



# Standardized transect walks outperform pan traps in assessing wild bee community in a Mediterranean protected area (Asinara National Park, Italy)

Matteo Lezzeri<sup>1</sup> · Vanessa Lozano<sup>1,2</sup> · Giuseppe Brundu<sup>1,2</sup> · Ignazio Floris<sup>1,2</sup> · Michelina Pusceddu<sup>1,2</sup> · Marino Quaranta<sup>3</sup> · Alberto Satta<sup>1,2</sup>

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## Abstract

The decline of pollinator insects in various parts of the world has increased the interest in studies on bee sampling. Because pollinator management and conservation policies depend on data representing their communities, knowledge of the systematic bias of sampling methods in relation to surrounding habitat is fundamental. This study examined the performance of standardized transect walks and colored pan traps considering differences in bee species richness, diversity, and composition, and the potential bias due to a different availability of floral sources throughout seasons. The study was conducted in a minor outlying island (Asinara, Italy), a natural park characterized by the prevalence of natural or semi-natural habitats and an Oceanic Pluviseasonal Mediterranean bioclimate. We found that transect and pan trap methods reflected different bee communities based on taxonomic families, genera, and species. In general, the transect method captured more varied samples than pan traps, despite the significantly lower number of individuals captured on transects. Moreover, pan traps captured more bees as floral abundance decreased, whereas transects showed greater bee abundance, richness, and diversity with increasing floral resource availability. In addition, 18 species out of the 68 observed in total were caught exclusively with pan traps, especially in July–October, when transect catches decreased due to substantial bloom reduction. Therefore, alternating the two methods according to season (i.e., transect in late winter-spring and pan trap in summer-autumn) might be the most suitable trade-off to obtain a better representation of the bee community by limiting sampling efforts and the negative impact on bee fauna.

**Keywords** Apoidea · Biodiversity · Conservation · Mediterranean islands · Phenology · Pollinators

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Matteo Lezzeri and Vanessa Lozano These authors contributed equally and are joint first authors.

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Extended author information available on the last page of the article

## Introduction

The population of many pollinator insects is declining in various parts of the world (Biesmeijer et al. 2006; Ollerton 2017; IPBES 2019; Kunin 2019; Powney et al. 2019), mainly due to landscape alteration and consequent habitat loss (Naug 2009; Potts et al. 2010; Ollerton et al. 2014; Goulson et al. 2015; Millard et al. 2021). Because pollinator insects are essential for natural ecosystems and human food security by ensuring the reproduction of wild and cultivated plants (Ashman et al. 2004; Aizen et al. 2009; Rodger et al. 2021), monitoring their populations is an emerging global need aimed at understanding the relationships between changes in pollinator diversity and abundance and the main factors causing these changes (European Commission 2018; Secretariat of the Convention on Biological Diversity (CBD/COP/DEC/14/6, 2018).

To obtain comparable results, pollinator monitoring schemes should be based on standardized methods that represent the sampled population with sufficient accuracy. Considering that the proportion of individuals sampled is extremely variable, depending on the behavior, size and micro-habitat of the species studied, a consistent bias in relation to the sampling method used is acceptable as long as a relevant trend can still be inferred (Walther and Moore 2005).

The most common sampling methods employed for pollinator surveillance are transect walks combined with hand net and pan traps (colored plastic bowls) (Banaszak 1980; Sutherland 1996; Cane et al. 2000; Southwood and Henderson 2000; Westphal et al. 2008). The former method is time-consuming and has a significant collector bias, but it allows identification of the insect-plant association (Westphal et al. 2008). Pan trapping is a passive method that lacks surveyor bias but can underestimate honey bees and some large species such as *Bombus* spp. and *Xylocopa* spp. (Toler et al. 2005; Roulston et al. 2007; Hutchinson et al. 2021).

In a study comparing six sampling methods to measure bee diversity in various European regions, pan trapping was the most efficient method in agricultural and seminatural habitats because it had the highest sample coverage, collected the highest number of species (similar to that observed with the transect method), and was the best indicator of overall bee species richness (Westphal et al. 2008). A similar study conducted on a Mediterranean island (Lesvos, Greece) achieved comparable results (Nielsen et al. 2011). Differently, in a study conducted in southern Sweden, the results obtained with pan traps were not comparable to those of transect walks for any of the taxonomic groups investigated (Lepturinae, Cetoniidae, Syrphidae and both social and solitary Apoidea) (Berglund and Milberg 2019). In particular, pan traps underestimated Syrphidae and both social and solitary bees and overestimated Lepturinae and Cetoniidae in terms of number of species and individuals collected. Similar differences between the two methods were reported in previous studies aimed at characterizing the bee fauna of creosote bush in Arizona (Cane et al. 2000) and spontaneous flora in northern Virginia (Roulston et al. 2007).

A possible, but poorly understood, bias that can affect the effectiveness of pan traps is the attractiveness of the surrounding flora to pollinator insects, which in turn can vary with the scent, color and abundance of the flowers present. Toler et al. (2005) concluded that a predominant flower color in the plant community did not influence the relative attractiveness of pan trap colors, but the effect of floral abundance per se was not studied. In fact, a study of Baum and Wallen (2011) clearly showed that pan traps underestimated richness and abundance of bee fauna when floral resources were abundant. In contrast, Wood et al. (2015) reported that pan traps caught more bumblebees in areas with a greater flower

density. Recently, this finding was confirmed for social bees (honey bees and bumblebees) only at a small spatial scale (25 m<sup>2</sup> around the pan traps) (Westerberg et al. 2021), but not for solitary bees, which were not affected by flower frequency, and for other pollinator groups (Cetonoinae, Lepturinae, Syrphidae and Vespoidea), which showed negative biases (fewer catches when flowers were more abundant) (Westerberg et al. 2021). These conflicting results could be, at least in part, due to the diversity of the habitats investigated because target insects respond to color differently across environmental contexts and within taxonomic groups (Saunders and Luck 2013; Templ et al. 2019). Another reason could be differences in the methodology applied for pan traps (e.g., placed on or above the ground) and transect walks (e.g., sampling done only on flowers or on the whole vegetation). To our knowledge, the influence of floral resource availability on the effectiveness of pan traps and transect walks has been compared only in agricultural contexts (Templ et al. 2019).

To acquire data on the effectiveness of the two sampling methods (pan traps and transect walks) in areas characterized by the prevalence of natural or semi-natural habitats, we conducted a study in the Asinara National Park, which includes Asinara Island located off northwestern coast of Sardinia (Italy). This island, recognized as SCI (ITB 1010082: Isola dell'Asinara, Bardi et al. 2015), has not been permanently inhabited since the park was established in 1997, and since then does not have any agricultural and industrial activities. In this context, we compared standardized transect walks and colored pan traps for two years. We examined the differences in bee species richness, diversity, and composition and the potential bias due to differences in the availability of floral sources throughout the season.

## Methods

### Study area and study sites

The island of Asinara, located at a sailing distance of less than 2 nautical miles from the Sardinian (Italy) northeastern coast, has a surface of 51.92 km<sup>2</sup>. Its highest peak reaches 408 m a.s.l. (Supplementary materials, Figure S1). It is characterized by an Oceanic Pluvi-seasonal Mediterranean bioclimate, upper thermo-Mediterranean phytoclimatic belt (Canu et al. 2014). The flora of the Asinara Island comprises 630 species and subspecies, out of which 35 are endemic exclusively to Sardinia or to Sardinia and Corsica or show an endemic range restricted to Sardinia and Sicily, Sardinia and the islands of the Tuscan archipelago, Sardinia and the Balearic Islands, or combinations of Sardinia and more than one of these areas (Pisanu et al. 2014; Nimis et al. 2015). The plant landscape of the island has been significantly shaped throughout history by the presence of a high-security prison (closed in 1997), and by extensive human activity, and it is slowly recovering after the establishment of the Asinara National Park, although facing intense grazing pressure due to the presence of large flocks of abandoned feral or domestic introduced animals (e.g., donkeys and goats). However, the island still hosts a residual fragment of *Quercus ilex* forest, in some northern sectors of the island, which is one the habitats listed in the “Habitat Directive” (Council Directive no. 92/43/EEC) under habitat 9340. Details on the flora, vegetation, habitat types and the island in general can be found in Bocchieri (1988) and Pisanu et al. (2014).

In January 2021 two sites on the island, with similar floristic composition and vegetation physiognomy and cover, were selected and geolocated by on-site survey and digital

orthophotos analysis. The first site was in the north (WGS84 8.3377° E, 41.0837° N) and the second site in the south of the island (WGS84 8.2432° E, 40.9998° N), with a distance of 13 km between the two areas (Supplementary materials, Figure S1). In both sites, the vegetation is a thermophilous shrubland with a prevalence of *Olea europaea* var. *sylvestris*, *Pistacia lentiscus* and *Euphorbia dendroides*, and large patches of herbaceous plant communities. In both sites there are some endemic species, albeit not particularly abundant, such as *Bryonia marmorata*, which is a Sardinian-Corsican endemic vine. The floristic analysis conducted over the period of two years demonstrated the absence of significant differences in terms of floristic composition between the selected transects and sites (see Statistical analysis section for more details).

## Bee sampling

In two consecutive years (2021 and 2022), from February to November, bees were sampled monthly by using standardized transect walks (hereafter transect) and pan traps (Kirk 1984; Westphal et al. 2008). For the transect walks, a permanent corridor (200 m long × 2 m wide), divided into four equal subunits of 50 m each (A, B, C and D), was geolocated with GPS in each of the two sampling sites.

Sampling was done only when flight conditions for pollinators were favorable (i.e., minimum temperature 15 °C, wind strength less than 3 on the Beaufort scale, and maximum 50% cloud cover), according to a predefined duration (15 min for each sub-unit) and repeating sampling twice during the same day in predefined time slots (from 10:30 to 12:00 and from 14:00 to 16:00), because not all bee species are active at the same time of the day (Pisanty et al. 2016). The surveillance time, tracked with a stopwatch, was stopped during specimen handling to exclude the time needed to securely transfer bees to vials. Sampling in the two transects was always done by the same collector within a maximum time interval of 48 h. All specimens of wild bees seen on the flowers were captured with an entomological net and associated with the plant species visited at that moment.

Wild bees were also captured using pan traps, i.e., plastic bowls with a diameter of 14 cm and a volume of 500 ml (Westphal et al. 2008; Westerberg et al. 2021), painted with ultraviolet (UV)-bright yellow, white and blue colors (Sparvar Leuchtfarbe, spray-color Gm-bH, Merzenich, Germany) to increase their efficiency (Westphal et al. 2008). Each cluster of pan traps consisted of three pans, one of each color, placed at the top of a wooden stick at the average vegetation height (1.50 m). At each sampling date, three clusters of pan traps were placed spaced 4–6 m apart from the transect at regular distances, i.e., 50, 100 and 150 m from the beginning of the transect. Each pan trap was filled with 400 ml of water and one drop of a common detergent and was left active for 48 h. All wild bee specimens collected, whether by transect walk or pan trap method, were temporarily stored in freezer at -20 °C until subsequently determination at the species level.

To better compare the two sampling techniques, the 10-month sampling period was conducted not only when the peak flowering of the dominant plant species occurred, but also when flowering was very low at our sites.

## Floristic survey

The floristic survey was conducted contemporaneously with the wild bee sampling, in each subsection (A, B, C, D) of the two transects of the study area. It should be noted that the flora survey was carried out, limited to tracheophytes with entomophilous pollination, with

single flowers (or inflorescences) larger than 0.5 cm. To determine the relative abundance of a plant species in each subsection, we first visually assessed the total flower cover (of all species summed) of a transect and then the relative proportion of each plant species to the total cover at four levels, as follows: 0–25%; 25.1–50%; 50.1–75%; 75.1–100%.

We counted flowering individuals by species. To calculate floristic richness, the data was then pooled across sites and years due to similarities in the composition of the sampled flora across sites and because sample sizes did not warrant further examination of spatial and temporal patterns (Supplementary materials, Figure S2).

## Statistical analysis

### Wild bee species richness, diversity, and abundance

We used Pearson's chi-squared test to evaluate statistical differences between species richness, abundance, and diversity (Shannon's index see Legendre and Legendre 1998) within the two sampling methods. The function *corrplot* was used to visualize the correlations. Before performing the analysis, wild bee richness (i.e., the total number of wild bee species) and abundance (i.e., the total number of individuals per species) were recorded per site and month for both pan trap and transect methods. To focus our analysis on the subset of visited flowering plant species, all plant species not visited by wild bees during the sampling survey were omitted from the statistical analysis (Supplementary materials, Table S1).

We used generalized linear models (GLMs), using function *glm*, for two main reasons. Firstly, to evaluate the effects of using the two different sampling methods on wild bee species richness, diversity (Shannon diversity index  $H'$ ) and abundance. Secondly, to investigate how species richness, diversity and abundance of wild bees are affected monthly and across blooming seasons, by keeping the models used with the different sampling methods separate. We used the richness, diversity, and abundance of wild bees as response variables. Data were then pooled across sites and for years. Then, the sampling method, month of sampling and the richness of the total visited flora were considered as predictors. We used Poisson error distributions and selected models with different distributions using AIC.

### Composition of the wild bee and plant community

We used permutational multivariate analysis of variance (PERMANOVA) to evaluate similarities on community composition of wild bees within the two sampling methods, using the *adonis* function, with 999 permutations. In the *adonis* permutational multivariate analysis, the Bray–Curtis distance matrix of wild bees' abundance (i.e., the number of individuals per species) was the response variable, with sampling methods as fixed factors. We then used Principal Coordinates Analysis (PCoA) to visualize the variation in the bee community composition and dispersion of sampling between the two different methods (i.e., pan traps and transect).

In a separate *adonis* model, we used a matrix with the total flora (i.e., number of plants visited and not visited by wild bees) to evaluate similarities in plant species composition across sites (Supplementary materials, Figure S2).

The relationship between bee community composition across the two different sampling methods was analyzed using canonical correspondence analysis (CCA) of the wild bee's

matrix (70 species  $\times$  2 methods  $\times$  10 months). CCA is a gradient analysis that shows the relationship amongst bee community patterns under the influence of seasonality and sampling methods. The function *envfit* was used to relate the sampling methods and seasonality to the CCA ordination. Correlation tests were done to evaluate the associations among variables before performing CCA. The significance of each variable in the CCA was evaluated using ANOVA.

All statistical analyses were performed in the R software environment (R Core Team 2021) with the use of the vegan library (Oksanen et al. 2020) and corrplot (Wei and Simko 2021).

## Results

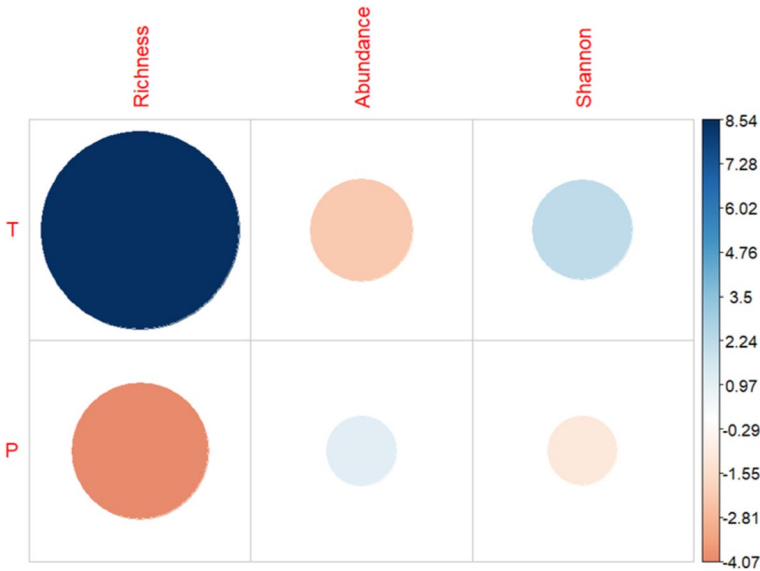
### Floristic survey

A total of 93 plant species were recorded in the sampling sites belonging to 27 families, including one Sardinian-Corsican endemic species (*Bryonia marmorata*), two invasive alien species (*Glebionis coronaria* and *Oxalis pes-caprae*) and one cryptogenic (*Glebionis segetum*). Fabaceae and Asteraceae (24 and 22) were the most represented families in the sampling sites. The recorded plant species were mainly therophytes (65), hemicryptophytes (17), chamaephytes (2), geophytes (5), nano-phanerophytes (2) and phanerophytes (2). The most common lifespan was annual (65). (Supplementary materials, Table S1). Annual legumes were among the herbaceous species with the greatest coverage, including several species of the genera *Trifolium* and *Medicago*. The greatest diversity and highest vegetation cover have been detected during spring. Plant species composition did not differ significantly between sites, which confirmed the a priori visual selection of comparable sites (adonis;  $F=0.829$ ,  $P=0.433$ ,  $R^2=0.044$ ; Figure S1).

### Wild bee species richness, diversity, and abundance

Overall, 68 bee species were identified within the 1486 wild bees collected during this study (Table S2). The highest species richness was recorded by the transect method compared to the pan trap method (50 species versus 42) despite the lowest number of individuals collected (235 specimens versus 1251). The Pearson chi-square statistics demonstrated the independence between sampling methods with a significant difference in terms of species richness, number of individuals captured and diversity ( $X^2=144.96$ ;  $P<0.05$ ). Figure 1 shows a strong positive association between species richness and Shannon index with the transect method and a negative association with the pan trap method. However, the number of individuals is positively associated with the pan trap and negatively with the transect method.

Wild bees' species richness, diversity Shannon index and abundance varied across months and between the two sampling methods (Fig. 2; Table S3). We found a significant positive correlation between wild bees' richness, abundance, diversity, and plant richness using the transect method ( $P<0.05$ ). Additionally, this positive correlation was found during spring months when plant richness was higher. It was clear that the period of maximum presence of wild bees corresponded to the spring months, during maximum blooming, whereas catches were smaller or null from August to November (Supplementary materials, Table S4).



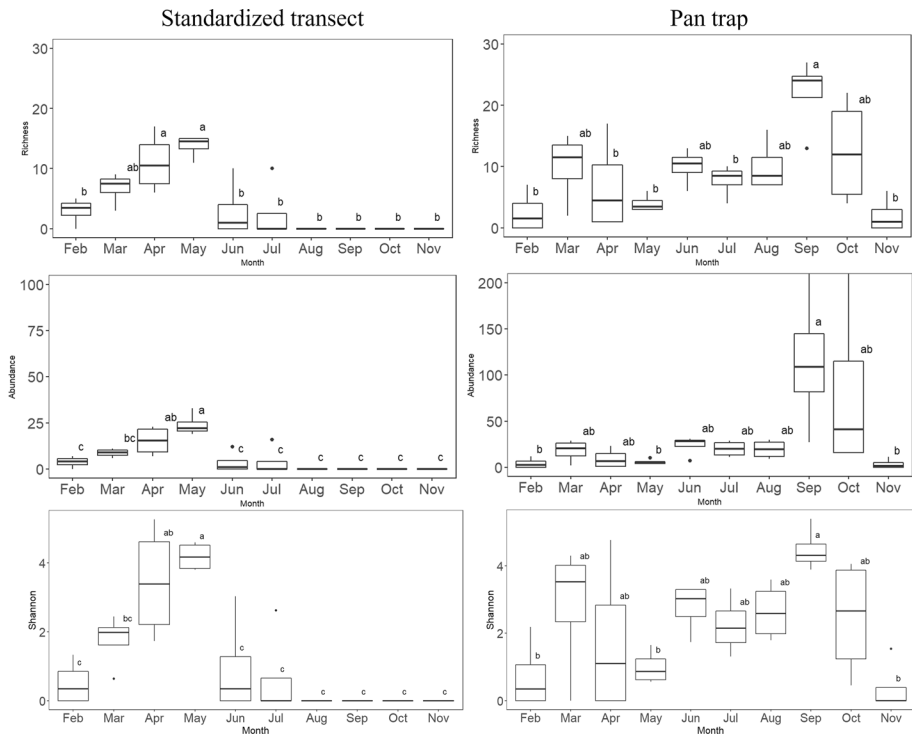
**Fig. 1** The Pearson chi-square plot shows for a given cell that the size of the circle is proportional to the amount of the cell's contribution. Positive residuals are blue and negative residuals are in orange. Positive and negative values in cells specify a positive or negative association between the corresponding row (i.e., transect walk [T] and pan trap [P] methods) and columns (richness, abundance, and Shannon diversity index)

Using the pan trap method, we found a significant negative correlation between species richness or abundance of wild bees and plant richness ( $P < 0.05$ ). The richness and abundance of wild bees were negatively correlated in spring and highly and positively correlated in the months without flowering (Supplementary materials, Table S4).

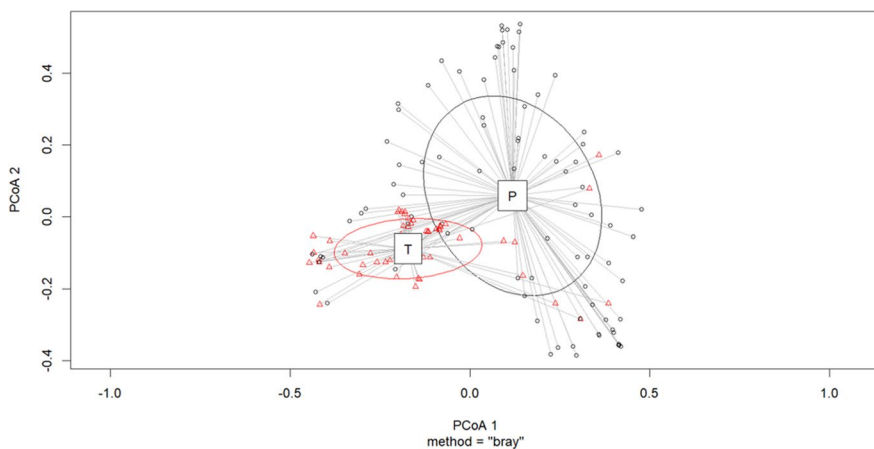
### Composition of the wild bee community

The permutational multivariate analysis of variance revealed a significant difference in bee species composition between sampling methods (adonis;  $F = 10.69$ ,  $P = 0.001$ ,  $R^2 = 0.65$  Fig. 3). This was probably due to the high number of single records, i.e., species caught exclusively by one of the two methods, which was 26 for the transect and 18 for the pan traps (Supplementary materials, Table S2). In addition, when compared to transect walk, pan traps featured particularly attractive to halictid bees (Halictidae) but showed much lower performance in relation to andrenid (Andrenidae) and megachilid (Megachilidae) bees. In fact, pan traps detected 88.9% of all halictid species captured with both methods but only 33.3% and 42.1% for andrenid and magachilid, respectively (Supplementary materials, Table S2). In contrast, the transect method detected 100%, 55.5%, and 78.9% of all observed species of andrenid, halictid and megachilid bees, respectively.

Using canonical correspondence analysis, we analyzed how the method used and the season influenced the capture of wild bees. The CCA model with two sampling methods across seasons was significant (ANOVA: 2.6537,  $P = 0.001$ ), explaining the observed variation based on the relative abundances of wild bee species within our study area. The overall results of the CCA ordination plot are shown in Fig. 4. Through the axis it is possible

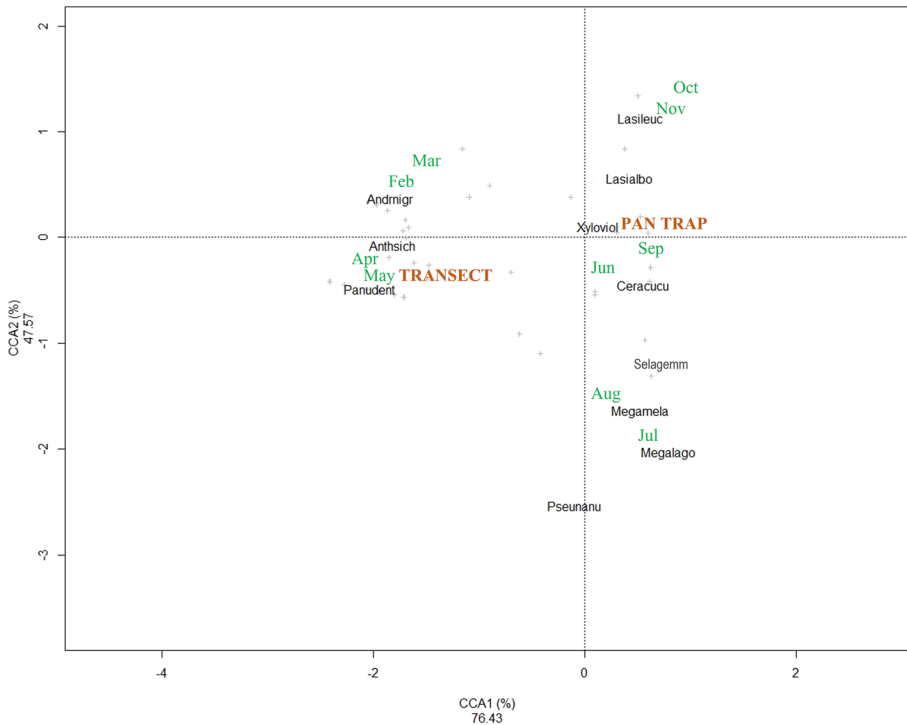


**Fig. 2** The figure shows the wild bees species richness, diversity (Shannon index) and abundance observed monthly using the standardized transect walk (left) and the pan trap (right) method, pooling samples from both sites and the two years (2021–2022). Different lowercase letters indicate significant differences between months (ANOVA; Tukey post-hoc test,  $P < 0.05$ )



**Fig. 3** The figure shows the Principal Coordinates Analysis (PCoA) of a Bray–Curtis dissimilarity matrix for analysis of the community composition in terms of number of individuals of wild bees achieved with the standardized transect walk (T) and pan trap (P) methods. The red triangles and black circles are each observation and were projected to get a coordinate value along the PC-line





**Fig. 4** The figure shows an ordination bi-plot of canonical correspondence analysis (CCA) of wild bees' species distribution with respect to the first 2 axes, using two different sampling methods, transect walk and pan traps (each method is written in brown), across seasons (each month is written in green). The grey crosses represent the species with the lowest abundance. Species codes: Andrnigr=*Andrena nigroaenea*; Anthsich=*Anthophora sichelii*; Ceracucu=*Ceratina cucurbitina*; Selagemm=*Seladonia gemma*; Lasiabo=*Lasioglossum albocinctum*; Lasileuc=*Lasioglossum leucozonium*; Megalago=*Megachile lagopoda*; Megamela=*Megachile melanopyga*; Panudent=*Panurgus dentipes*; Pseunanu=*Pseudoanthidium nanum*; and Xyloviol=*Xylocopa violacea*

to observe that the spring season was associated with the transect sampling method and with the species *Andrena nigroaenea*, *Panurgus dentipes*, *Pseudoanthidium nanum* and *Anthophora sichelii*. On the contrary, the pan trap method seems to be more sensitive in summer-autumn seasons and to the species, *Lasioglossum albocinctum*, *Lasioglossum leucozonium*, *Seladonia gemma*, *Megachile lagopoda*, *Megachile melanopyga*, *Ceratina cucurbitina* and *Xylocopa violacea*.

## Discussion

The decline in diversity and abundance of bees, widely documented in recent decades (Biesmeijer et al. 2006; Ollerton 2017; Kunin 2019; IPBES 2019; Powney et al. 2019), demands careful and constant surveillance of their populations for conservation purposes. For this reason, low-cost, reliable and unbiased sampling methods that accurately reflect the richness, diversity and abundance of bee communities are needed.

The present study showed that standardized transect walks outperformed pan traps in assessing species richness of the wild bee community in the Asinara National Park. Importantly, about 76% of the total bee species detected ( $n=68$ ) were captured along the transect, while 60% were captured with pan traps. This result appears quite surprising considering that 84% of the total specimens collected ( $n=1,486$ ) were captured with pan traps. Slightly lower ratios, i.e., 76% and 82%, can be obtained by checking data reported by Westphal et al. (2008) and Nielsen et al. (2011), respectively, as seen in the supplementary materials, without considering *Apis mellifera*, which we never observed along the transects or captured with pan traps. This finding is justified by the absence of hives on the island which is also unattractive to honeybees. In fact, blooms are concentrated almost exclusively in spring and the island is often windy.

The lower efficiency of pan traps in detecting species richness observed in our study area is likely related to the confounding effect of plant richness. In fact, the period when pan traps made the lowest catches overlapped with the spring season, when flowers were more abundant. In contrast, catches with pan traps increased when flowers were lower or absent. These results support the hypothesis that there is a kind of competition between pan traps and flowers (Cane et al. 2000). Given that many studies report higher pollinator numbers when more flowers are present (Potts et al. 2003; Westphal et al. 2003; Hegland and Boeke 2006; Ebeling et al. 2008), our findings document a considerable underestimation bias of pan traps, suggesting that pan traps do not accurately reflect the species richness and diversity of wild bee community under those conditions.

Consistently with our results, some studies reported a decrease in pan trap catches as the number of flowers around increased (Mayer 2005; Baum and Wallen 2011; Chamorro et al. 2023). Differently, Westerberg et al. (2021) found that pan traps suffer from a negative bias due to surrounding flower frequency for different taxa of pollinator insects (Vespoidea and Lepturine) but not for solitary Apoidea. Wood et al (2015) also reported a close and positive association between the number of bumblebees caught and flower density. These conflicting or partially conflicting results suggest that the bias related to the flower abundance is context- and taxon-dependent. In Mediterranean climate regions, where most plant species have a single-peaked flowering period in late winter and early spring (Bosch et al. 1997), the effectiveness of pan traps is likely to be significantly affected. However, it is relevant to highlight that out of the 18 species caught exclusively with pan traps, 14 were detected in the July–October period, which is when catches along the transect decline due to a substantial reduction in flowering. Therefore, to obtain a better representation of the bee community, the implementation of complementary sampling methods is necessary, as highlighted in several other studies (O'Connor et al. 2019; Kuhlman et al. 2021; Chamorro et al. 2023; Pei et al. 2022).

Westphal et al. (2008) stated that pan traps are the most efficient method for estimating bee species richness in long-term and large-scale monitoring programs across various agricultural and seminatural habitats of European regions, based on their high percentage of sample coverage (> 80%). A similar conclusion was reported by Nielsen et al. (2011) for a Mediterranean area with higher species richness, although in this case sample coverage was much lower (50%). The different results we obtained, with respect to these two studies, could be due to some differences in the sampling protocol used. In fact, we spent more time walking the transect (1 h for a transect 200 m long  $\times$  2 m wide in our study *versus* 50 min for a transect 250 m long  $\times$  4 m wide in their studies), which we also walked twice at different time slots in the same sampling round (*versus* one in their studies). In addition, we used three clusters (sets) of pan traps per transect compared to five clusters per transect used in the other two studies. It is very likely that if we had increased the number of pan trap sets

at each round of observation, we would have increased the sampling coverage previously observed for this method (Nielsen et al. 2011). However, it is legitimate to ask whether a significant increase in the multitude of specimens captured by the passive traps would go against the protection and conservation actions which are, in general, the main reasons why the monitoring plans are implemented, particularly inside protected areas. There is a risk that higher catches by pan traps could furthermore accelerate the decline of bee species, especially those that are uncommon (Tepedino and Portman 2021).

We also found the pan trap sampling was positively biased toward halictid bees whereas underestimated andrenid and megachilid bees when compared with the transect method. The strong association between halictid and pan traps was already observed in other studies (Gollan et al. 2011; Gonçalves and Oliveira 2013; Chamorro et al. 2023). In general, the high attractiveness of pan traps for halictids is explained by their small size compared to other bee groups, which makes it more difficult to observe them with other sampling method such as the transect method, for which the observer's skill in detecting bees on flowers by sight is crucial (Hutchinson et al. 2021; Chamorro et al. 2023). Our observations showed that some big halictids caught with pan traps were not observed along the transect (e.g., *Halictus brunnescens*), unlike some small species (e.g., *Lasioglossum griseolum*). Unfortunately, it was not possible to make an accurate statistical comparison between the sizes of the halictid species detected by the two sampling methods due to the small number of individuals caught of some species. Despite that, our results suggest that body size alone cannot explain the association between pan traps and halictids. Moreover, this rationale cannot explain why pan traps were less attractive to andrenids and megachilids compared to transect method.

The primary limitation of the transect method, as observed with other active techniques relying on direct visual observations, is that results vary with the expertise of the collector. Traditional standardized transect methods involve a walk where the surveyor collects bees separately. To avoid killing all individuals, only those that cannot be identified in the field are kept for identification later in the lab. This method is susceptible to biases stemming from the surveyor's knowledge of food plant specialization, microhabitats, and nesting sites, as well as the tendency to avoid killing abundant and easily identifiable bee species. However, despite extensive knowledge of the local fauna, field identification errors are inevitable, and some bees may evade capture during the identification process. In contrast, pan traps, as other passive methods, capture specimens indiscriminately, thus showing negligible collector bias (Westphal et al. 2008; Hutchinson et al. 2021; Packer and Darla–West 2021). Therefore, to partially fill the bias gap between transects and pan traps, we encourage surveyors to collect all individuals observed on the flowers, excluding only the easily identifiable honeybees, along transects during the collection time. This approach should not substantially affect the bee fauna, because of the substantially lower number of insects sighted on the flowers on transects compared to those captured by pan traps within 48 h. This particular version of the transect method was developed during the European project BIOBIO—(Indicators for biodiversity in organic and low-input farming systems—EC FP7 project BioBio Contract KBBE-2B-227161) (Dennis et al. 2012) and used in the BeeNet project (Giovannetti et al. 2023). On the other hand, when the aim of sampling is to obtain the most exhaustive list of bee species, net sampling also allows for the collection of cuckoo bees, which are relatively well detected when they are searched for in the vicinity of the nesting sites of their host species.

Finally, this survey has greatly increased our knowledge of the bee fauna of Asinara island, which had been studied only occasionally in the past. In fact, according

to the summary of previous reports made by Nuvoli et al. (2007), the number of bee species reported for Asinara island was 34, out of which only 15 were found in our survey. Therefore, 53 out of the 68 species we surveyed were new reports for the Asinara island. It is clear that, to obtain a more complete list of bee species for the island, sampling should be extended to habitats other than those in which our transects fell such as the *Centaurea horrida* and *Astragalus terraccianoii* garigue (habitat 5410, Directive 92/43/EEC, Farris et al. 2007), or the annual vegetation of drift lines along the coasts of Asinara (habitat 1210, *Salsolo kali-Cakiletum maritimae*, Pisanu et al. 2014). Nevertheless, if we take into account the 19 bee species previously reported but that we did not find, we can state that the total bee fauna of Asinara island includes 87 species. It is also interesting to note that, among the 53 species considered as new reports for Asinara, two of them (*Lasioglossum lineare* and *Lasioglossum punctatissimum*; Table S2) had not been reported in other areas of Sardinia, which currently has 332 bee species (Nobile et al. 2021; Reverté et al. 2023). These results suggest that exchanges of individuals between the two islands are not frequent, despite their proximity, as indeed confirmed by the absence of *Apis mellifera* in Asinara island.

The biodiversity of islands is peculiar compared to continental areas due to their isolation and the distance effect between the islands and colonization sources. In fact, it is variably influenced by factors such as size, latitude, altitude, and the availability of nesting sites. These conditions generate phenomena of competitive interactions among species and evolutionary adaptations, leading to diverse and unique pollinator communities on islands compared to continental ones. Such conditions may also favor speciation and result in endemic species. In our study, we identified many plant species that are highly attractive to wild bees, such as the hemicryptophytes *Carlina corymbosa*, *Echium plantagineum* and *Galactites tomentosus*, and the therophytes (i.e., annuals) *Glebionis segetum*, *Raphanus raphanistrum*, and *Trifolium nigrescens* with particular importance for bees depending on the season. The first flowers of *E. plantagineum*, *G. tomentosus*, and *R. raphanistrum* open in February and the plants bloom until mid-summer, whereas the annual trifolium, *T. nigrescens*, bloom until mid-spring. Thus, these are important host plants across all seasons for bee activity and this seasonality should be considered in bee conservation. Whereas *E. plantagineum*, and *R. raphanistrum* were visited by many bee species, *G. tomentosus* was strictly visited by *Bombus terrestris*. *Carlina corymbosa* was the most abundant and most visited plant species during summer, when potential food sources for the wild bees were limited to a few herbaceous plant species. Thus, hemicryptophytes such as spiny thistles, whose presence is favored by grazing, are very important because they are highly visited by wild bees, and because they continue to bloom in summer when other plants have already finished their cycle. Therefore, the conservation of natural and protected areas that offer diverse floral resources should be a top priority for the conservation of wild bees (Kuppler et al. 2023).

In conclusion, our study showed that in Mediterranean areas, where blooms are concentrated in late winter and early spring, the transect method better represents the wild bee community than the pan trap method, despite the significantly lower number of individuals captured on transects. However, considering that pan traps allow the detection of bee species even when blooms are scarce or absent (summer-autumn), alternating the two methods in the two distinct sampling seasons (i.e., transect in late winter-spring and pan trap in summer-autumn) might be the most suitable trade-off to obtain a better representation of the bee community by limiting sampling effort and negative impact on bee fauna.

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## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

**Conflict of interest** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Authors and Affiliations

Matteo Lezzeri<sup>1</sup> · Vanessa Lozano<sup>1,2</sup> · Giuseppe Brundu<sup>1,2</sup> · Ignazio Floris<sup>1,2</sup> ·  
Michelina Pusceddu<sup>1,2</sup> · Marino Quaranta<sup>3</sup> · Alberto Satta<sup>1,2</sup>

✉ Vanessa Lozano  
vlozano@uniss.it

✉ Alberto Satta  
albsatta@uniss.it

<sup>1</sup> Department of Agricultural Sciences, University of Sassari, Viale Italia 39/A, 07100 Sassari, Italy

<sup>2</sup> National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133 Palermo, Italy

<sup>3</sup> CREA Research Centre for Agriculture and Environment (CREA-AA), Via Di Corticella 133, 40128 Bologna, Italy