scientific reports

OPEN

Environmental influence and species occurrence of yellowjacket drones in an invaded area

Agustina P. Porrino ✉, Maité Masciocchi & Andrés S. Martínez

During the mating season, reproductive individuals of numerous insect species gather in rendezvous areas, which increases mating opportunities. Male hymenopterans often have to move considerable distances during a particular season, searching or waiting for receptive females. Such behavior is likely driven by a complex combination of individual and species-specific traits, environmental influence, and landscape cues. Our field study aimed to determine factors affecting the occurrence of *Vespula* spp. drones, focusing on the influence of vegetation traits, atmospheric factors and diel effects, and the species occurrence proportion in an invaded area in Patagonia. Our results indicate that the probability of drone presence over different types of vegetation is affected both by plant species and height. Also, weather and time of day influence the number of individuals simultaneously gathering, as higher abundances of flying drones are found in early hours, warmer days and at low cloud cover. Lastly, through mid-flight drone captures, we determined that both *V. germanica* and *V. vulgaris* drones are found concurrently in the same rendezvous areas. This constitutes the first exploratory field study reporting the heterospecific occurrence of *Vespula* spp. drones and overall, our results contribute to the understanding of yellowjackets mating systems.

The congregation of reproductive individuals in locations where potential sexual partners are likely to be found, is a widespread strategy among numerous insect species, which leads to increased mating probabilities[1]. Males of many hymenopterans, that abandon their parental nest to copulate, move over considerable distances searching or waiting for receptive females, usually during a particular season and specific times of the day. Consequently, the spatiotemporal distribution of receptive females and distribution of natal nests can shape male mating behavior and determine rendezvous sites. Since finding females in areas where nests are widespread and their emergence from colonies is extended across several weeks, males usually roam and cluster in specific transit areas where females are likely to pass[2–4]. Under these circumstances, males can gather in transient amorphous aerial aggregations in which they consistently patrol in order to locate females, rather than being fixed at one specific site[4–6].

The gathering of reproductive individuals not only increases mating opportunities by bringing sexual partners together at rendezvous sites but can also reduce predation risks due to early predator detection or decreased per capita risk of predation, facilitate mate choice, and aid polyandrous females to effectively mate with multiple males, promoting outcrossing, hence diminishing inbreeding probabilities[3,4]. Moreover, inbreeding avoidance is well-documented in arrhenotokous haplodiploid hymenopterans (i.e., many eusocial ant, bee and wasp species) which are more susceptible to inbreeding depression due to their complementary sex determination system in which homozygous eggs, resulting from inbred matings, develop into sterile or non-viable diploid males[1,7–9]. Thus, drone congregations could allow the mixing of local genes, consequently reducing inbreeding probabilities[5].

Ultimately, the underlying process of reproductive encounters is the individuals' active movement from natal to, or between, reproduction sites[10]. This is likely driven by a complex combination of individual and species-specific traits, environmental influence, and landscape cues[11]. For instance, every mating season, honeybee *Apis mellifera* (L.) drones gather at the same sites, influenced by flight abilities correlated with body size, distance to natal colonies, and environmental conditions like sun irradiance and wind direction[6,12,13]. Furthermore, rendezvous sites can be interconnected through flyways formed adjacent to terrestrial features such as tree

Grupo de Ecología de Poblaciones de Insectos, Instituto de Investigaciones Forestales y Agropecuarias Bariloche (IFAB) (CONICET – INTA), Modesta Victoria N°4450, San Carlos de Bariloche, Río Negro 8400, Argentina. ✉email: agustina.porrino@gmail.com

lines, which correlate with visual and olfactory cues including the presence of receptive females or sexual pheromones[14]. Also, for species that do not mate in flight, vegetation can provide a grasping substrate and shelter at the same time[15]. Thus, these reports are indicative that male mating behavior is simultaneously influenced by the complex interaction of intrinsic and extrinsic factors.

Consequently, reproductive assemblages are generally expected to be composed of individuals from the same species (i.e., monospecific) as selective pressures should optimize the search and encounter of conspecific partners to avoid interspecific mating[16]. However, in eusocial hymenopterans, more than one species –usually closely related– can gather at the same rendezvous sites[2,16,17]. Numerous hypotheses have been proposed to explain this, such as the attraction to a common, highly volatile chemical compound or males of different species sharing chemical profiles based on nonvolatile cuticular compounds[16].

Commonly known as yellowjackets, *Vespula germanica* (F.) and *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) are sympatric haplodiploid eusocial wasps native to Eurasia, which in the last decades have invaded many parts of the globe, becoming significant pests in many countries[18]. The first detection of *V. germanica* in Argentina date from 1980 in north-western Patagonia[19] while *V. vulgaris* was first detected in 2010 in the same region[20]. Since registered, both species have greatly increased their abundance spreading throughout southern Argentina and exhibit overlapping ranges[21,22]. Previous reports indicate that in invaded areas, *Vespula* wasps can cause economic and environmental impacts, negatively affecting industries like beekeeping, cattle, forestry, and tourism[23–25]. Furthermore, these species threaten human health as they can be a nuisance and cause physical harm to humans, even anaphylaxis due to their venomous sting[26].

Vespula germanica and *V. vulgaris*, share similar external morphology, behavior, habits and life cycle. Colonies in temperate regions are annual, with the foundress queen awakening in early spring after overwintering, time in which starts laying eggs. During summer, colonies can grow to several thousand individuals (mostly non-reproductive workers) reaching their population peak in early autumn, time in which reproductive individuals (i.e., gynes and drones) begin to emerge from parental nests to mate. Shortly after the reproductive season, the foundress queen, workers, and drones die, and only newly fertilized queens overwinter in sheltered places to continue the cycle the following spring, with the establishment of new colonies[27,28].

Previous reports indicate that when leaving the parental nest, gynes and drones have sex-biased emergence timing, with drones gathering in loose conspecific aggregations around trees and shrubs, waiting for females to arrive[28,29]. Besides, reproductive aggregations might be conditioned by the presence of kin (nestmates) and drone-density[30]. Moreover, previous studies suggest that both *V. germanica* and *V. vulgaris* gynes are polyandrous (i.e., gynes mate multiple times) and produce an airborne sex pheromone that attracts drones and stimulates copulation[31–33].

Our main goal was to elucidate the influence of biotic and abiotic factors on *Vespula* spp. drone occurrence through a field study. Specifically, during the yellowjacket reproductive flight period in an invaded area in Northern Patagonia, we evaluated (i) the effect of woody vegetation on drone presence likelihood; (ii) the influence of weather and daytime on the abundance of flying individuals; and (iii) the proportion of species occurrence (*V. germanica* and *V. vulgaris*) in drone high-density areas.

Results
Effect of vegetation on drone presence
The probability of drone occurrence on woody vegetation correlated positively with vegetation height (GLMM Binomial distribution, ANOVA, $\chi^2 = 42.8$, df = 1, $P < 0.001$; Supplementary Table S1), and was also affected by vegetation species (GLMM Binomial distribution; ANOVA, $\chi^2 = 320.6$, df = 13, $P < 0.001$; Table S1) with the highest predicted probabilities of presence on *Schinus patagonicus* ($\bar{x}$ predicted = 0.833; $\bar{x}$ height: 2.64 ± 0.09 m; Supplementary Table S2), followed by *Austrocedrus chilensis* ($\bar{x}$ predicted = 0.719; $\bar{x}$ height ± SE: 4.00 ± 0.46 m), *Maytenus boaria* ($\bar{x}$ predicted = 0.631; $\bar{x}$ height ± SE: 3.40 ± 0.16 m), *Pinus* spp. ($\bar{x}$ predicted = 0.618; $\bar{x}$ height ± SE: 5.73 ± 0.41 m), *Lomatia hirsuta* ($\bar{x}$ predicted = 0.610; $\bar{x}$ height ± SE: 4.04 ± 0.30 m), *Discaria chacaye* ($\bar{x}$ predicted = 0.529; $\bar{x}$ height ± SE: 2.89 ± 0.07 m) and *Bulnesia retama* ($\bar{x}$ predicted = 0.402; $\bar{x}$ height ± SE: 2.87 ± 0.14 m). Lower presence probabilities were recorded on *Nothofagus antarctica* ($\bar{x}$ predicted = 0.377; $\bar{x}$ height ± SE: 2.61 ± 0.13 m) and *Juniperus communis* ($\bar{x}$ predicted = 0.352; $\bar{x}$ height ± SE: 1.91 ± 0.15 m), while null ($\bar{x}$ predicted = 0.000) on *Salix fragilis* ($\bar{x}$ height ± SE: 4.00 ± 0.00 m), *Rosa rubiginosa* ($\bar{x}$ height ± SE: 1.60 ± 0.16 m), *Aristotelia chilensis* ($\bar{x}$ height ± SE: 2.5 ± 0.00 m), *Berberis darwinii* ($\bar{x}$ height ± SE: 1.47 ± 0.09 m) and *Retama sphaerocarpa* ($\bar{x}$ height ± SE: 1.82 ± 0.11 m) (Tukey post-hoc pairwise comparisons, $P < 0.05$; Supplementary Table S3) (Fig. 1).

Effect of weather and daytime
Throughout the sampled period, temperatures varied between 5.0 and 18.8 °C ($\bar{x}$ temperature = 10.7 °C) and were positively correlated with drone abundance (GLMM Negative Binomial error distribution, Z = 2.55, df = 1, $P = 0.010$; Supplementary Table S4) (Fig. 2a). Additionally, drone abundance was negatively affected by time of day (GLMM Negative Binomial error distribution, Z = -2.66, df = 1, $P = 0.007$; Supplementary Table S4) (Fig. 2b). Different levels of cloud cover negatively correlated with drone abundance regardless temperature and time of the day (GLMM Negative Binomial error distribution, 0%: Z = 6.04, $P < 0.001$; 50%: Z = 4.79, $P < 0.001$; 100%: Z = 2.24, $P = 0.024$, df = 2; Supplementary Table S4). There were also significant differences between clear (0%) and cloudy skies (50–100% cloudiness) (Tukey post-hoc pairwise comparisons, $P < 0.01$; Supplementary Table S5) (Fig. 2).

Species occurrence in snapshot points

During the surveyed period, fourteen drone samples were obtained from snapshot points, yielding a total of 306 drones captured mid-flight ($\bar{x}$ = 20 drones/sample). All captured individuals were identified as drones from *V. germanica* or *V. vulgaris* while none were workers. Twelve samples (86%) contained individuals from both species in different proportions (Fig. 3). The proportion of *V. germanica* drones found in samples significantly decreased over time (GLMM Beta error distribution, Z = -3.26, df = 1, P = 0.001), while the proportion of *V. vulgaris* drones significantly increased over the same period (GLMM Beta error distribution, Z = 3.26, df = 1, P = 0.001).

Discussion

The objective of our study was to determine the effect of biotic and abiotic factors on the occurrence of *Vespula* spp. drones during their reproductive flight period in an invaded area in Patagonia. We focused on the effect of vegetation traits on drone presence probability along with the influence of atmospheric variables and diel effects on drone abundance. We also characterized the relative occurrence of *V. germanica* and *V. vulgaris* drones in rendezvous areas. Our results indicate that the probability of drone presence was affected by vegetation species and also correlated positively with the height of the vegetation. Furthermore, our analysis indicates that weather and time of day influence the number of individuals present in the sampled areas, with higher abundances of flying drones found during early hours, on warmer days with less cloud cover. We also found that drone captures resulted in both *V. germanica* and *V. vulgaris* drones gathering concurrently in rendezvous areas. Moreover, the proportion of occurrence of each species resulted in variations throughout reproductive season. To our knowledge this is the first study to report mixed (i.e., heterospecific) reproductive gatherings in *Vespula* wasps.

Previous studies indicate that males from species in which reproductive individuals emerge from widespread natal nests over several weeks and must disperse to ensure mating, will tend to roam around broad areas where receptive females are more predictable, in order to maximize their reproductive success[2,3]. Our results indicate that *Vespula* spp. drones are more likely to patrol around the highest vegetation along wide areas –rather than being fixed at specific locations–, which might represent a mate-searching strategy based on the interception of dispersing females. Many other hymenopteran species are known to head towards the highest local points during reproductive flights, generally surrounding the canopy or gathering around locations nearby high vegetation[2,15,34]. Features such as hilltops, rivers and vegetation patches alternated with gaps, have been found to likely represent navigation aids for orienting males and females in their flight[35,36]. For example, tree lines or differences in adjacent vegetation heights have been proposed as conspicuous markers establishing common flyways[14]. At the same time, vegetation can also provide shelter from predators and unfavorable atmospheric conditions, as well as offering substrates where individuals can rest and engage in mating events (AP personal observation)[2,6,15]. Furthermore, some types of vegetation could potentially supply food resources (e.g. nectar, honeydew) needed to meet the energetic costs during the reproductive period. However, this aspect needs further research since these differences may also be given by attributes not considered in the present work, such as the architecture of each vegetation type.

Our findings also point out that the abundance of *Vespula* spp. drones correlates with diel patterns and atmospheric conditions. Generally, higher abundances of drones were recorded from mid-morning to noon, progressively decreasing towards sunset, which also corresponds with the hours of highest solar radiation. *Vespula* spp. drone activity period is within the range described for other hymenopteran species sharing similar mating behavior and matches the previously reported nest-departure behavior of *V. germanica* reproductive individuals[30,34]. Recent experimental studies have demonstrated that temperature mediates insect flight performance, as muscle activity rates are a direct function of temperature (i.e., greater flight performance at higher temperatures), which strongly depends on environmental heat in ectothermic or heterothermic insects[37]. Furthermore, sunlight and light distribution in temperate regions have been proved to be important for geographical orientation during flight in hymenoptera, related to visual stimuli perception[6,38]. Consequently, daily fluctuations in *Vespula* spp. drone densities observed during the reproductive flight season are probably subject to the interaction between the species circadian cycles and changing environmental conditions related to the characteristic seasonal timing of a temperate region.

Our study also indicates that drones of both species are found concurrently in the same areas. Previous studies have also reported that different species within ants, bees and bumblebees can share reproductive rendezvous sites[2,16,17,34]. Despite that many hypotheses have been proposed to explain the underlying mechanisms that result in the concurrence of different species to the same places, this heterospecificity in mating areas could be explained by behavioral phylogenetic homologies, related to similar evolutionary histories[39]. Selective pressures of widely dispersed receptive females –a key ecological trait– and environmental fluctuations might have shaped a set of flexible mating strategies shared by many Hymenoptera species, which range from the conformation of usually ephemeral, conspicuous reproductive aggregations to long mate-searching flights in species where females produce a sex-pheromone to attract males[2,15,40]. Consequently, similarities in mating strategies and reproductive periods may contribute to the co-occurrence of invasive species within the same rendezvous areas in invaded regions.

Temporal variations in the proportion of occurrence of *V. germanica* and *V. vulgaris* drones was also found in an invaded area. Specifically, we observed higher proportion of *V. germanica* at the beginning of the sampling period and lower at the end, with the contrary occurring for *V. vulgaris*. This temporal variation could minimize the co-occurrence of these two species during their mating period. Contrasting reproductive phenologies could be driven by differences in development and maturation timing of the colonies of each species (*V. germanica* worker activity during the season –number of wasps entering and leaving the nests– declines before of *V. vulgaris*; AM & MM personal observation). Previous studies indicate that sympatric and related species that have

similar life histories, such as the reproductive period, could exhibit strategies aimed at reducing competition and reproductive interference, thus allowing coexistence[41]. Temporal partitioning could also minimize behavioral reproductive interspecific interactions, known as reproductive interference, which can lead to a fitness reduction and affect species coexistence[42,43]. For instance, previous studies reported that when sharing the same host simultaneously, the mixing of female pheromones of two tick species, *Botrhiocroton hydrosauri* (Ixodida: Ixodidae) and *Amblyomma albolimbatum* (Ixodida: Ixodidae), suppresses the reproductive behavioral response of males[44]. We hypothesize that species-specific seasonal phenology differences could lead to temporal partitioning of reproductives of both *Vespula* species, hence avoid the potential detrimental consequences of reproductive interference at the mate acquisition stage, such as inaccurate visual recognition or pheromonal interference. However, it is important to note that in native areas temporal drone occurrence could have different patterns further contributing to reducing drone overlap, for example each species having a shorter flight period. Consequently, more studies are needed to understand the drivers for these contrasting levels of abundance between the two species.

This constitutes the first field study assessing the occurrence of *Vespula* spp. drones during their reproductive flight period and contributes to the understanding of yellowjacket mating system. Understanding the mating behavior of Hymenopterans and addressing the drivers for successful encounters in the field, is a valuable resource for biodiversity conservation and pest management programs. The fact that two invasive species in the same geographical region share reproductive rendezvous sites synchronically, comprises a valuable input for control strategies development aimed at the reproduction of these species. However, conducting further research on such interspecific interactions is fundamental, as this topic has not been addressed within *V. germanica* and *V. vulgaris* native range, nor in other invaded areas.

Methods
Study area
This study was conducted in an uninhabited area spanning ca. 30 ha in the region of San Carlos de Bariloche, Argentina (41° 09' S, 71° 18' W) where both *Vespula* species are well established. The area, located between 800 and 850 m a.s.l., is characterized by an assemblage of trees and shrubs represented by the native species *Maytenus boaria* (Fam: Celastraceae), *Austrocedrus chilensis* (Fam: Cupressaceae), *Lomatia hirsuta* (Fam: Proteaceae), *Nothofagus antarctica* (Fam: Nothofagaceae), *Schinus patagonicus* (Fam: Anacardiaceae), *Discaria chacaye* (Fam: Rhamnaceae), *Bulnesia retama* (Fam: Zygophillaceae), *Berberis darwinii* (Fam: Berberidaceae) and *Aristotelia chilensis* (Fam: Elaeocarpaceae); combined with exotic species such as *Salix fragilis* (Fam: Salicaceae), *Pinus* spp. (Fam: Pinaceae), *Juniperus communis* (Fam: Cupressaceae), *Rosa rubiginosa* (Fam: Rosaceae) and *Retama sphaerocarpa* (Fam: Fabaceae). Surveyed area was inspected for *Vespula* spp. nest presence, with ca. 0.7 nest/ha (*V. germanica* = 12, *V. vulgaris* = 10).

Field survey design
During the onset of the reproductive flight period of *Vespula* wasps in the austral autumn (April–May 2022) we established ten transects in the study area, through a field observational process in which ten locations (i.e., snapshot points, 1 × 1 m) were defined a priori in places where high densities of drones were found. Snapshot points demarcated the center of the transect, with an area of 100 m long x 20 m wide, projected 50 m in opposite directions (Fig. 4). Transects did not overlap spatially, with at least 200 m between them. On these transects, we (i) assessed the presence/absence of flying drones over all woody species of vegetation found, while on snapshot points we (ii) counted the number of passing drones at the same time and (iii) captured flying individuals to establish the relative occurrence proportion of *V. germanica* and *V. vulgaris* drones. All surveys were carried out once or twice a week between 10 and 19 h, over a span of 43 days. As this study involved focal animal groups in the field, data blind was not recorded.

Effect of vegetation on drone presence
To assess the effect of vegetation species on the probability of drones flying over, we registered drone presence/absence on all woody species found in subareas of 5 × 20 m within transects (Fig. 4). Drones were distinguished from other flying insects with the aid of an entomological net (see below), although it is important to acknowledge that the trained observer could identify drones in mid-flight. Regardless of the number of individuals of a certain vegetation species present in the same transect, if at least one drone was found flying over one specimen it counted as presence. Absences were determined as such when no drones were found flying over any of the specimens within the same transect. Each transect was surveyed 5–6 times on consecutive days and vegetation species along with the height of specimens (m) were recorded. All heights of woody vegetation were measured with the aid of a laser range finder (Nikon Forestry Pro) and averaged for individuals of the same species within the same transect.

Effect of weather and daytime
To determine the influence of abiotic factors including weather variables and time of day on the number of drones simultaneously gathering on snapshot points (i.e., drone abundance), a single observer counted the individuals flying past at each snapshot point within a height of 2–8 m. Each count was performed for three minutes, three subsequent times, with 10 min in between each measurement and then averaged to estimate drone abundance. Time of day (hour) and cloudiness (%) were registered in situ. The percentage of cloud cover was visually estimated at the center of the transect with the aid of a spherical densiometer[45]. Temperature (°C), wind speed (km h^{-1}), atmospheric pressure (hPa) and relative humidity (%) were obtained from the Argentinean National Meteorological Service closest station (Bariloche Airport). Drone abundance was measured at least 6 times at each snapshot point between 29 March and 5 May.
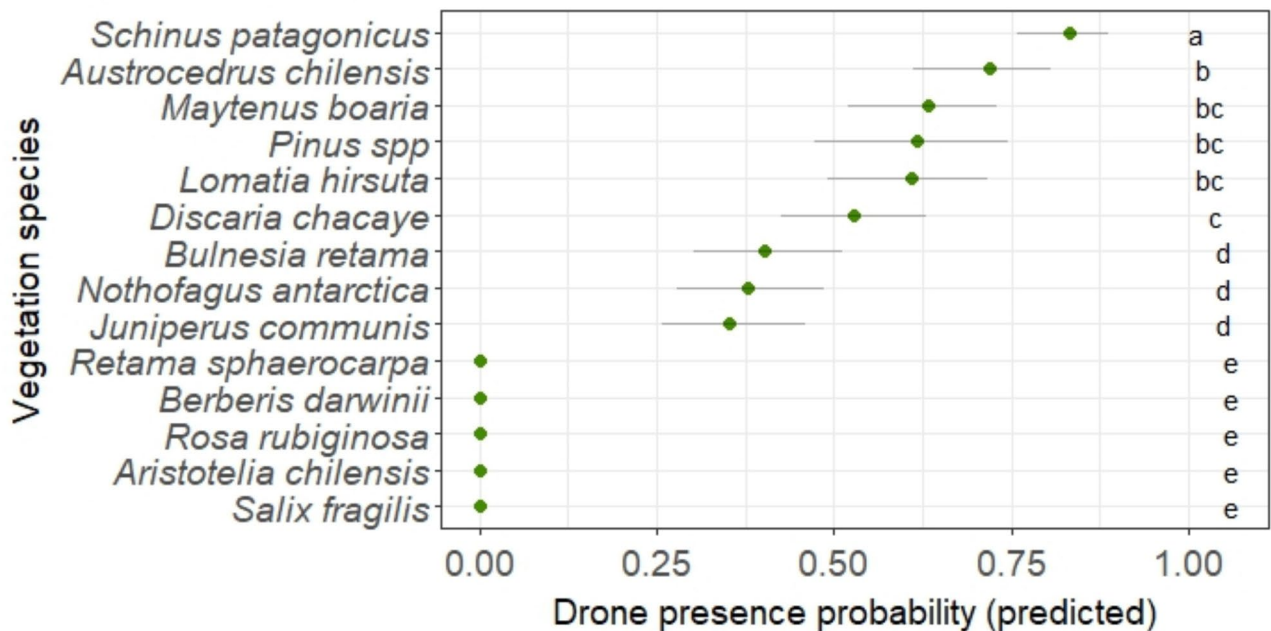
Fig. 1. Predicted *Vespula* spp. drone presence probabilities for different vegetation species found in the sampled area according to the GLMM Binomial error distribution. Different letters beside species indicate significant differences (Tukey post-hoc pairwise comparisons, $p < 0.05$; Table SI3). Horizontal gray lines represent 0.95 confidence intervals. For clarity purposes, model predictions over the response variable were back-transformed to its original scale.

Species occurrence in snapshot points

To determine the proportion of occurrence of each species (*V. germanica* and *V. vulgaris)* in snapshot points, flying drones were captured mid-flight using an entomological net (polyfilament, 36T x 36U per cm², 45 cm diameter) attached to a telescopic pole (2–7 m long). The net was swept during 30 min at least once in each snapshot point on different sampling days. Captured individuals were preserved individually in ethanol (70% vol/vol) Eppendorf tubes (1.5 mL), taken to the laboratory and dissected under a stereoscopic microscope (Stemi 305, Carl Zeiss Microscopy GmbH, Germany) for caste and species identification. *Vespula germanica* and *V. vulgaris* drones are distinguishable by differences in the morphology of the genitalia (i.e., aedeagus)[46] (Fig. 5). To validate the recognition technique, we identified 56 drones from known origin (i.e., extracted from different nests of known species; *V. germanica* = 28 and *V. vulgaris* = 28) with all individuals successfully classified.

Data analysis

We evaluated the effect of vegetation species and height on drone presence probability via a generalized linear mixed model (GLMM) with a Binomial error distribution. The full model included 'drone presence' as response variable (binary: 1/0), 'vegetation species' and with 'vegetation height' as explanatory variables, 'transect' as random effect and 'abundance of vegetation species per transect' as a set of weights. Post-hoc pairwise comparisons between vegetation species were conducted using Tukey contrasts. Model validation was assessed with a receiver operating characteristic (ROC) curve and area under curve (AUC) estimation.

The effect of abiotic factors on drone abundance in snapshot points were analyzed with generalized linear mixed models (GLMM) using Negative Binomial error distributions. The choice of fitting a Negative Binomial distribution, instead of a Poisson error distribution to the count data was based on likelihood ratio tests ($\alpha = 0.05$) which allowed testing of their relative statistical fit[47]. 'Time of day', 'temperature', 'wind speed', 'atmospheric pressure', 'cloud cover' and 'relative humidity' were considered as explanatory variables and 'snapshot point' as random effect. As 'relative humidity' showed high correlation with 'time of day' ($r > 0.6$) it was excluded prior to running the models following a collinearity threshold criteria[48]. Afterwards, 'wind speed' and 'atmospheric pressure' were removed from the models following a backward selection process in which the most non-significant variables were excluded one at a time checking for statistically significant differences between distinct models. The final model included 'temperature', 'time of day' and 'cloud cover' as explanatory variables. Model validation was carried out through residual analysis.

The proportion of drones of each *Vespula* species present in the samples taken at snapshot points throughout the season was analyzed via generalized linear mixed models (GLMM) with Beta error distribution. 'Proportion of individuals' was considered as response variable, while 'sampling day' was deemed as the explanatory variable and 'snapshot point' as random effect.

All statistical analyses were performed with R Software version 4.4.1[49]. Generalized linear mixed models were fitted using the *glmmTMB* function from the *glmmTMB* package version 1.1.9[50]. Model predictions were estimated with the *emmeans* function from the *emmeans* package version 1.10.4[51]. Post-hoc comparisons were
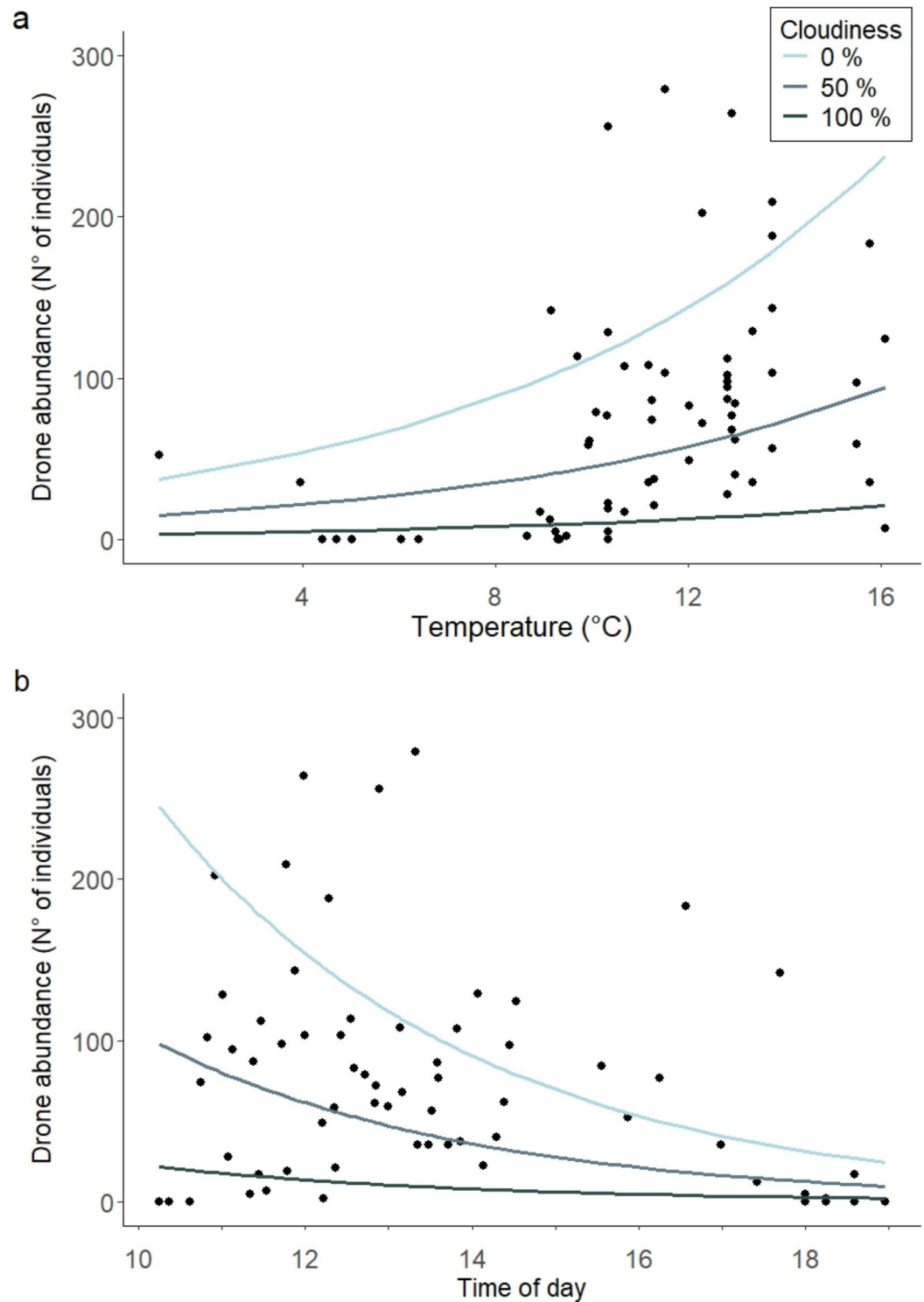
Fig. 2. Effect of (**a**) temperature and (**b**) time of day on *Vespula* spp. drone abundance in snapshot points. The lines represent predicted drone abundance in relation to (**a**) temperature and (**b**) hour for different levels of cloudiness according to the Negative Binomial error distribution GLMM (temperature: $Z = 2.55$, $df = 1$, $p < 0.05$; time of day: $Z = -2.66$, $df = 1$, $p < 0.05$; cloudiness: 0%: $Z = 6.04$, $p < 0.05$; 50%: $Z = 4.79$, $p < 0.05$; 100%: $Z = 2.24$, $p < 0.05$, $df = 2$).
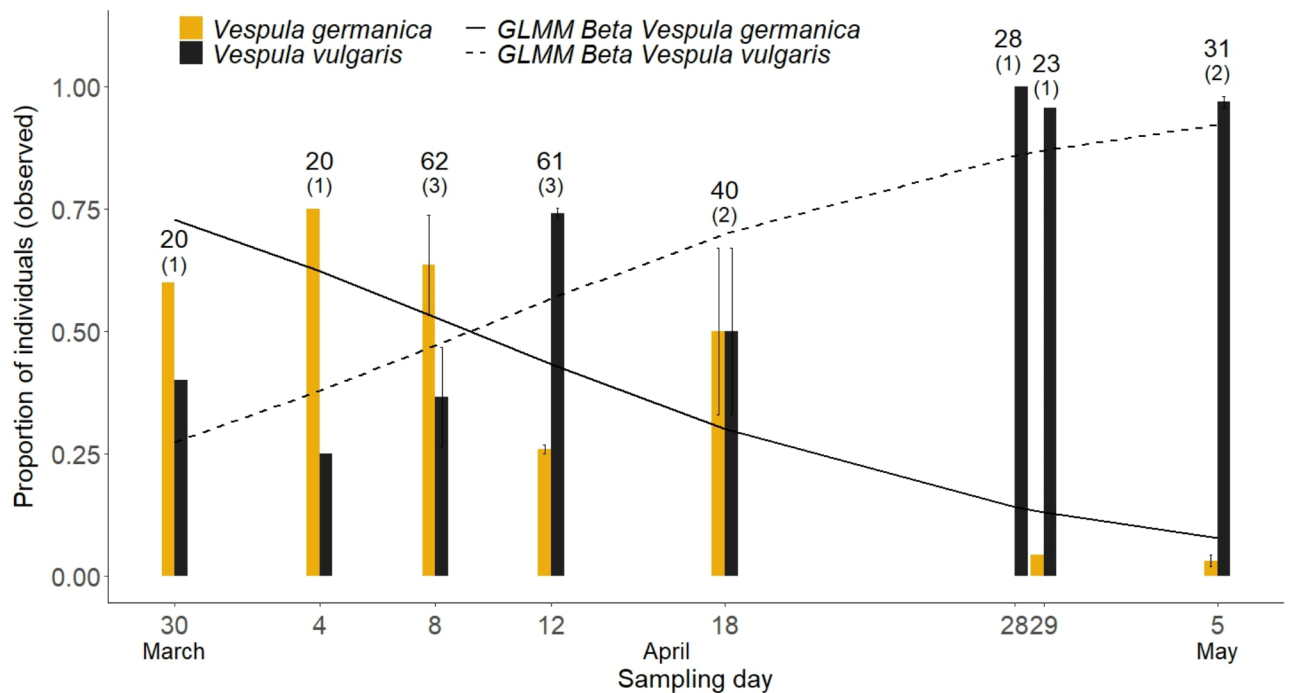
Fig. 3. Proportion of *Vespula germanica* and *Vespula vulgaris* drones sampled throughout the reproductive flight period. The absolute number of drones captured mid-flight per day is indicated above each bar while the number between parentheses indicates the amount of samples taken. The black solid curve represent the predicted proportion of V. germanica individuals according to the GLMM Beta error distribution (Z = -3.26, df = 1, $p < 0.05$). The black dashed curve represent the predicted proportion of V. vulgaris individuals according to the GLMM Beta error distribution GLMM (Z = 3.26, df = 1, $p < 0.05$).
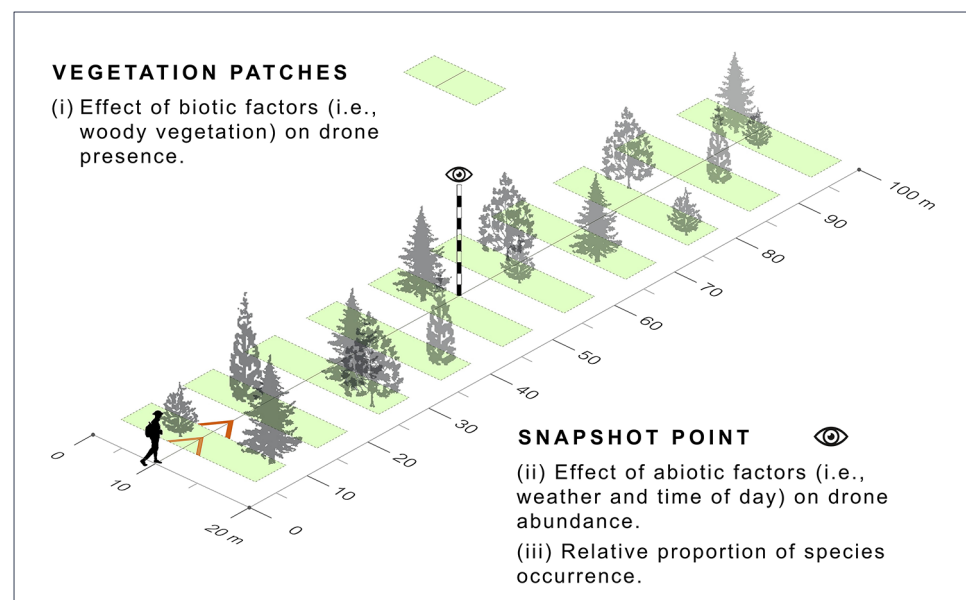
Fig. 4. Transects defined in the field and surveyed during the reproductive flight period of *Vespula* wasps in the austral autumn in Northern Patagonia. The transects were defined a priori in locations where high densities of drones were found. Within transects we registered (**i**) drone presence/absence on different types of woody vegetation, while on snapshot points we estimated (**ii**) the influence of weather and time of day on drone abundance and (**iii**) the relative proportion of species occurrence (*Vespula germanica* and *Vespula vulgaris*).
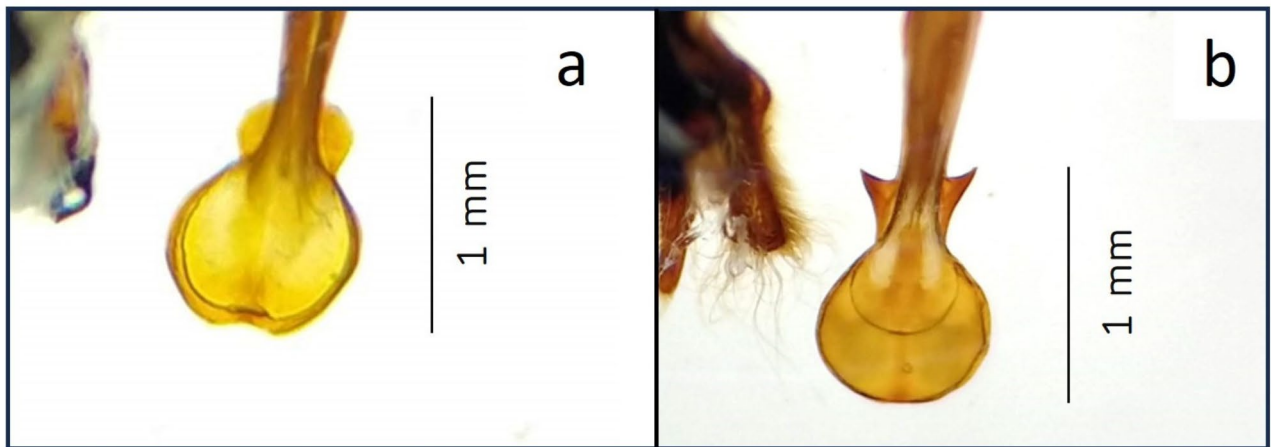
Fig. 5. Detail of (**a**) *Vespula germanica* and (**b**) *Vespula vulgaris* drone aedeagus morphology. Observed under a stereoscopic microscope (40X). Image credit AP.

assessed through the *glht* function and grouping letters were assigned through the *cld* function, both from the *multcomp* package version 1.4.26[52].

Data availability

References
1. Thornhill, R. & Alcock, J. *The Evolution of Insect Mating Systems* (Harvard University Press, 1983).
2. Goulson, D., Sangster, E. L. & Young, J. C. Evidence for hilltopping in bumblebees? *Ecol. Entomol.* **36**, 560–563 (2011).
3. Paxton, R. J. Male mating behaviour and mating systems of bees: an overview. *Apidologie* **36**, 145–156 (2005).
4. Shelly, T. E. & Whittier, T. S. Lek behavior of insects. in *The Evolution of Mating Systems in Insects and Arachnids* (eds. Choe, J. C. & Crespi, B. J.) 273–293Cambridge University Press, (1997).
5. Dos Santos, C. F., Francisco, F., de Imperatriz-Fonseca, O., Arias, M. C. & V. L. & Eusocial bee male aggregations: spatially and temporally separated but genetically homogenous. *Entomol. Exp. Appl.* **158**, 320–326 (2016).
6. Galindo-Cardona, A. et al. Landscape analysis of drone congregation areas of the honey bee, *Apis mellifera. J. Insect Sci.* **12**, 1–15 (2012).
7. Gandon, S. Kin competition, the cost of inbreeding and the evolution of dispersal. *J. Theor. Biol.* **200**, 345–364 (1999).
8. Heimpel, G. & Boer, J. Sex determination in the Hymenoptera. *Annu. Rev. Entomol.* **53**, 209–230 (2008).
9. Bortolotti, L. et al. Ploidy determination in *Bombus terrestris* males: cost-efficiency comparison among different techniques. *J. Apic. Res.* **61**, 180–189 (2022).
10. Clobert, J., Baguette, M., Benton, T. G. & Bullock, J. M. *Dispersal Ecology and Evolution* (Oxford University Press, 2012).
11. Bowler, D. E. & Benton, T. G. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biol. Rev.* **80**, 205–225 (2005).
12. Hayashi, S. & Satoh, T. Landscape features causing the local congregation of honeybee males (*Apis mellifera* L). *Ethology* **127**, 582–591 (2021).
13. Hayashi, S. & Satoh, T. Body size and age of drone honeybees (*Apis mellifera*) affect the structure and characteristics of mating congregations via dispersal. *Apidologie* **54**, 3 (2023).
14. Loper, G. M., Wolf, W. W. & Taylor, O. R. Honey bee drone flyways and congregation areas: radar observations. *J. Kans. Entomol. Soc.* **65**, 223–230 (1992).
15. Alcock, J. & Dodson, G. The diverse mating systems of hilltopping insects. *Am. Entomol.* **54**, 80–87 (2008).
16. Dos Santos, C. F., Ferreira-Caliman, M. J. & Nascimento, F. S. An alien in the group: eusocial male bees sharing nonspecific reproductive aggregations. *J. Insect Sci.* **15**, 157 (2015).
17. Koeniger, N. & Koeniger, G. Reproductive isolation among species of the genus *Apis. Apidologie* **31**, 313–339 (2000).
18. Beggs, J. R. et al. Ecological effects and management of invasive alien Vespidae. *BioControl* **56**, 505–526 (2011).
19. Willink, A. Sobre La Presencia De *Vespula germanica* (Fabricius) en La Argentina (Hymenoptera: Vespidae). *Neotropica* **26**, 205–206 (1980).
20. Masciocchi, M., Beggs, J. R., Carpenter, J. M. & Corley, J. C. Primer registro de *Vespula Vulgaris* (Hymenoptera: Vespidae) en La Argentina. *Rev. Soc. Entomol. Arg.* **69**, 267–270 (2010).
21. Masciocchi, M. & Corley, J. Distribution, dispersal and spread of the invasive social wasp (*Vespula Germanica*) in Argentina. *Austral Ecol.* **38**, 162–168 (2013).
22. Sola, F. J., Valenzuela, J., Anderson, A. E., Martínez Pastur, C. B., Lencinas, M. V. & G. & Reciente invasión del Archipiélago de tierra del fuego por la avispa *Vespula germanica* (Hymenoptera: Vespidae). *Rev. Soc. Entomol. Arg.* **74**, 197–202 (2015).
23. Clapperton, B. K., Alspach, P. A., Moller, H. & Matheson, A. G. The impact of common and German wasps (Hymenoptera: Vespidae) on the New Zealand beekeeping industry. *N Z. J. Zool.* **16**, 325–332 (1989).
24. Yeruham, I., Schwimmer, A. & Brami, Y. Epidemiological and bacteriological aspects of mastitis associated with yellow-jacket wasps (*Vespula Germanica*) in a dairy cattle herd. *J. Vet. Med. Ser. B.* **49**, 461–463 (2002).

25. MacIntyre, P. & Hellstrom, J. *An Evaluation of the Costs of Pest Wasps in New Zealand* (Department of Conservation and Ministry for Primary Industries, 2015).
26. Golden, D. B. K. et al. Clinical and entomological factors influence the outcome of sting challenge studies. *J. Allergy Clin. Immunol.* **117**, 670–675 (2006).
27. Spradbery, J. P. *Wasps. An Account of the Biology and Natural History of Social and Solitary Wasps, with Particular Reference to those of the British Isles* (University of Washington, 1973).
28. Greene, A. *Dolichovespula* and *Vespula* in *The social biology of wasps* (eds Ross, K. G., & Matthews, R.W.) 263–305Cornell University Press, Ithaca, NY, USA, (1991).
29. Martínez, A. S., Rousselot, N., Corley, J. C. & Masciocchi, M. Nest-departure behaviour of gynes and drones in the invasive yellowjacket *Vespula Germanica* (Hymenoptera: Vespidae). *Bull. Entomol. Res.* **111**, 174–181 (2020).
30. Masciocchi, M., Angeletti, B., Corley, J. C. & Martínez, A. S. Drone aggregation behavior in the social wasp *Vespula Germanica* (Hymenoptera: Vespidae): Effect of kinship and density. *Sci. Rep.* **10**, 7143 (2020).
31. Ayasse, M., Paxton, R. J. & Tengö, J. Mating behavior and chemical communication in the order Hymenoptera. *Annu. Rev. Entomol.* **46**, 31–78 (2001).
32. Goodisman, M. A., Matthews, R. W. & Crozier, R. H. Mating and reproduction in the wasp *Vespula Germanica. Behav. Ecol. Sociobiol.* **51**, 497–502 (2002).
33. Brown, R. L., El-Sayed, A. M., Suckling, D. M., Stringer, L. D. & Beggs, J. R. *VeVulgarislgaris* (Hymenoptera: Vespidae) gynes use a sex pheromone to attract males. *Can. Entomol.* **145**, 389–397 (2013).
34. Leprince, D. J. & Francoeur, A. Hilltop swarming by ants (Hymenoptera: Formicidae) in southwestern Quebec and interspecific competition at the swarm marker. *Ann. Entomol. Soc. Am.* **79**, 865–869 (1986).
35. Alcock, J. Male reproductive tactics in the libellulid dragonfly *Paltothemis Lineatipes*: temporal partitioning of territories. *Behaviour* **103**, 157–173 (1987).
36. Veldtman, R., Daly, D. & Bekker, G. F. H. Spatio–environmental analysis of *Vespula Germanica* nest records explains slow invasion in South Africa. *Insects* **12**, 732 (2021).
37. Kenna, D., Pawar, S. & Gill, R. J. Thermal flight performance reveals impact of warming on bumblebee foraging potential. *Funct. Ecol.* **35**, 2508–2522 (2021).
38. de Ibarra, N. H., Philippides, A., Riabinina, O. & Collett, T. S. Preferred viewing directions of bumblebees (*Bombus terrestris* L.) when learning and approaching their nest site. *J. Exp. Biol.* **212**, 3193–3204 (2009).
39. Wenzel, J. W. Behavioral homology and phylogeny. *Annu. Rev. Ecol. Syst.* **23**, 361–381 (1992).
40. Shik, J. Z., Donoso, D. A. & Kaspari, M. The life history continuum hypothesis links traits of male ants with life outside the nest. *Entomol. Exp. Appl.* **149**, 99–109 (2013).
41. Chase, J. M. & Leibold, M. A. *Ecological Niches: Linking Classical and Contemporary Approaches* (University of Chicago Press, 2009).
42. Kyogoku, D. Reproductive interference: ecological and evolutionary consequences of interspecific promiscuity. *Popul. Ecol.* **57**, 253–260 (2015).
43. Hood, G. R., Blankinship, D., Doellman, M. M. & Feder, J. L. Temporal resource partitioning mitigates interspecific competition and promotes coexistence among insect parasites. *Biol. Rev.* **96**, 1969–1988 (2021).
44. Burdfield-Steel, E. R. & Shuker, D. M. Reproductive interference. *Curr. Biol.* **21**, R450–R451 (2011).
45. Corley, J., Sackmann, P., Rusch, V., Bettinelli, J. & Paritsis, J. Effects of pine silviculture on the ant assemblages (Hymenoptera: Formicidae) of the Patagonian steppe. *Ecol. Manag.* **222**, 162–166 (2006).
46. Jacobson, R. S., Matthews, R. W. & Macdonald, J. F. A systematic study of the *Vespula vulgaris* group with a description of a new yellowjacket species in eastern North America (Hymenoptera: Vespidae). *Ann. Entomol. Soc. Am.* **71**, 299–312 (1978).
47. Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A. & Smith, G. M. *Mixed Effects Models and Extensions in Ecology with R*Springer New York, New York, USA,. (2009).
48. Dormann, C. F. et al. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* **36**, 27–46 (2013).
49. R Development Core Team. *A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2024).
50. Brooks, M. E. et al. glmmTMB balances speed and flexibility among packages for zero-inflated generalized Linear mixed modeling. *R J.* **9**, 378–400 (2017).
51. Lenth, R. Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.4.900001, (2024).
52. Hothorn, T., Bretz, F. & Westfall, P. Simultaneous inference in general parametric models. *Biom J. Biom Z.* **50**, 346–363 (2008).

Acknowledgements

Author contributions

AP, AM and MM conceptualized the study and designed the methodology. AP performed the field sampling. AP, MM and AM carried the formal analysis of the data. AP wrote the first draft of the manuscript. All authors reviewed and edited on previous versions of the manuscript. All authors have approved the submitted version.

Funding

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-85851-0.

Correspondence and requests for materials should be addressed to A.P.P.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.