpine Lepidoptera have been conducted on European fauna (Henriques *et al.* 2022; Hill *et al.* 2021; Le Roy *et al.* 2019). This poses huge challenges in predicting the effects of climate change on the Australian alpine lepidopterans, owing to a lack of quantification and understanding of their diverse functional traits and corresponding interactions in alpine environments. With rapid and pronounced reactions to climate change often exhibited by species communities in mountainous regions, it is vital to address this gap in ecological research urgently (Bertrand *et al.* 2011; Kerner *et al.* 2023).

Here, we were interested in exploring the interactions between the lepidopteran's functional traits and the structural characteristics of its habitat. Specifically, we aimed to examine whether the flight behaviour of lepidopterans – in terms of their flapping rate – differs across elevations and/or plant communities. We hypothesised that Lepidoptera found at high elevations would have higher flapping rates than Lepidoptera found at low elevations, since increased flapping rate could compensate for reduced lift in an environment of lower air density. Moreover, we hypothesised that Lepidoptera found within heathlands would have higher flapping rates than Lepidoptera found within woodlands. This is in accordance with studies in dense understorey habitats noting more powerful flapping phases (Le Roy *et al.* 2021). Additionally, we hypothesised an interaction effect between elevation and plant community, since the composition of plant communities are highly dependent on environmental parameters shaped by elevation.

Methods

Study Area

Lepidopterans are widespread, inhabiting almost all terrestrial environments ranging from tropical rainforests to arid deserts and even high mountain peaks (Legal 2022). Most lepidopterans occupy specialised ecological niches, with many niches confined to a limited selection of host plants – potentially narrowed down to one single plant part (Grehan and Mielke 2018; Legal *et al.* 2015). We collected data across four sites at Kosciusko National Park, New South Wales, Australia in late November 2023, with generally sunny conditions and an average temperature of 18.5°C during data collection between 0930hrs and 1630hrs. Two low elevation sites comprising a montane woodland (36°35'17"S, 148°56'47"E) and an open heathland (36°20'55"S, 148°31'22"E) were located near Sawpit Creek (~ 1260m elevation), whereas two high elevation sites comprising a sub-alpine woodland (36°25'55"S , 148°19'41"E) and a closed heathland (36°25'57"S, 148°19'36"E) were located near the summit trail on Charlotte's Pass (~ 1835m elevation).

Data Collection

In the respective study sites, we sampled randomly selected 30m by 30m blocks of heathland or woodland for an average of 19.6 minutes per site – with each block sampled at different times between 0930hrs and 1630hrs – making sure to maintain a distance of at least 10m away from the track to minimise the influence of edge effects (Eldegard *et al.* 2015). Blocking was also applied by sampling the low and high elevation sites in a different order across the three days, with low elevation sites sampled first on the first and third day. To ensure that the observations were independent, we conducted sampling in one direction and limited the number of samples recorded per person to around six samples. This allowed us to avoid recording individual Lepidoptera more than once.

Using our smartphones, we took video recordings of lepidopteran observed in flight, concurrently noting down the relative size of each individual (small; <15mm, medium; 15mm – 30mm, large; >30mm); size was used as a proxy for species type. We then sifted through the recorded videos, retaining footage which contained at least one second of continuous visible flight. 20 independent recordings of lepidopterans in flight were ultimately obtained per site. From these videos we recorded the flapping rate (flaps per second) of each individual. We started off by cropping and slowing down the video segment that displayed the individual in clear visible flight; this was achieved using the smartphone's inbuilt video editing software. Thereafter, we counted the number of wing flaps – which we defined as an upstroke-downstroke motion – and divided this by the duration of the cropped video segment. For each video, we also recorded the elevation (metres) and temperature (°C) when the video was taken using the 'Garmin Earthmate' app and the Kestrel weather meter respectively.

Table 1. Average temperature (°C) and corresponding standard error recorded at the four study sites

		Plant Community	
		Woodland	**Heathland**
Elevation	**High**	19.250 ± 0.109	17.800 ± 0.000
	Low	16.200 ± 0.000	21.950 ± 0.085

Analytical Approach

All the analyses were performed using JMP version 17.2. To test both hypotheses that Lepidoptera found at higher elevations have higher flapping rates than those at lower elevations, and that Lepidoptera found within heathlands have higher flapping rates than those within woodlands, two-sample t-tests were respectively performed. A two-way ANOVA also enabled the examination of an interaction effect between elevation and plant community on flapping rate.

Additionally, we attempted to account for the effects of both differing Lepidoptera species and temperature on flight performance. To control for the diverse functional traits of Lepidoptera species on flight performance, a two-way ANOVA was performed to examine the interaction effects of size with elevation and plant community respectively. Moreover, the size differences across all four sites were analysed. Two-sample t-tests were also performed to determine the effect of size on flapping rates.

To control for the effects of temperature on flight performance, a two-way ANOVA was also performed to examine the interaction effects of temperature with elevation and plant community respectively. However, temperature could not be controlled for in the factorial design; there were insufficient degrees of freedom that could be supported by the model as indicated by the error "LostDFs" on JMP.

Results

Out of the total sample size of 78 Lepidoptera video footages, 38 samples were obtained at both high elevations and heathlands respectively, whereas 40 samples were obtained at both low elevations and woodlands respectively (Table 1).

Table 2. Lepidoptera sample size at the four study sites

		Plant Community	
		Woodland	**Heathland**
Elevation	**High**	18	20
	Low	22	18

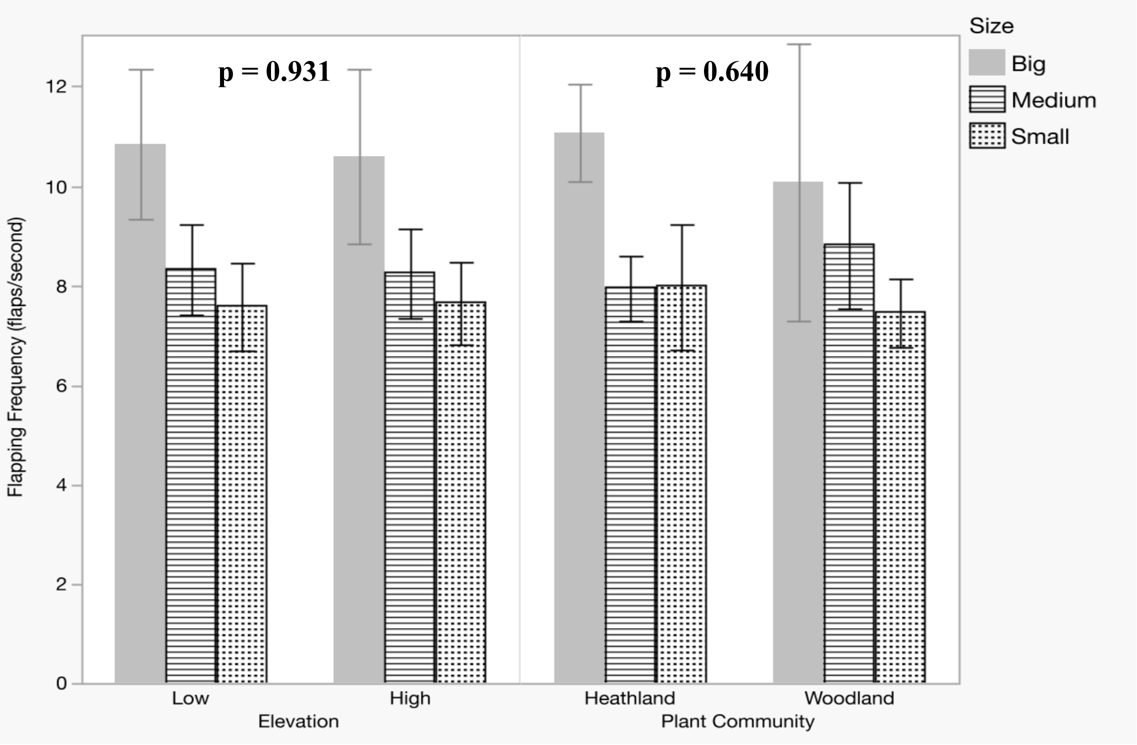

Figure 1. Bar graph of the interaction effect between size and elevation or plant community respectively on Lepidoptera's mean flapping rate. Error bars indicate the standard error for the respective means.

Table 3. Lepidoptera size counts across the four study sites

		Size Count		
		Small	**Medium**	**Large**
Site	**Low Woodland**	11	7	4
	High Woodland	11	6	1
	Low Heathland	6	9	3
	High Heathland	3	11	6

Table 4. Lepidoptera mean flapping rates (flaps per second) and corresponding standard errors based on size

Size	Mean Flapping Rates
Small	7.607 ± 3.353
Medium	8.285 ± 3.627
Large	10.721 ± 4.175

There was no effect of the interaction between size and elevation (p = 0.931, F = 0.072, DF = 2), or between size and plant community (p = 0.640, F = 0.449, DF = 2) on flapping rate (Fig 1). However, there were evident differences in Lepidoptera sizes measured across the four sites (Table 3). When analysing the effects of size on flapping rates using two-sample t-tests, we found that large-sized lepidopterans had significantly higher flapping rates than medium-sized lepidopterans (p = 0.035, t = -1.900, DF = 21.752) and small-sized lepidopterans (p = 0.011, t = -2.457, DF = 20.905), whilst flapping rates of medium-sized lepidopterans did not differ significantly from small-sized lepidopterans (p = 0.220, t = -0.778, DF = 61.986) (Table 4). As such, to minimise the confounding effects of different number of Lepidoptera sizes across the four sites, we took an average value for each size group at the respective four sites for further analysis. This ensured that every size group would be equally represented across all the sites, thereby minimising the possible effects of species differences on our variables of interest.

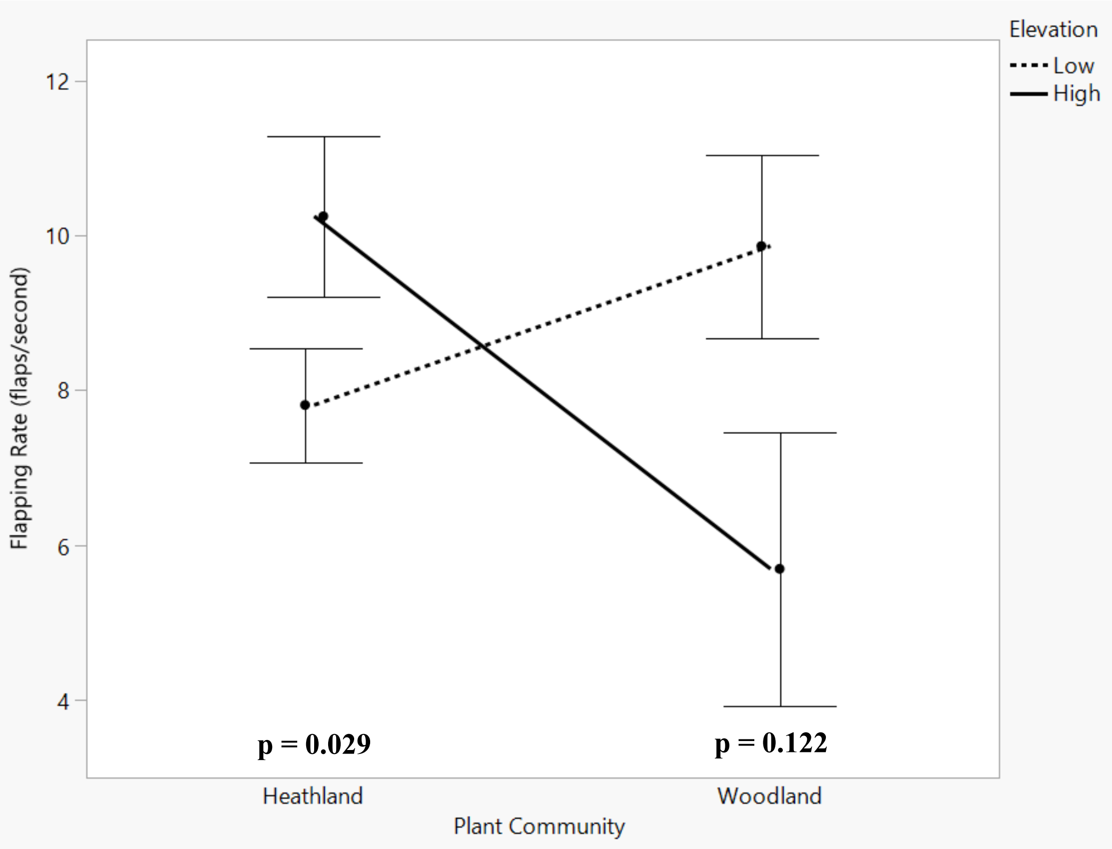

Figure 2. Interaction plot of Lepidoptera's mean flapping rate at high and low elevations in heathland and woodland respectively. Error bars indicate the standard error for the respective means.

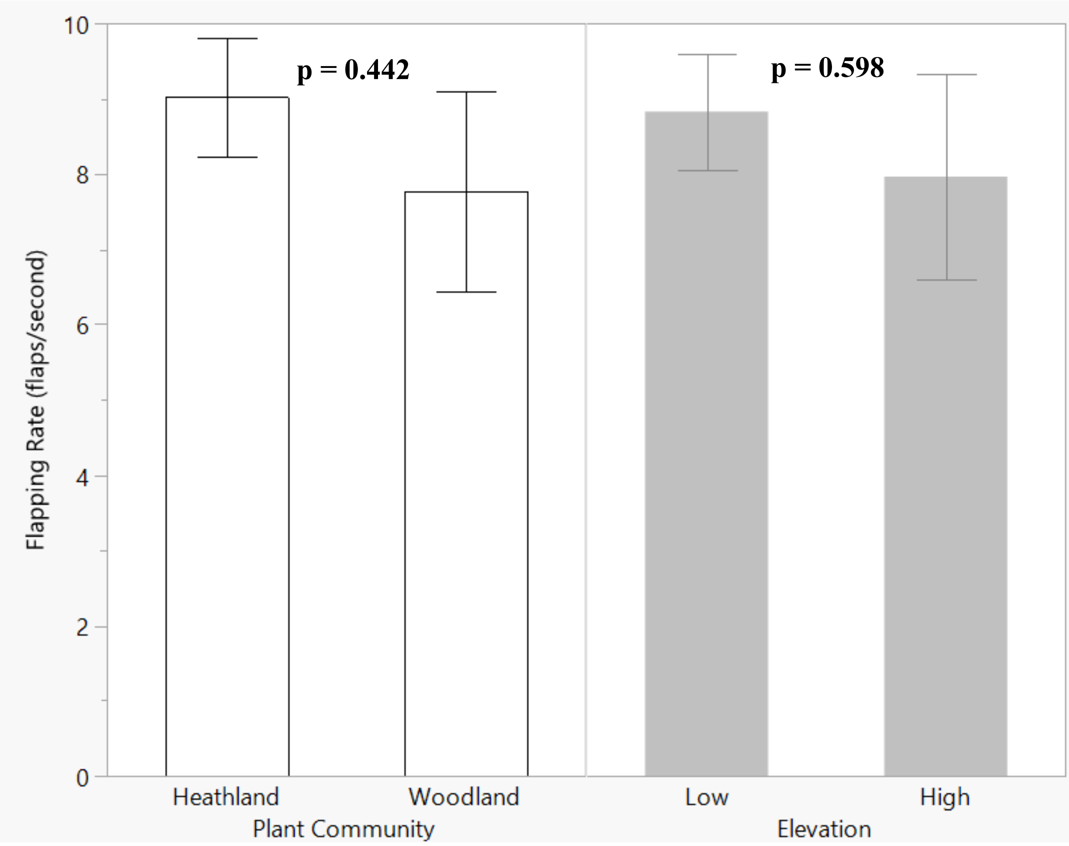

Figure 3. Bar graph of Lepidoptera mean flapping rate with elevation and plant community respectively. Error bars indicate the standard error for the respective means.

When looking at the effects of elevation and plant community on flapping rate we found that there was a significant interaction effect involving elevation and plant community (Fig 2). Within the heathland community the mean flapping rate for lepidopterans found at higher elevations (10.244 ± 1.802 flaps/second) was greater than lepidopterans found at lower elevations (7.807 ± 1.269 flaps/second) ($p = 0.029$, $F = 2.863$, $DF = 4$). However, there was no effect of elevation within woodland communities ($p = 0.122$, $F = 3.829$, $DF = 4$).

Overall, there were no significant main effects of elevation ($p = 0.598$, $t = -0.550$, $DF = 7.883$) or plant community ($p = 0.442$, $t = -0.807$, $DF = 8.115$) (Fig 3).

Discussion

This study's purpose was to determine whether the flapping rate of Lepidopterans differed across elevations and/or plant communities. The results did not support our predictions regarding the main effects – that lepidopterans found at high elevations will have higher flapping rates than those found at low elevations, and that lepidoptera found within heathlands would have higher flapping rates than those found within woodlands. However, the results supported our predictions regarding the interaction effect, in which the effect of elevation is dependent on the plant community that lepidopterans are flying in.

Various experimental studies have been conducted to evaluate the effects of altitude on flapping flight. For instance, an experimental study on the lift generation of monarch butterflies *Danaus plexippus* (Linnaeus) across elevational gradients was undertaken by altering air densities inside a large pressure chamber (Sridhar *et al.* 2021). Sridhar *et al.* (2021) concluded that the increased lift coefficient generated to maintain altitude was not correlated to higher flapping rates, but was instead attributed to an efficient attack angle associated with the wing to body velocity ratio. These findings are supported by Tsuchiya *et al.* (2023), whose study investigated the high-altitude flight performance of an aerial robot with a tailless flapping-wing. The results similarly highlighted that flight ability under low density conditions was not due to a "simple increase in the flapping frequency" (Tsuchiya *et al.* 2023, p. 9), but rather an altered angle of attack. Evidently, existing literature around the effect of elevation on flapping frequencies generally corresponds with our results, indicating that elevation alone does not affect flapping rates. Crucially, it emphasises the interaction effect that elevation only affects flapping rates for lepidopterans in heathland communities.

This brings about the main point of discussion: What characteristics of heathland communities vary at different elevations to influence the Lepidoptera's flapping rate? A possible explanation could be linked to the denser shrub-dominated heathland understories as compared to that of woodland understories. In a denser environment, the ability of lepidopterans to alter their angle of attack is potentially restricted, hence forcing them to rely on an increased flapping rate to maintain altitude.

Another possible explanation lies in the variation in adult-specific food sources within heathland communities across the elevational gradient. Plant species richness in varying growth forms such as shrubs have been known to vary with elevation, where different spatial niches are likely to provide varying food sources (Bhattarai and Vetaas 2003; Lomolino 2001). Even within plant species themselves, differentiation in floral-traits and nectar abundance arise throughout plant populations alongside pollinator shifts. For instance, in a study on the *Trollius ranunculoides* in the east Qinghai-Tibet Plateau, a greater number of smaller nectariferous petals were found in alpine populations as compared to sub-alpine populations (Zhao and Huang 2013); the stronger selection on petal numbers in alpine populations was in accordance with the nectar preference of flies which were the primary pollinators. In response to varying food sources, consequent feeding habits could thereby impose specific constraints on flight behaviour (Le Roy *et al.* 2019). The sphingid moth's extraordinary hovering abilities for example have been linked to a co-evolution with their nectar-feeding habits that support high metabolic rates (Arms *et al.* 1974). Ultimately, increased flapping rates within heathland communities could be explained by a corresponding increase in nectar-rich plant species at high elevations, hence offering an avenue for further study.

Conversely, the non-significant effect of elevation within woodland communities could be attributed to the relative lack of access to sunlight at the field and ground layers. Lepidopterans bask in the sun for direct heat absorption via their wings (Liao et al. 2019). This is essential for their thoracic temperature to be raised higher than the surroundings – ultimately serving as a key prerequisite in facilitating autonomous flight (Kemp and Krockenberger 2004; Mattila 2015). Compared to heathlands which are bright open spaces, woodlands consist of distinct layers of vegetation which filters light to varying extents. As such, lepidopterans could be exposed to differing amounts of sunlight depending on their position with respect to different canopy heights. Despite greatest sunlight exposure nearing the canopy layer, corresponding measurements were inaccessible and hence most observations were recorded at the field or ground layer where access to light was limited. With flight behaviour strongly affected by light intensity (Liao et al. 2017), the uneven exposure to sunlight within woodlands could therefore affect flapping rates at both high and low elevations, hence explaining the non-significant interaction.

Limitations

A limitation of this study was the lack of control for extraneous environmental variables such as temperature and wind that could have affected flapping rates. For instance, external temperature is a potential extraneous variable that is regarded as a key selective factor influencing a butterfly's behaviour, thus strongly constraining an insect's flight activity (Clench 1966; Nève and Després 2020). Furthermore, wind exposure could lead to air movement instabilities as aforementioned, hence impacting flight behaviour. Ideally, future studies should strive to control for these external factors by ensuring that similar binned measurements are recorded across the different sites.

Another limitation was the inability to identify the species of each individual to determine their wing morphology and behaviour. The diverse range of lepidopteran species possess unique wing morphologies which could heavily influence their flight behaviour and hence performance (Le Roy *et al.* 2019). Though we accounted for size differences by taking the average of each size, size alone is not the most representative proxy to differentiate Lepidoptera species - especially with more than 160000 described species (Cranston and Gullan 2009). As such, future extensions could include the use of geometric morphometrics to quantity wing shape (Le Roy *et al.* 2021), and must include accurate identification of species or genera studied. Furthermore, identification of the contrasting wing shapes could allow us to study the relationship between wing morphology and flight behaviour in Australian alpine Lepidoptera. This would enable us to better understand lepidopteran evolutionary adaptations and consequently predict their response to climate change. Further studies should also be conducted to investigate the wider associations between elevation and flapping rate, particularly the biotic interactions between flowering plants and Lepidoptera. For instance, surveying plant species richness and abundance could serve to identify potential adult lepidopteran food sources that would influence feeding habits and hence flight behaviour.

Conclusion

This study found that Lepidoptera at high elevation in heathland communities have higher flapping rates than those found at low elevation, hence demonstrating key insights into an interaction between elevation and heathland communities on flapping rates. The study of flight behaviours with regards to flapping rates suggests an energetic cost used for flying. This entails trade-offs in terms of energy available for other crucial functions such as reproduction. Consequent changes in reproduction could have population-level effects on lepidopterans; over time, this may change pollination structures. Other reduced energetic costs could involve foraging for nectar, thereby affecting species interactions with plant communities (Nespolo *et al.* 2008). For instance, reduced foraging durations would likely decrease pollination and hence fertilisation rates of mutualistic flowering plants (Gagic *et al.* 2021). This could also lead to a self-reinforcing cycle, in which the declining abundance of flowering plants due to reduced pollination would increase interspecific competition for limited food resources, thus resulting in reduced fitness and potentially cascading effects on the ecosystem at large. However, as elevation effects appear restricted to heathland communities, the impact of climate change on Lepidoptera is likely to be minimal with most generalist species able to survive in other habitats.

Ultimately, a more in-depth understanding of lepidopteran’s functional traits is not only crucial in determining their change in distribution in the Australian Alpine, but also in predicting their response to rapidly changing climates. Due to their high temperature sensitivity, quick generational turnovers and short life cycles, lepidopterans are highly sensitive to the effects of climate change (Hill *et al.* 2021; Lenoir *et al.* 2020). With warmer temperatures likely to shift lepidopteran's distribution to regions of higher elevation, understanding their corresponding flight behaviours would facilitate better-informed conservation actions if required.

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