



## Bat diversity boosts ecosystem services: Evidence from pine processionary moth predation

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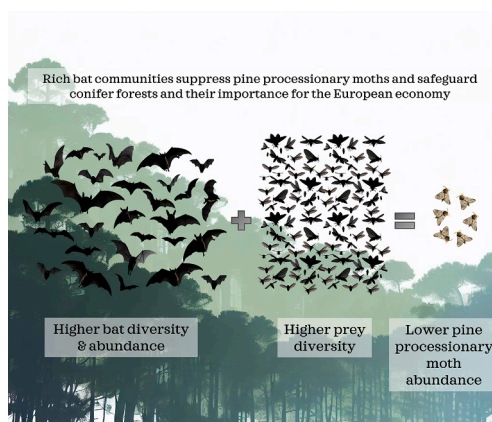
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### HIGHLIGHTS

- Unveiled new PPM bat predators, emphasizing bats' pest suppression role
- Cluttered and open space foragers: bat guilds with high pest consumption potential
- Bat abundance, species richness and bat diet richness link with pest consumption.
- Diverse bat communities enhance pest consumption, reinforcing biotic resistance.
- Niche segregation fosters bat coexistence, enhancing ecosystem stability

### GRAPHICAL ABSTRACT



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## ABSTRACT

Coniferous forests contribute to the European economy; however, they have experienced a decline since the late 1990s due to an invasive pest known as the pine processionary moth, *Thaumetopoea pityocampa*. The impacts of this pest are increasingly exacerbated by climate change. Traditional control strategies involving pesticides have had negative effects on public health and the environment. Instead, forest managers seek a more ecological and sustainable approach to management that promotes the natural actions of pest control agents. This study aims to evaluate the role of bats in suppressing pine processionary moths in pine forests and examine how the bat community composition and abundance influence pest consumption. Bats were sampled in the mountainous environment of the Serra da Estrela in central Portugal to collect faecal samples for DNA meta-barcoding analysis. We assessed the relationship between a) bat richness, b) bat relative abundance, c) bat diet richness, and the frequency of pine processionary moth consumption. Our findings indicate that sites with the highest bat species richness and abundance exhibit the highest levels of pine processionary moth consumption. The intensity of pine processionary moth consumption is independent of insect diversity within the site. The highest occurrence of pine processionary moth presence in bat diets is primarily observed in species that forage in cluttered habitats. A typical predator of pine processionary moths among bats is likely to be a forest-dwelling species that specialises in consuming Lepidoptera. These species primarily use short-range echolocation calls, which are relatively inaudible to tympanate moths, suitable for locating prey in cluttered environments, employing a gleaning hunting strategy. Examples include species from the genera *Plecotus*, *Myotis*, and *Rhinolophus*. This study enhances our understanding of the potential pest consumption services provided by bats in pine forests. The insights gained from this research can inform integrated pest management practices in forestry.

## 1. Introduction

Coniferous forests make a significant contribution to the European economy. In 2020, the gross value added (GVA) of wood-based industries in the EU was €136 billion, 7.2 % of the total manufacturing industry with 3.5 million jobs associated (Eurostat, 2022; UNECE/FAO, 2021). Yet, the forestry sector has been suffering severe economic losses since the late 1990s due to the presence of the pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775). This forest pest is native to the southern Mediterranean area, North Africa, the Middle East, and southern Europe (Ferracini et al., 2020). In the Mediterranean region, it is known as the greatest defoliator of the tree genera *Pinus* and *Cedar*. Several studies support that the caterpillar of the pine processionary moth feeds on the pine needles causing a decrease in radial growth, and therefore major economic losses (Campôa et al., 2021; Çatal, 2011; Sangüesa-Barreda et al., 2014). However, it is worth noting that varying results exist in the literature, as illustrated by Linares et al. (2014), which did not find a significant reduction in radial growth after pine processionary moth defoliation, underscoring the complexity of the topic. For example, in Portugal, pine forests cover an area of over 10,000 km<sup>2</sup> (ca. 32 % of the national forest coverage) and between 1999 and 2010, >2630 km<sup>2</sup> of pine forests were lost mainly due to damages caused by the pine processionary moth. This weakening of the tree also makes it more susceptible to other pests (Castaño et al., 2020; Dewey et al., 1974; Setiawan and Verheyen, 2013; Wargo, 1981). However, it is important to note that while this concept is widely cited, direct evidence supporting this claim is currently limited.

In addition to the damage caused to the trees, the urticant hairs of the pine processionary moth caterpillar pose a serious health risk, causing allergies in animals and humans (Valdoleiros et al., 2021). Some of the pest management strategies used against the pine processionary moth primarily involve the use of pesticides (Cebeci et al., 2010). As the use of pesticides to treat the pine processionary moth decreases due to their impacts on the environment, human health, and animal health (Köhler and Triebkorn, 2013), it becomes necessary to develop more sustainable support tools for forest management that promotes the action of natural pest control agents. In the context of pest management, traditional approaches to control often focus on suppressing or significantly reducing pest populations through various means, including chemical interventions. However, unlike a definite reduction in pest populations, the presence of bats introduces a dynamic element where we cannot ascertain conclusively if they consume enough to ensure an absolute and significant decrease in the pest numbers. In this context, it is more fitting

to adopt the concept of “suppression”, indicating that bats may contribute to reducing pest numbers, although we cannot guarantee it is sufficient to prevent an uncontrolled surge in population.

Therefore, when considering the intervention of bats, we acknowledge that while their presence may result in the suppression of the pest, it is crucial to continue monitoring and adjusting strategies to ensure effective and sustainable management.

The risks associated with this pest are heightened by the impacts of climate change leading to further economic losses for the forestry sector. Therefore, it is necessary to develop a support tool for forest management that promotes the action of natural pest control agents. Integrated pest management is an ecosystem-based strategy that focuses on the long-term prevention of pests or their damage through a combination of techniques such as biological control, habitat manipulation, modification of cultural practices, and use of resistant varieties (Vignola et al., 2015). Effective pest suppression conservation copes with the increasing natural enemy populations to reduce forestry and agricultural losses caused by pests (Shields et al., 2019). Higher levels of biodiversity are thought to increase the provision of ecosystem services (Melo and Piratelli, 2023; Russo et al., 2018). However, such studies usually rely on an inference of co-occurring species (predator and prey) and rarely include diet analysis of pest predators.

Many bat species are voracious predators of insects, consuming more than half of their body mass in prey every night (Kalka and Kalko, 2006). Considering this, numerous studies present insectivorous bats as very effective consumers of agriculture and forestry pests (Baroja et al., 2021; Russo et al., 2018). These vertebrates have the potential to provide crucial ecosystem services, particularly in pest suppression as they feed on multiple arthropod pests (Mata et al., 2021).

Some bat species have already been identified as potential key candidates for consuming the pine processionary moth (Mata et al., 2021). These species are known to be important predators of adult moths (Aizpurua et al., 2018; Garin et al., 2019). However, limited knowledge exists regarding the overall contribution of the bat community to this ecosystem service and the specific species that provide pine processionary moth pest control services. The coexistence of ecologically similar bat species in the same area can lead to competition or niche partitioning among them (Denzinger and Schnitzler, 2013; Russo et al., 2018). Niche partitioning promotes sensory specialisation among species, resulting in higher foraging success for specialised predators (Safi and Siemers, 2010). This is particularly relevant for bats that may prey on pests or specialise in pest control.

The classification of different bat species into various foraging guilds

based on feeding associations, habitat use patterns, and foraging behaviours has been well established (Fenton, 1990). This guild classification system facilitates the identification of community structure patterns (Denzinger and Schnitzler, 2013). Coexistence of different bat species is possible if they occupy distinct foraging niches, enabling them to prey on a wider range of arthropods when coexisting with other bat species compared to when niche segregation is absent (Mata et al., 2021). To investigate the ecological conditions and hunting strategies of bats, we adopt a habitat-oriented approach that categorises them into guilds or functional groups with similar ecological conditions and hunting patterns (Denzinger and Schnitzler, 2013). This guild-based framework has proven effective in understanding how different functional bat groups respond to factors such as habitat connectivity (Frey-Ehrendbold et al., 2013), forest structure (Froidevaux et al., 2016), presence of roads (Medinas et al., 2019), types of street lighting (Bolliger et al., 2020), and levels of urbanization (Villarroya-Villalba et al., 2021).

Based on their hunting strategies, bat species can be categorised into four foraging guilds: clutter, open space, edge, and aquatic foragers (Baroja et al., 2021; Denzinger and Schnitzler, 2013; Garin et al., 2019). Bats that forage in cluttered environments employ a gleaning foraging technique characterised by short-range echolocation and wing morphology that enables slow but highly manoeuvrable flight and easy take-off from the ground (Froidevaux et al., 2016; Puig-Montserrat et al., 2021). Furthermore, it is worth noting that the nature of the echolocation calls used by bats in this guild renders their calls inconspicuous to certain moths (Nakano et al., 2015). The pine processionary moth, for instance, belongs to the Notodontidae family, which possesses auditory cells sensitive to ultrasound (Surlykke, 1984). Nevertheless, the auditory sensitivity of the pine processionary moth remains unknown (Garin et al., 2019). This guild of bats is particularly vulnerable to habitat fragmentation (Frey-Ehrendbold et al., 2013) and faces a high risk of extinction, necessitating significant conservation efforts (Jones et al., 2003). Bats that forage in open spaces encounter challenges in locating prey distributed over vast areas. They have evolved long-range echolocation systems, enabling them to detect insect echoes and glints using prolonged signals (Denzinger and Schnitzler, 2013). Their wing morphology is adapted to achieve high flight speeds, optimizing efficiency during long-distance flights but compromising manoeuvrability (Norberg and Rayner, 1987). Bats specialising in edge environments utilise the external canopy surface, providing access to open habitats while excluding closed ones. They exhibit functional versatility, capable of functioning in both edge and open situations, as observed in forest gaps where they show a preference for areas with denser vegetation (Baroja et al., 2021; Froidevaux et al., 2016). These bats typically emit calls comprising both broadband and narrowband components (Fenton, 1990). Their wing morphology, characterised by long and narrow wings with high aspect ratios, suggests an adaptation for sustained flight during foraging activities, whether within the edge or from the edges of tree canopies into more open habitats (Fenton, 1990). Lastly, bats that forage in aquatic environments, known as trawling foragers, fly at low heights above water. They capture drifting insects or fish, encountering similar echolocation scenarios as edge foragers when hunting near the shore. These foragers possess specialised morphological adaptations, such as hind legs and interfemoral pouches designed for capturing prey from water surfaces, while piscivorous species possess sharp claws (Denzinger and Schnitzler, 2013).

While the ecology of bats and pests and community structure have been well researched, little is known about the effect of pest consumption services by bat communities as a whole and how bat species coexist in the presence of a pest. In this study, we analyse a rich insectivorous bat community with different ecological requirements sampled along a mountainous area whose heterogeneous environmental conditions support different bat assemblages. Assessing the bat assemblage in its entirety will facilitate the identification of the primary providers of ecosystem services at the species level. We establish which bat guilds possess a greater potential for consuming the pine processionary moth,

thereby enabling the generalization of the findings to other regions.

Therefore, we hypothesise that:

a) Bat species with diverse foraging strategies will exhibit varying degrees of prey consumption of pine processionary moths. We predict that bats foraging in cluttered habitats, which are commonly located within forested areas where the pests reside, are more likely to provide pine processionary moth consumption services to pine stands.

b) Communities with higher species richness of bats will consume a greater number of pests, leading us to predict a positive association between bat richness and pine processionary moth consumption.

c) Communities with a higher abundance of bat individuals will consume a greater quantity of pine processionary moths. Hence, we anticipate a positive association between bat relative abundance and pine processionary moth consumption.

d) Bats with more diverse diets are likely to include a greater variety of pests, leading us to predict a positive association between bat diet richness and pine processionary moth consumption.

## 2. Material and methods

### 2.1. Study area

Our work was set in central-north Portugal, within the mountain chain of Serra da Estrela – (WGS84 lat.: 40.319833, long.: -7.607664) (Fig. 1). Its peak is the highest point in continental Portugal (1993 m). The area has distinct biogeography within the Mediterranean region with unique physical and biological characteristics (Costa et al., 1998).

The mountain range is distinguished by three different bioclimatic belts (ICNB, 2008; Pinto da Silva and Teles, 1999). Below 900 m a.s.l. a meso-Mediterranean zone occurs, with remnant forests of oak (*Quercus rotundifolia*, *Q. suber* and *Q. pyrenaica*), Portuguese laurel (*Prunus lusitanica*), plantations of *Pinus pinaster*, maquis vegetation, vineyards, olive groves and agricultural fields. Between 900 and 1600 m a.s.l., a supra-Mediterranean belt occurs with remnant woods of *Q. pyrenaica*, chestnut groves, shrublands and some rye cultivation. Above 1600 m a.s.l., the oro-Mediterranean belt is found, with matgrass lawns, heaths, juniper scrub, pastures, and aquatic communities (Costa et al., 1998; Pinto da Silva and Teles, 1999; Raposeira et al., 2023).

### 2.2. Bat sampling

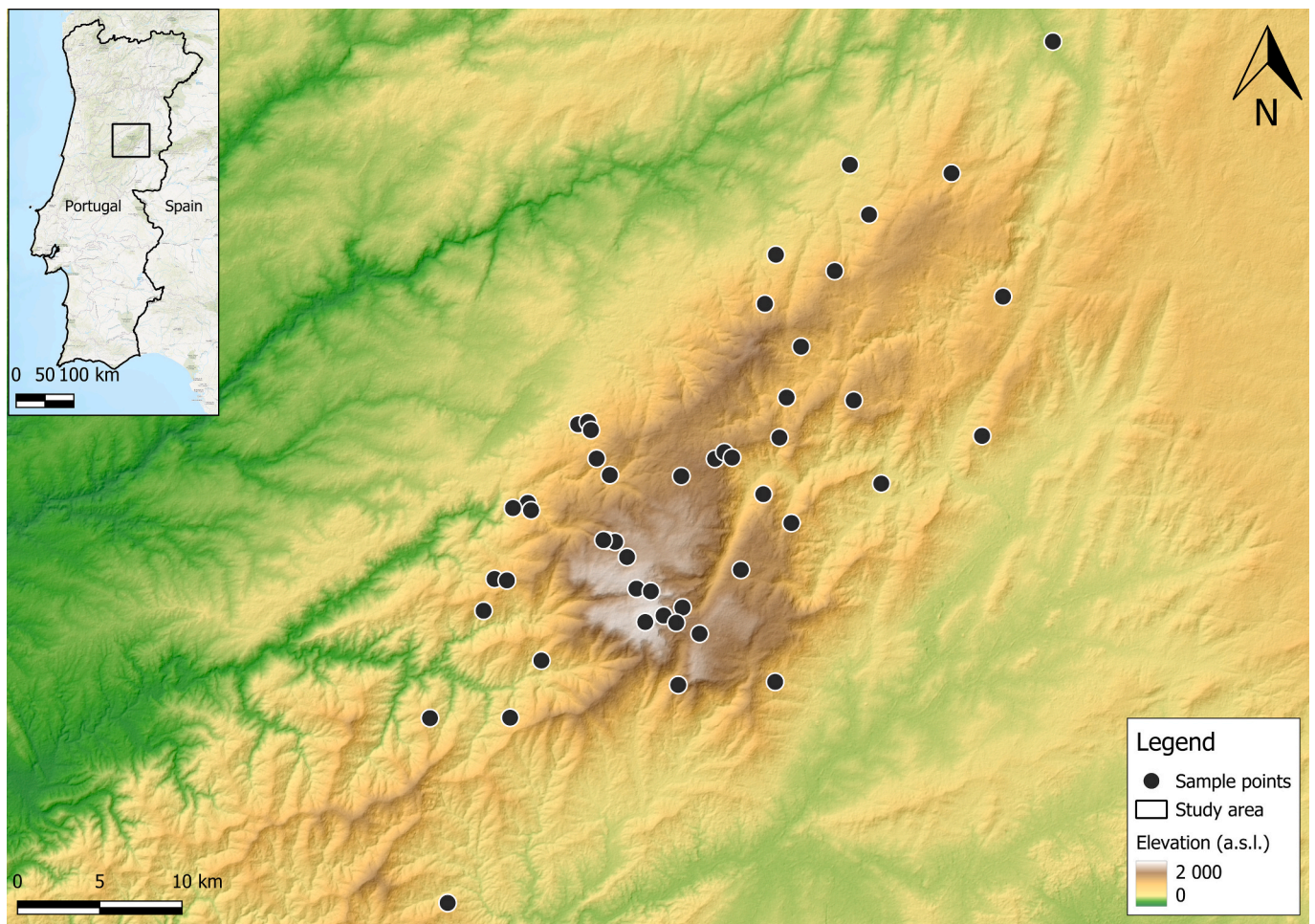
We conducted bat sampling between May and October from 2014 to 2018 to align our sampling period with the phenology of the pine processionary moth. These pest moths typically emerge between June and September (ICNF, 2015).

Our sampling methodology aimed to ensure the representativeness of bat populations within the study area. It is important to note that our primary objective was not to encompass the entire lepidopteran community but rather to explore the extent of pine processionary moth predation in the context of bat co-occurrence. Sampling sites were selected in areas known with high number of bat species and individuals, according to best practices (Raposeira et al., 2023; Russo and Jones, 2002) on habitat preferences of bats in Portugal. We targeted habitats known to be favourable for bats, such as remnant oak forests (*Quercus* spp.), chestnut groves, shrublands, and pastures. For a more comprehensive overview of the study area's environmental attributes with the bat distribution and abundance patterns at specific sampling points, additional histograms can be consulted in the Supplementary Material.

Bats were sampled at 39 sites representing the primary habitats, altitudinal ranges, and climatic conditions in the study area.

Bats were captured in mistnets at foraging and drinking sites (Fig. 1). In the sites where multiple sampling events were conducted, the nets were placed in the same position for 5 h, starting 30 min after sunset (Walsh et al., 2004). The sampling occurred when conditions were suitable for bat activity: low wind, low or no rainfall, good visibility and





**Fig. 1.** Sampling sites where bats were captured to collect faecal pellets for diet analysis along the study area in the Serra da Estrela mountain range, Portugal.

temperatures above 2.8 °C. Upon capture, bats were temporarily held in clean cotton bags for up to 30 min. During this time, we collected their faecal pellets, which were subsequently stored in 96 % ethanol for molecular analyses.

Bat capture and handling adhered to applicable guidelines and regulations and received approval from the Ethical Committee at the ICNF (Instituto da Conservação da Natureza e das Florestas).

### 2.3. DNA meta-barcoding protocols and data analysis

#### 2.3.1. Laboratory procedures (DNA extraction and amplification)

DNA from individual faecal pellets was extracted using the E.Z.N.A tissue DNA kit (Omega Bio-Tek, Norcross, Georgia, USA) as described by Mata et al. (2021), except that no Inhibitex tablets were used. DNA was isolated in randomised batches of 23 samples along with one negative control with no faecal pellet and stored at −20 °C until further processing.

DNA was amplified using general arthropod primers fwhF2-R2n (Vamos et al., 2017) that target a small fragment (254 bp) of the cytochrome oxidase subunit 1 (COI) region. For each reaction, we used 5 µL of QIAGEN Multiplex PCR Master Mix, 0.25 µL of each primer (modified with Illumina overhangs), 2.5 µL of ultra-pure water, and 2 µL of DNA extract in a total volume of 10 µL. We performed an initial denaturation step at 95 °C for 15 min, followed by 42 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 60 °C for 10 min. We confirmed the quality and success of the amplification by checking the PCR products in 2 % agarose gels stained with GelRed. These primers also produce reads that allow the

identification of bat species (Mata et al., 2021).

#### 2.3.2. Library preparation

We diluted PCR products 1:4 with water and subjected them to a second amplification step to incorporate P5 and P7 Illumina adaptors, each tagged with distinct 7 bp long barcodes and used in unique combinations. The PCR mixture consisted of 7 µL KAPA HiFi HotStart ReadyMix (Rocher, KAPA Biosystems, Basel, Switzerland), 2.8 µL of ultra-pure water, 0.7 µL of each P5 and P7 index, and 2.8 µL of diluted PCR product. For the indexing PCR, we denatured the mixture at 95 °C for 3 min, followed by 9 cycles of 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a last extension at 72 °C for 5 min.

Next, we cleaned the indexed PCR products with Agencourt AMPure XP beads (Beckman Coulter, Brea, California, USA) using a 1:0.8 ratio according to the manufacturer's instructions to eliminate remaining nucleotides, primers, and primer dimers. We quantified and normalised the libraries with Epoch (dsDNA) and pooled them. We then confirmed the mean amplicon length and lack of small non-target amplicons or primer dimers that could interfere with sequencing by running the pool in TapeStation (Agilent, California, USA) with a High Sensitivity D1000 ScreenTape.

Finally, we confirmed the DNA concentration using qPCR (KAPA Library Quant Kit qPCR Mix; Rocher) and diluted the pool to 4 nM. We sequenced the libraries on a MiSeq desktop sequencer (Illumina) using a MiSeq Reagent Kit v3 (2x250bp) to achieve a target depth of ~40,000 paired reads per sample.

### 2.3.3. Bioinformatic analysis

We processed the raw sequencing data using Obitools (Boyer et al., 2016) and VSEARCH (Rognes et al., 2016). We used the command ‘illumina-paired-end’ to combine paired reads and removed primer sequences and tagged reads with ‘ngsfilter’. We dereplicated reads per sample with the command ‘obuniq’. To remove potential PCR and sequencing artifacts, as well as sequences shorter than predicted, we applied the commands ‘-cluster\_unoise’ and ‘-uchime3\_denovo’. The remaining sequences were clustered at 99 % similarity with ‘-cluster\_size’. We mapped initial reads back to the retained Operational Taxonomic Units (OTU) with ‘-usearch\_global’ using an identity level of 99 %. To remove nuclear segments of the mitochondria, PCR and sequencing artifacts, we used the R package LULU (Froslev et al., 2017). Remaining OTUs were taxonomically assigned using BOLDigger (Buchner and Leese, 2020). OTUs identified to the order level or below, while belonging to classes Insecta or Arachnida, except for known parasites like Trombidiformes, Mesostigmata, Ixodida, and Siphonaptera, were categorised as diet and kept for analysis, while the remaining ones were removed. We discarded samples with <100 arthropod reads from further analysis. We removed OTU reads found in extraction and PCR negative controls from the samples associated with those controls. To calculate diet richness in each sample, we considered only OTUs with >1 % of the total diet reads. We considered pine processionary moth to be present when 5 or more reads were observed in a sample, even if that represented <1 % of the total diet reads of that sample for pine processionary moth. We found this number to be a good compromise between trying to maximise the number of detections, while still reducing tag-jumping and contamination events, as no control samples showed the presence of pine processionary moth with five or more reads. These procedures reduced the false positives related to lab procedures and tag-jumping from sequencing.

### 2.4. Statistical analyses

After an initial exploratory analysis, we refined our sampling strategy and only analysed data collected between July and September due to the higher presence of the pine processionary moth in bat diets during these months compared to the rest of the year.

The fieldwork data consisted of counts of captured bat species, and we standardised the bat richness and relative abundance based on the surface of mistnets used on each sampling night (number of bat species/bats captured per square metre) following Ferreira et al. (2021). We then compared these standardised data with the bat diet data.

The meta-barcoding analysis of the faecal pellets resulted in two categories of data. First, we addressed the presence or absence of the pine processionary moth in bat diets and calculated the frequency of its occurrence by dividing the number of pellets with pine processionary moth presence by the total number of pellets from these species. For prey richness data (number of prey OTUs in each sample), we calculated the average per site, year, month, and fortnight and then standardised the data (Ferreira et al., 2021).

We performed statistical analyses in RStudio (v.2022.07.2 Build 576 2009-2022 RStudio, PBC), using ecological models, including a Negative Binomial GLM, to identify which localities, years, and months were associated with bat activity and predation upon pine processionary moth. Because bat species richness, abundance and some subgroups of the diet richness were not influenced by sampling year, data from all years were grouped together in the subsequent GLM analyses. We used analysis of variance to test the significance of the model employing the ‘glm’ function with the ‘.nb’ distribution from the ‘MASS’ package (Venables and Ripley, 2002). In the subgroups of bat diet richness that were significantly influenced by the sampling year, we conducted GLMM analysis only for this hypothesis. We used analysis of variance to test the significance of the model employing the ‘glmmTMB’ function with the ‘nbinom2’ distribution from the ‘glmmTMB’ package (Brooks et al., 2017). We considered statistical significance at an alpha value of

0.05.

To analyse what was influencing the level of pine processionary moth consumption, we used the frequency of occurrence of pine processionary moths in the diet of each bat species as an independent variable. Subsequently, we produced GLMs to examine the relationships between both bat diversity (number of species) and the frequency of occurrence of pine processionary moths, as well as bat relative abundance (number of individuals) and the frequency of occurrence of pine processionary moths. We also generated GLMMs to examine the relationships between species richness of bat diets (both total and per species) and the frequency of occurrence of pine processionary moths.

To test the hypothesis that only some bat species and specific guilds will provide a pine processionary moth consumption service to pine stands, we determined which bats and guilds consumed the pine processionary moth. For a more refined analysis, we grouped these species into four guilds of bats according to their foraging specialisation strategy in cluttered, open space, edge and aquatic habitats. The clutter foragers included eleven species, which accounted for 48 % of the total species captured in the study: *Myotis emarginatus*, *Plecotus austriacus*, *Plecotus auritus*, *Rhinolophus euryale*, *Myotis bechsteinii*, *Rhinolophus ferrumequinum*, *Myotis mystacinus*, *Myotis escalerae*, *Myotis myotis/blythii*, *Barbastella barbastellus* and *Rhinolophus hipposideros*. The open space foragers included six species, which accounted for 26 % of the total species sampled in the study: *Nyctalus leisleri*, *Eptesicus serotinus*, *Eptesicus isabellinus*, *Eptesicus serotinus/isabellinus*, *Nyctalus lasiopterus* and *Tadarida teniotis*. The edge foragers included five species, which accounted for 22 % of the total species sampled in the study: *Miniopterus schreibersii*, *Pipistrellus kuhlii*, *Pipistrellus pipistrellus*, *Hypsugo savii* and *Pipistrellus pygmaeus*. *Myotis daubentonii*, which is the only species we caught characterised by an aquatic hunting strategy, accounted for 4 % of the total species sampled in the study, therefore this category was excluded from the GLM analysis. Subsequently, we calculated the frequency of occurrence of the pine processionary moth in the diet for each bat species by relating the number of pellets containing the presence of the pine processionary moth to the total number of pellets. We used the Clopper-Pearson exact binomial interval to calculate the binomial 95 % confidence intervals for the frequency of occurrence (%). This method provides a range estimating the potential variation around the observed percentage, even with a single value. The resulting interval represents a reasonably likely range for the true percentage of samples with the pest, with wider intervals indicating greater uncertainty and narrower intervals suggesting a more precise estimate. While typically used for larger datasets, it still provides insight into the variability around the single observed percentage. These intervals were then used to identify more relevant bat species in terms of pine processionary moth consumption.

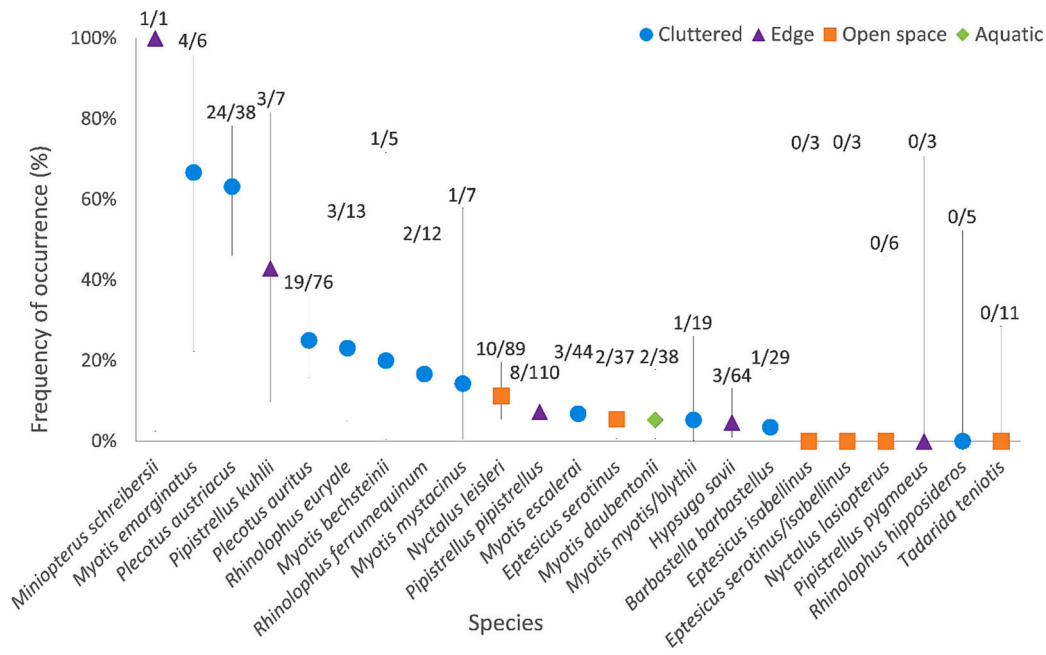
## 3. Results

We captured 876 individuals belonging to 23 bat species between July and September, from which we collected and analysed 626 faecal pellets. The frequency of occurrence of the pine processionary moth in bat diet varied between species and ranked as follows: *Myotis emarginatus* (67 %), *Plecotus austriacus* (63 %), *Pipistrellus kuhlii* (43 %), *Plecotus auritus* (25 %), *Rhinolophus euryale* (23 %), *Rhinolophus ferrumequinum* (17 %) and *Nyctalus leisleri* (11 %). This assemblage of species with >10 % pine processionary moth in their diet is referred to as the top 7 in subsequent analyses (Fig. 2). For additional information regarding bat species diet, please refer to the Supplementary Material.

### 3.1. Is bat richness positively associated with higher pine processionary moth consumption?

We found a significant relationship between bat species richness and frequency of consumption of pine processionary moth when including all sampled bats ( $p$ -value: 0.002;  $R^2$ : 0.104) and clutter foraging bat





**Fig. 2.** Bat species predation of pine processionary moth per guild in Serra da Estrela, Portugal. The frequency of occurrence (%) relates to the number of pellets with the pine processionary moth in the total number of pellets. The numerical values positioned above each species refer to the number of samples containing pine processionary moth per total number of samples. Guilds: clutter foragers, edge foragers, open space foragers, and aquatic foragers.

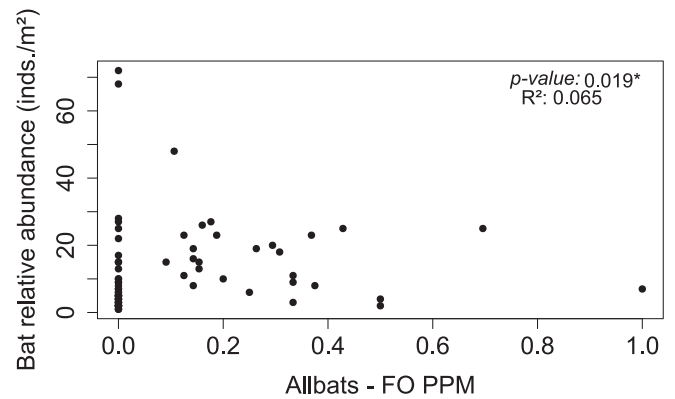
species (*p*-value: 0.024;  $R^2$ : 0.069) (Fig. 3). In these cases, with the increasing number of bat species, the consumption of the pine processionary moth also rises. For additional information regarding the data and models' metrics and results, please refer to the Supplementary Material.

**3.2. Is bat relative abundance positively associated with higher pine processionary moth consumption?**

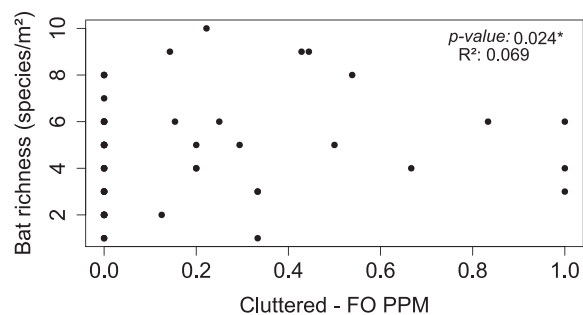
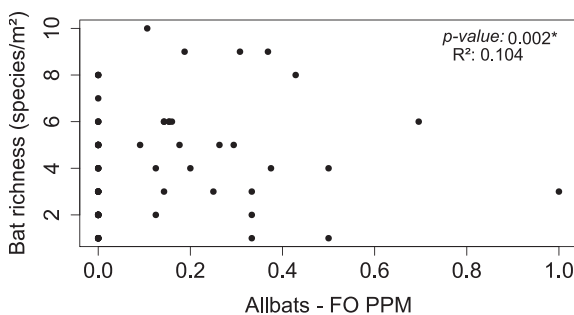
We observed a significant relation between bat relative abundance and the frequency of pine processionary moth consumption, for all sampled bats (*p*-value: 0.019;  $R^2$ : 0.065) (Fig. 4). There was a positive association, indicating that as bat abundance increased, so did the consumption of pine processionary moths.

**3.3. Is bat diet richness positively associated with higher pine processionary moth consumption?**

The relationship between bat diet richness and the consumption frequency of pine processionary moths was statistically significant in the analyses involving all sampled bats (*p*-value: 0.005;  $R^2$ : 0.043), and the clutter foragers (*p*-value: 0.006;  $R^2$ : 0.088) (Fig. 5). In these instances, with the increasing richness of bat diets, the consumption of pine



**Fig. 4.** Relationship as shown by GLMs between the percentage of bat species relative abundance within the overall bat communities and the frequency of occurrence of the pine processionary moth consumption. *p* significance values: \* < 0.05.



**Fig. 3.** Relationship as shown by GLMs between the percentage of bat species richness within the overall bat communities and clutter foragers, and the frequency of occurrence of the pine processionary moth consumption. *p* significance values: \* < 0.05.

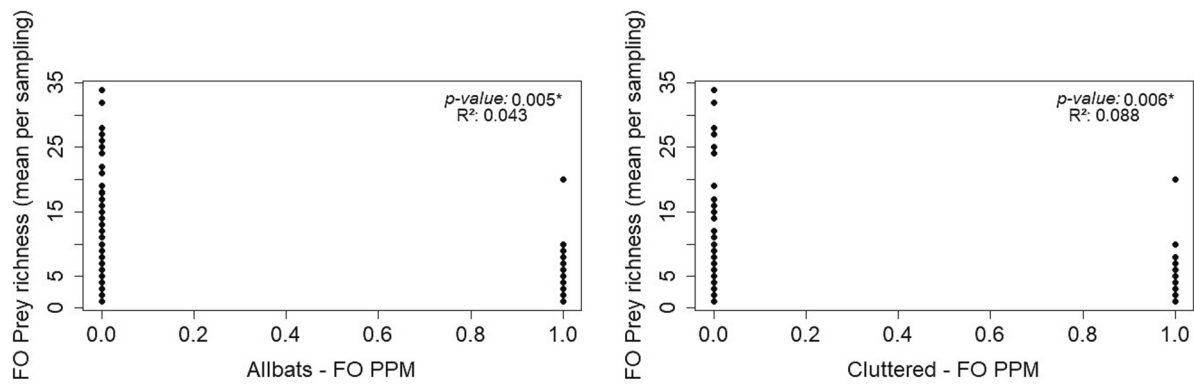


Fig. 5. Relationship as shown by GLMMs between the percentage of bat diet richness within the overall bat communities and clutter foragers, and the frequency of occurrence of the pine processionary moth consumption.  $p$  significance values: \*  $< 0.05$ .

processionary moth also rises.

#### 4. Discussion

Our study assessed the impact of bats on the consumption of pine processionary moths in pine forests while considering the influence of bat community composition and abundance on pest consumption. We determined the bat guilds with the greatest potential for consuming pine processionary moths.

Additionally, we analysed bat diet metrics to understand how bat diet composition varies with factors such as bat species, abundance, and prey availability, facilitating ecological niche comparisons within bat diets. It is important to note that this study does not seek to represent the entire lepidopteran community but focuses on investigating the intensity of pine processionary moth predation due to bat co-occurrence. The research concentrates on bats, encompassing their diversity, relative abundance, and dietary diversity, with a focus on understanding the factors influencing pest consumption. The primary novelty of our study lies in demonstrating that sites with the highest number of bat species, the greatest bat abundance and high bat diet richness provided the highest degree of pest consumption service.

##### 4.1. Which bat species consume the pine processionary moth?

Our results corroborate the hypothesis a) that bat species with diverse foraging strategies will exhibit varying degrees of pine processionary moth consumption. We confirmed that 23 bat species, belonging to four guilds forage in the study area, including clutter, open space, edge, and aquatic foragers. The great majority of the seven species with  $>10\%$  pine processionary moths in their diet were clutter foragers belonging to the genera *Myotis*, *Plecotus*, and *Rhinolophus*. These bats are typically found in forests and broadcast weak, short-range detection echolocation calls, often using prey-generated noise, such as *Plecotus* spp. (Norberg and Rayner, 1987), or, for rhinolophids, acoustic glints (amplitude and frequency shifts) are generated in their constant frequency by fluttering insects (e.g., Vanderelst et al., 2011). It is important to note that these echolocation calls are not conspicuous to moths sensitive to ultrasound, including notodontids. However, specialised edge foragers such as *Pipistrellus kuhlii* and open space foragers such as *Nyctalus leisleri* also featured significant numbers of pine processionary moths in their diets. In this specific study, *Miniopterus schreibersii*, *Myotis bechsteinii* and *Myotis mystacinus* cannot be considered important pest consumers because they have a very low frequency of occurrence of pine processionary moths in their diets. It is noteworthy that, while the contribution of *Miniopterus schreibersii* to the consumption of this pest could not be definitively confirmed in our study due to the capture of only a single individual, this individual did indeed consume pine processionary moth. This finding aligns with other studies that have

identified this species as a significant predator of the pine processionary moth (Aizpurua et al., 2018; Garin et al., 2019).

Furthermore, a study conducted by Alberdi et al. (2020), with a primary focus on *Miniopterus schreibersii*, revealed that this species shapes its diet in response to the most abundant pests in each locality. These findings underscore the dynamic nature of bat diet preferences in response to local ecological conditions.

The forest bat species mentioned above specialise in preying on lepidopterans (Baroja et al., 2021). Therefore, they are likely to target the more abundant Lepidoptera species. Previous studies analysing the diet of bats have already recognised some bat species as predators of the pine processionary moth (Garin et al., 2019; Mata et al., 2021). However, by conducting a comprehensive study encompassing the whole bat community and employing a community-based approach, we were able to identify new potential contributors to pest consumption services and assess the impact of bat richness on this service. This study revealed, for the first time, additional predators of the pine processionary moth, including *Pipistrellus kuhlii*, *Myotis bechsteinii*, *Myotis mystacinus*, *Nyctalus leisleri*, *Pipistrellus pipistrellus*, *Eptesicus serotinus*, *Myotis daubentonii*, and *Hypsugo savii*, regardless of their high or low contribution to pest consumption.

In summary, our findings indicate that forest bats specialising in Lepidoptera predation and possessing short-range echolocation abilities, such as species from the genera *Plecotus*, *Myotis*, and *Rhinolophus*, are key predators of the pine processionary moth.

##### 4.2. Bat community richness and abundance and pine processionary moth

Our results support hypotheses b) and c), indicating that communities with higher species richness and higher abundance of bats consume a larger quantity of pine processionary moths.

Among all the bat species that were confirmed to prey on the pine processionary moth, the Lepidoptera specialists were the main predators. We show that these specialists prey more intensely on pine processionary moths when other bat species are present in the area, highlighting the importance of biodiversity for the delivery of ecosystem service.

Theoretically, these findings lend credence to the hypothesis that the association between pine processionary moth consumption and the relative abundance of bats may be elucidated by the dual mechanisms of numerical and functional responses exhibited by bats in reaction to pine processionary moth activity. The limited ability of our model to account for variations in the data suggests that there are likely other factors beyond pine processionary moth consumption that influence the regulation of pine processionary moth populations. These factors may encompass habitat diversification, the role of parasitoids, and the impact of bat predation, all of which contribute to the suppression of pine processionary moth population levels.

Although it is generally believed that a higher bat richness is advantageous for pest consumption, it can also lead to competition among bats (Russo et al., 2018). However, our findings demonstrate that as the number of bat species increases, the consumption of pine processionary moths also increases. This may seem counterintuitive unless we consider the possibility that, to alleviate competition for scarce prey, different bat species increase their feeding on non-limiting prey species, such as the pine processionary moth. We propose that some bats may avoid competition by selecting prey that is typically not consumed by most bat species, such as the pine processionary moth, which is found in high densities in our study area. It is important to note that it is challenging to demonstrate that bats use niche partitioning to avoid competition (Razgour et al., 2011; Salinas-Ramos et al., 2020).

Our findings reveal that, in general, sites with higher bat species richness and abundance exhibited higher consumption of pine processionary moths. More specifically, an increase in bat species richness resulted in a significant upsurge in consumption by all bats and clutter foragers. Furthermore, our analyses demonstrated that an increase in the number of bats led to a significant rise in pine processionary moth consumption by all bats. Previous studies have indicated a positive relationship between bat abundance and pine processionary moth abundance (Baroja et al., 2021; Charbonnier et al., 2014).

Another possible explanation is that diverse bat communities consisting of clutter, open space, edge, and aquatic foragers are capable of capturing pine processionary moths at various life stages, including adults and larvae, resulting in increased overall consumption. Although in our study bats may have captured the pine processionary moth at different life stages, either as adults (resting on leaves or in flight) or as caterpillars, it is likely that clutter foragers employing a gleaning hunting strategy targeted both stages. However, the predation peak of the pine processionary moth in our study coincided with the peak in adult moth's abundance (Arnaldo et al., 2011), when the larval stage was less prevalent. This suggests that predation primarily focuses on the adult stage. It is important to note that pine processionary moth caterpillars have urticating hairs (Valdoleiros et al., 2021). Despite some bat species being known to tolerate venomous prey, such as scorpions (Holderied et al., 2011), our findings indicate that bats avoid the urticating phase of the pine processionary moth, despite the high availability of this prey in the region (Schmidt et al., 1999).

#### 4.3. Dietary niche and pine processionary moth

Related to hypothesis d) that bats with more diverse diets are likely to include higher consumption of pine processionary moths, our results showed a significant relationship between this prey consumption and diet diversity among all bats and clutter foragers.

Typically, sites with greater levels of arthropod abundance exhibit higher bat activity in terms of abundance and diversity (Wickramasinghe et al., 2004). This suggests that bats can track food resources (Kunz et al., 2011). Bats have diverse diets and consume prey species from various families and orders, even those species that are considered specialists do not solely focus on a single prey species (Clare et al., 2011).

Our results reveal that the higher the bat diet richness, the more pine processionary moth consumption, especially among all bats and clutter foragers. Consequently, we provide conclusive evidence supporting the coexistence of different bat species through niche segregation. Their predatory behaviour may encompass a wider range of arthropods than expected in the absence of niche segregation (Mata et al., 2021).

Bats may actively avoid competition by engaging in niche segregation, which involves targeting highly abundant prey that is not preferred by most other sympatric species. This form of niche partitioning likely facilitates the coexistence of multiple bat species within the same foraging grounds. The tendency of bat species to coexist through trophic niche partitioning holds significant importance, particularly in disturbed systems where adaptations of predator species to reduce

dietary overlap may disrupt traditional predator-prey relationships (Burgar et al., 2014).

#### 4.4. Limitations of the study

Our results suggest that higher predation on pine processionary moth may help to mitigate impacts of interspecific competition by converging on the most abundant (non-limiting) prey, though this pattern still needs to be confirmed in detail.

One limitation of this study is the lack of direct sampling to estimate pine processionary moth abundance in the study area, which limits our understanding of the actual impact of bats on pine processionary moth populations. Pine processionary moth hatch in outbreaks, therefore, at some point there is a high density of individuals in the same space (Campôa et al., 2021). However, the pattern of imago dispersal is still unknown, meaning that it is difficult to validate the effectiveness of bats in consuming pine processionary moth (Garin et al., 2019). However, this study identifies the bat species and seasons when pest consumption services may be most intense. It should be noted that other bat species may also prey on pine processionary moth, and additional information on the abundance of pine processionary moth in the area could strengthen these data. The landscape and habitats where bats occur play an essential role in shaping their ecology and diet (Amorim et al., 2018; Medinas et al., 2021). While both features are intertwined, our study focused only on bat diet composition, which allowed us to concentrate on biotic interactions and the co-existence of bat species.

#### 4.5. Management implications and future studies

Higher consumption of pine processionary moth associated with greater levels of bat species richness emphasises the importance of biodiversity. Therefore, it is crucial to promote conditions that allow relevant pest consumers to coexist and forage in the area. The presence of a higher number of pest predators leads to an increased delivery of ecosystem services.

Diverse bat assemblages are essential to facilitate the targeted positioning of bat species that consume pine processionary moths. It is imperative to elucidate strategies for landscape management that promote high bat diversity, ultimately ensuring the effective relocation of pest-consuming bats to appropriate locations.

An important recommendation, particularly in the context of the pine processionary moth, is to provide artificial roosts for forest bats, as pine trees do not offer suitable roosting sites due to their resin content. It is also vital to maintain and improve habitats to sustain higher levels of biodiversity (Tortosa et al., 2023). Additionally, implementing various management and conservation actions can help reduce or even replace the need for pesticide use. Future studies should focus on investigating the influence of landscape factors on the consumption of pine processionary moths. Spatial planning can then be used to optimise the landscape and enhance pine processionary moth consumption (Vaz et al., 2021).

By identifying the specific nature-based solutions, as well as green and blue infrastructures, that are associated with the consumption of pine processionary moths, we can provide valuable information to forest practitioners and land managers regarding the most effective ecological restoration actions for promoting pest consumption services by bats. Incorporating spatial planning that considers climate change scenarios can further enhance the robustness of these efforts. Such an approach would enable the identification of areas that may be more vulnerable to climate change, thus guiding targeted interventions and maximizing the impact of conservation measures. Ultimately, this comprehensive approach can contribute to more effective and informed decision-making in managing pine processionary moth populations and promoting the ecological services provided by bats.



## 5. Conclusions

In conclusion, our study explored the intricate relationship between bats and the consumption of pine processionary moths. Through a comprehensive analysis of bat communities across different guilds and using dietary metrics, we identified key predators of the pine processionary moth, highlighting the significance of forest bats specialised in Lepidoptera predation, such as those from the genera *Plecotus*, *Myotis*, and *Rhinolophus*.

Our findings support the hypotheses that diverse bat communities, characterised by higher species richness and abundance, play a pivotal role in efficiently consuming pine processionary moths. Importantly, we revealed that as bat species richness and abundance increased, so did the consumption of these pests.

Furthermore, our study highlighted the importance of dietary niche diversity among bats, illustrating how bats with more diverse diets exhibit higher consumption of pine processionary moths. Apparently, there was a niche segregation, suggesting that bats actively avoid competition by targeting abundant prey that may not be preferred by most other sympatric species.

Despite study limitations, including a lack of direct sampling for pine processionary moth abundance estimation, our results suggest higher predation on these pests may mitigate interspecific bat competition, particularly targeting the most abundant prey. Management implications stress promoting biodiversity, providing suitable roosting sites for forest bats, aiding ecological restoration, and informing pest management.

Future research should explore landscape factors' influence on moth consumption, integrating climate change scenarios for conservation effectiveness. Identifying nature-based solutions and climate-resilient interventions contributes to informed decision-making, enhancing sustainable pine processionary moth population management through bats in forest ecosystems.

## CRediT authorship contribution statement

**A.M. Augusto:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **H. Raposeira:** Resources. **P. Horta:** Resources. **V.A. Mata:** Writing – review & editing, Writing – original draft, Formal analysis. **O. Aizpurua:** Writing – review & editing, Conceptualization. **A. Alberdi:** Writing – review & editing, Conceptualization. **G. Jones:** Writing – review & editing, Conceptualization. **O. Razgour:** Writing – review & editing, Conceptualization. **S.A.P. Santos:** Writing – review & editing, Conceptualization. **D. Russo:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **H. Rebelo:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ana Margarida Augusto reports financial support was provided by Foundation for Science and Technology. Ana Margarida Augusto reports a relationship with Foundation for Science and Technology that includes: funding grants. The authors have no competing interests.

## Data availability

The dataset from the fieldwork is accessible at <https://datadryad.org/stash/dataset/doi:10.5061/dryad.d2547d84w>.

The dataset from the laboratory analyses of faecal pellets is accessible at <https://www.ebi.ac.uk/biostudies/studies/S-BSST1261>.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.169387>.

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