Biological Conservation 305 (2025) 111089

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

When pets go wild: Integrating DNA metabarcoding and morphological analyses to investigate the impacts of free-ranging cats (*Felis catus*) on oceanic islands

Alexandra Galão [a], Elena J. Soto [b,c], João Nunes [c], Nuno M. Pedroso [d], Ricardo Rocha [e,*,1], Catarina Rato [f,g,1]

[a] *Departamento de Biologia, UBC - Unidade de Biologia da Conservação, Universidade de Évora, Pólo da Mitra, Ap.94, 7006-554 Évora, Portugal*
[b] *Faculty of Biology, University of Murcia, 30003 Murcia, Spain*
[c] *Câmara Municipal do Funchal, Parque Ecológico do Funchal, 9050-554, Madeira, Portugal*
[d] *MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal*
[e] *Department of Biology, University of Oxford, 11a Mansfield Rd, OX1 3SZ Oxford, UK*
[f] *CIBIO – Research Centre in Biodiversity and Genetic Resources, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas 7, 4485-661 Vila do Conde, Portugal*
[g] *BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal*

ARTICLE INFO

Keywords:
Felis catus
Invasive species
Diet analyses
Next-generation sequencing
Madeira Island

ABSTRACT

Oceanic island ecosystems are particularly vulnerable to the impacts of introduced mammalian predators. Among these, free-ranging cats (*Felis catus*) are especially detrimental, and have been linked to the extinction and population decline of numerous insular taxa. Yet, a more holistic understanding of the impacts of free-ranging cats has been hampered by an incomplete understanding of their diet and population densities. Using a peri-urban protected area in the subtropical Madeira Island as a case study, we combined DNA metabarcoding, morphological scat analyses, and camera trapping to investigate the magnitude of native and introduced prey consumption by cats. Based on 58 scats collected over the summer and autumn of 2021, we found that cats consume over 20 distinct taxa from ten orders, including native and non-native prey, as well as taxa associated with anthropogenic food. Cat diet was seasonally modulated, with a higher incidence of reptiles in the summer months. Although DNA metabarcoding revealed a higher diversity of prey taxa compared to traditional morphological scat analyses, both methods provided complementary insights, highlighting the benefits of their combined use. Combining our diet results with a free-ranging cat density of 1.4 cats/km^2, as revealed by the protected area-wide camera trap survey, we estimate an annual prey intake of over 18,000 vertebrates by cats in a protected area of 7.5 km^2, of which over one-third corresponds to native birds and reptiles. These findings underscore the complex interactions between cats and native wildlife, emphasising the need for targeted conservation strategies in ecologically sensitive areas.

1. Introduction

Insular ecosystems tend to have fewer species than their mainland counterparts. However, due to their evolutionary isolation, island biodiversity is usually characterised by high numbers of endemic taxa and communities that evolved largely sheltered from many of the ecological stressors affecting mainland ecosystems, such as high levels of interspecific competition, parasitism, and predation (Russell and Kueffer, 2019). As a consequence, insular biota are particularly sensitive to the pervasive effects of exotic species, especially invasive mammals (Russell et al., 2017).

For millennia, cats (*Felis catus*) have been important companions, pest controllers, and part of our cultural heritage, currently being found in most continental and island ecosystems inhabited by humans

---

* Corresponding author.
E-mail address: ricardo.rocha@biology.ox.ac.uk (R. Rocha).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.biocon.2025.111089
Received 2 October 2024; Received in revised form 27 February 2025; Accepted 7 March 2025

(Crowley et al., 2020; Magle and Crowther, 2023). However, due to competition with local species for food and resources, transmission of diseases, hybridization and predation, they constitute an important threat to native biodiversity (e.g., Fardell et al., 2023; Herrera et al., 2022a; Loss et al., 2022; Szentivanyi et al., 2024). Indeed, their impact as predators of native species is particularly problematic (Trouwborst et al., 2020). As opportunistic and generalist predators, cats prey on a wide variety of wild species, including mammals, reptiles, birds, and insects (Lepczyk et al., 2023), even while being regularly fed by humans (Hernandez et al., 2018; Plimpton et al., 2021). This has complex impacts on native ecosystems and has contributed to the populational decline of ca. 14 % of the mammals, birds and reptiles listed as Extinct by the IUCN Red List (Courchamp et al., 2003; Medina et al., 2011).

The impact of free-ranging cats is particularly problematic in island ecosystems, where they often assume the role of apex predators. Yet, although a growing body of literature sheds light on the consumption of insular vertebrates by cats (e.g., Rocha, 2015; Alves et al., 2024; Hernández and Rando, 2024; Russell et al., 2018), and to their links to declines in the abundance and, in some cases, to local and global extinctions of island taxa (e.g., Bonnaud et al., 2011; Moseby et al., 2015), we still have a poor understanding of cat predator-prey interactions. This is largely an artefact of studies on cat diet typically relying on morphological analysis of faecal samples, stomach or intestinal contents, which although useful, have limited resolution in terms of taxa assignment due to their dependence on the identification of undigested prey remains (Bonnaud et al., 2011; Carrión and Valle, 2018). However, next-generation sequencing (NGS) techniques, now extensively used in dietary analyses (de Sousa et al., 2019), allow for a more thorough understanding of free-ranging cat diet. Yet, despite commonly used to study the diet of a wide array of carnivorous mammals (e.g., Lino et al., 2023; Massey et al., 2021; McLennan et al., 2022; Woo et al., 2023), there is only one study published – from mainland USA - applying this technique to free-ranging cats (Plimpton et al., 2021).

Despite multiple benefits, molecular methods can overlook species due to the absence of suitable primer-binding sites (e.g., mutations in the primer-binding regions), and incomplete molecular databases (Schenk et al., 2020). Accordingly, some studies recommend the parallel use of molecular and morphological techniques, as complementary approaches in dietary studies (e.g., Gil et al., 2020). However, as far as we know, no study has yet combined the results obtained by molecular and traditional diet methods in the assessment of the impacts of free-ranging cats.

Here, we address this gap by jointly using DNA metabarcoding and morphological analyses, to investigate the trophic ecology of free-roaming cats in a peri-urban protected area in Madeira Island, Portugal. Furthermore, based on cat densities estimated by a protected area-wide camera trap survey and the daily dietary needs of cats, we use the combined results from molecular and morphological diet analyses to estimate the annual prey intake by free-ranging cats in the protected area. Specifically, we i) investigate how different are the dietary patterns obtained using DNA metabarcoding and morphological analysis; ii) investigate if the diet composition and richness of free-ranging cats is seasonally modulated; and, (iii) assess the magnitude of cat predation throughout the protected area. Based on previous studies contrasting both methodologies (e.g., Massey et al., 2021) and considering the relatively good representation of vertebrates from Madeira in molecular databases, we anticipate that DNA metabarcoding will provide a greater number of prey species consumed and a higher resolution identification compared to morphological analyses. Furthermore, we hypothesise that during the summer, cats will exhibit a higher propensity to prey upon birds and the Madeira wall lizard (*Teira dugesii*), due to the greater availability of more naive, immature birds and the increased activity of ectothermic species during this time of the year. In contrast, during autumn, we predict that there will be a higher incidence of introduced mammals. Lastly, based on the cat's dietary needs and on previous studies both in Madeira (Medina et al., 2010), and other oceanic islands (e.g., Carrión and Valle, 2018; Medina et al., 2021), we anticipate that despite the consumption of anthropogenic food sources, free-ranging cats will consume several thousand vertebrates per year, including both native and non-native species.

2. Material and methods

2.1. Study area

The Ecological Park of Funchal (7.29 km^2; 470 to 1818 m asl) is situated on the subtropical Madeira Island (Portugal), ca. 900 km from mainland Portugal and 600 km from Morocco (Fig. 1). It is located north of the island's capital, and roughly half of its area is included in the Natura 2000 Network. The park is home to over 25 breeding bird species, of which three are endemic to Madeira (*Regulus madeirensis*, *Fringilla maderensis*, and *Columba trocaz*), three bat species, and to the endemic Madeira wall lizard (*Teira dugesii*). Furthermore, the park hosts the only known breeding colony of the Manx shearwater (*Puffinus puffinus*) in Madeira Island, a regionally threatened seabird, whose population size and ecology in Macaronesia is poorly known (Nunes et al., 2010; Rodríguez et al., 2020). In addition to cats, the list of invasive mammals inhabiting the park include black and Norway rats (*Rattus rattus* and *R. norvegicus*, respectively), domestic mice (*Mus musculus*), European rabbits (*Oryctolagus cuniculus*) and ferrets (*Mustela furo*) (Soto et al., 2023, 2024).

2.2. Faecal sample collection

Between July and November 2021, we randomly collected a total of 58 cat scats throughout the park (Fig. 1). Only fresh scats (deposited <1 day) were considered for this study, as we aimed to obtain relatively good quality DNA for the molecular analyses. Twenty-five samples were collected during the summer, and 33 during autumn. These were stored in clean plastic bags or individual vials with silica gel and then refrigerated at −20 °C until DNA extraction. To avoid contamination and amplification of non-target taxa as much as possible, morphological scatology was always performed after DNA extraction.

2.3. DNA extraction and library preparation

For the extraction of DNA, we used the Stool DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada). A subsample of ca. 2.5 cm was extracted, taking care not to sample the external surface, as this will have a high concentration of predator epithelial cells (Waits and Paetkau, 2005). Then, it was homogenised in 4 to 7 ml of PBS Solution, vortexed and centrifuged at room temperature for 30 s to 1 min. The remaining steps followed the kit's recommendations, except that the DNA was eluted twice. This resulted in two 50 μL elutions per sample and three extraction control samples, which were stored in plates at −20 °C until amplification.

To analyse the vertebrate component of the cat's diet, the V5 variable region of the mitochondrial 12S gene (73–110 bp) was amplified using the 12SV5F/12VS5R primers, and the Polymerase Chain Reaction (PCR) protocol from Plimpton et al. (2021). Both primers were modified to include Illumina adaptors, and a 0–5 bp shift made of Ns was added between the adaptor and the primer to increase sequencing diversity and quality. PCR negative controls were included in the amplification to check for potential contaminations. Amplification success and quality were checked by running the PCR products in 2 % agarose gels stained with GelRed.

The library preparation began by conducting an initial PCR clean-up, utilising Agencourt AM-Pure XP beads (Beckman Coulter, Brea, CA, USA), to eliminate primer dimer. Subsequently, an indexing PCR was performed to accurately label each amplified product. The protocol described in Rato et al. (2022) was used to perform the PCR indexing. To eliminate any remaining primer dimer, nucleotides and enzymes that could interfere with the sequencing reaction, a second bead clean-up of

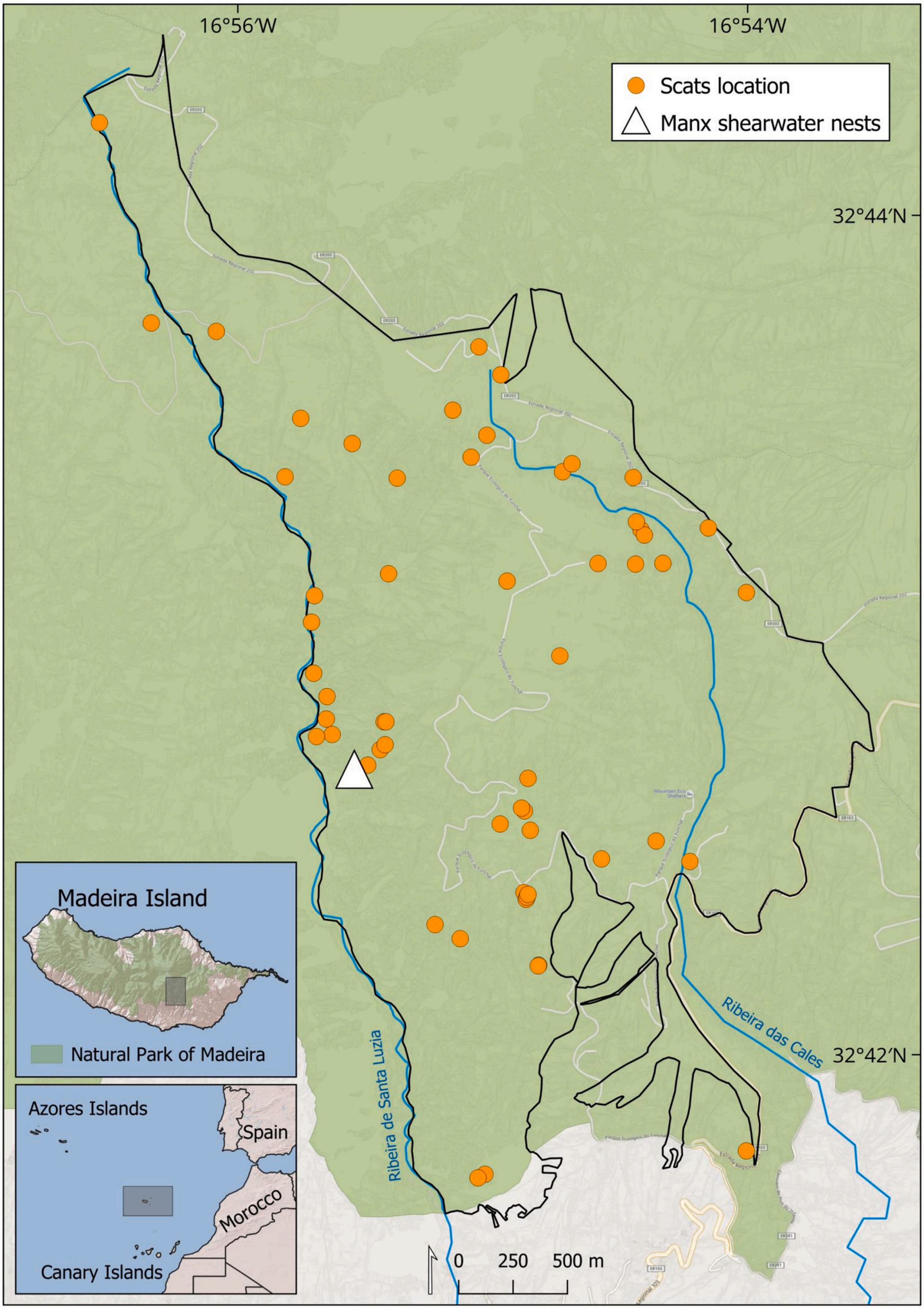

Fig. 1. Location of the Ecological Park of Funchal in Madeira Island, Portugal. The distribution of cat scats collected is shown with orange dots. The white triangle indicates the location of the only known nesting colony of Manx shearwaters (*Puffinus puffinus*) in the Madeira Archipelago.

the PCR products was carried out. Following these procedures, all purified PCR products were quantified using Epoch, normalised to 20 nM, and pooling all samples. From the purified and normalised pool, the library was quantified using qPCR (KAPA Library Quant Kit qPCR Mix; Bio-Rad iCycler) and then diluted to 4 nM. Ultimately, the library was equimolarly pooled and sequenced using a MiSeq Reagent Micro Kit v2 (300 cycles) to achieve an expected average of 25,000 paired-end reads per sample-marker combination.

2.4. *Bioinformatic pipeline*

Initially, the paired-end reads were aligned using PEAR (Zhang et al., 2013), where base-pairs with q-scores below 26 were discarded. Subsequently, reads were assigned to the respective sample, and primer sequences were eliminated employing the *ngsfilter* command from OBITools (Boyer et al., 2016) allowing a maximum of four mismatches to the expected primer sequence. Reads were then de-replicated into unique sequences or exact sequence variants (ESVs), and singletons were excluded using the *obiuniq* command. Fragments <73 bp (as in Plimpton et al., 2021) and those occurring ≤10 times were removed using the *obigrep* command. This command was then used to enhance data accuracy by eliminating potentially spurious sequences with an 'r' level of one. An Operational Taxonomic Unit (OTU) table was produced using the *obiannotate* function. Ultimately, the *–usearch_global* command from VSEARCH (Rognes et al., 2016) was employed to create a matchlist containing all internal matches of OTUs. The obtained OTU table and sequences were further cleaned using the R package LULU to remove potential mtDNA nuclear copies, persisting PCR and sequencing errors. ESVs with a read count <1 % of the total reads in each PCR product were removed (Mata et al., 2016), aiming to eliminate remaining PCR and sequencing errors that had passed the *obiclean* denoising step. Subsequently, reads identified in the extraction and PCR negative controls were subtracted from the respective sample batch (Evans et al., 2021).

We used NCBI database's BLAST function to taxonomically classify the OTUs. Sequences displaying >90 % similarity were categorised only at the class level, while those with 90–95 % similarity were classified at the family level. Sequences exhibiting <95 % similarity were identified at the species or genus level. In situations with multiple potential matches at the genus or species level, OTU were assigned based on the species/genus known presence in Madeira (Borges et al., 2008). For ESVs that could not be determined to the species level, a neighbour-joining tree was constructed using Geneious Prime software (Drummond et al., 2010). Subsequently, a visual inspection of the alignment was conducted, looking for co-occurrence patterns among similar ESVs to cluster them (~ 98 %) into distinct taxa, such as *Rattus* sp.1, *Rattus* sp.2, and so on, also known as Molecular Operational Taxonomic Units (MOTUs). After this, taxa that did not belong to the animal kingdom, as well as mammals (such as cats, dogs, and humans), were excluded.

2.5. *Morphological scatology*

Following Medina et al. (2010), scats were saturated in water, broken and inspected under the stereo microscope. Prey remains (e.g., bones, hair, feathers, scales, and exoskeletons), were extracted using tweezers. These were cross-referenced with reference collections and literature sources (Teerink, 2003) and identified to the lowest taxonomic level possible. Identification was complemented by specialist consultation whenever necessary. The number of occurrences of each specific prey category was recorded, considering the minimum number of individuals for each taxon, given the highly fragmented nature of the prey remains. All avian remains outside the passerine category were collectively classified as "non-Passeriformes". Non-Passeriformes OTUs were considered introduced species, as non-native partridges are the most abundant non-passerine birds in the park and, due to their behaviour, are particularly susceptible to cat predation.

2.6. *Statistical analyses*

Dietary analyses were performed at the order, family and OTU levels, with the latter referring to the most resolved taxonomic unit, even if the classification was limited to the family or order level. OTUs were categorised into two primary groups: wild and anthropogenic food, the latter encompassing pet food or food remnants (Tables S1, S2). The relationship between the amount of ingested wild and anthropogenic food was assessed through a linear regression, using the *lm* function from R (R Core Team, 2022).

We used General Linear Models (GLMs) to evaluate how the average number of prey taxa detected per faecal sample (i.e., richness) was influenced by season and diet type (wild or anthropogenic). When using season as the predictor, we excluded taxa associated with anthropogenic food as our objective was to determine if the richness of wild prey changed between seasons. For this, we employed the *glm* function with a Poisson distribution. We then used the *anova* function from the *car* package to assess the significance of the model. For morphological analyses, we only used season as an explanatory variable since, in this method, it was often challenging to identify prey taxa originating from anthropogenic food sources.

To calculate dietary niche-width across different seasons and methods (i.e., molecular versus morphological), we built prey rarefaction and extrapolation curves using the package *iNEXT*. These analyses were conducted with incidence frequencies of prey taxa. We compared the estimated richness considering completeness (i.e., sample coverage) instead of sample size (i.e., number of samples), to avoid biases of communities with different levels of richness requiring different sampling efforts to be sufficiently characterised (Chao and Jost, 2012). We considered that differences were significant if the 84 % confidence interval (a proxy $\alpha = 0.05$) of both estimates did not overlap, as the use of a 95 % confidence interval is a very conservative approach (MacGregor-Fors and Payton, 2013).

Permutational multivariate analysis of variance (PERMANOVA) was used to compare the diet composition between seasons in both methods, as well as the diet composition between methods with the function *adonis* from the *vegan* package. First, presence/absence of each prey in each sample was used to build a Jaccard dissimilarity matrix using the *vegdist* function. A homogeneity of dispersion test (function *betadisper*) was carried out to assess if the observed differences in PERMANOVA could be due to unequally dispersed values across the different groups (Anderson, 2006). Finally, the command *simper* was used to identify the prey items that contributed the most to dietary composition disparities across seasons and methods. The comparison of diet composition between the two methods was conducted exclusively at the order taxonomic level. This limitation occurred because many of the prey items in the morphological analysis could not be classified beyond the order level due to the absence of assignable species-specific prey remains.

The frequency of occurrence (FO) of each food item was calculated as the number of occurrences of the diet item (limited to a maximum of 1 occurrence per sample) divided by the total number of sequenced cat scats for molecular analyses. In the morphological analysis, the number of occurrences was divided by the total number of scats with prey remains. The FO for diet type (wild or anthropogenic) was calculated based on the results of the molecular analyses. Additionally, the FO for prey type (native or non-native) was determined for each approach.

2.7. *Estimation of annual prey intake*

To estimate the annual prey intake of free-ranging cats across the park, we combined the morphological and molecular datasets and counted the number of scat samples containing each of the OTUs. Because DNA metabarcoding is poorly suited for estimating quantitative data (Lamb et al., 2019), and since we could not reliably assess the number of individuals of each prey consumed per meal through morphological analysis, we adopted a conservative approach, assuming that each OTU appeared only once per sample. Hence, the total number of OTUs corresponded to the number of samples containing those OTUs.

The weights of the different prey species were extracted from the literature (mammals - Jones et al., 2009; *Tarentola mauritanica* - Martins et al., 2022; *Teira dugesii* - Rato et al., 2022; birds - Tobias et al., 2022) and used to calculate the ingested biomass. For each OTU, the total biomass was calculated by multiplying the weight of each prey by the number of samples. For large prey such as rabbits (*Oryctolagus cuniculus*), the biomass applied was 170 g, since they constitute more than the daily food intake by cats (Fitzgerald and Karl, 1979; Medina et al., 2010). Invertebrates (i.e., Orthoptera) were not considered in the calculation of biomass due to their minimal contribution in weight to the diet (Medina and Nogales, 2009). The percentage of biomass (%W) was calculated as the biomass of the OTU x 100 and divided by the total consumed biomass.

The annual prey intake of cats in the Ecological Park of Funchal was estimated based on a daily intake of 170 g (Fitzgerald and Karl, 1979; Medina et al., 2010). Additionally, following previous studies, a year was considered to have 365.25 days (Blancher, 2013; Loss et al., 2013; Woinarski et al., 2017, 2018). Therefore, the yearly biomass ingested by a single cat was calculated by multiplying 365.25 days by the daily intake (i.e., 62,092.5 g). The number of consumed prey was determined from the percentage of biomass (%W) each prey item contributed to the diet. This was done by multiplying (%W/100) by the annual biomass intake of a cat and then dividing this number by the weight of each prey item.

Between August and November 2021, free-ranging cat density was estimated by dividing the park into a 1 × 1 km grid and sampling each grid with three cameras over 16 trap-nights. Photographed cats were identified and their density per km^2 was estimated using spatial explicit capture–recapture models (see Soto et al. (2023) for additional details). The calculation of the annual prey intake was based on a mean estimated population size of 36 cats (95 % confidence interval: 25 to 55; Soto et al., 2023). To assess the minimum, mean, and maximum annual prey intake for the entire cat population, we multiplied the yearly biomass ingested by a single cat by each of the three estimated population sizes.

3. Results

3.1. Overall diet description

A total of 20 OTUs were identified using both methods, spanning five classes and ten orders (Table 1). Prey items from the orders Artiodactyla, Anseriformes, and Scombriformes, as well as chicken (*Gallus gallus*) from the Galliformes order, were likely derived from anthropogenic resources (such as pet food or food remnants) (Tables S1, S2). All Passeriformes and Orthoptera were considered native species, while Squamata included one introduced (*Tarentola mauritanica*) and one native species (*Teira dugesii*). Conversely, all prey from the orders Rodentia, Lagomorpha, and Testudines correspond to non-native species (Fig. 2).

3.2. Diet characterisation using DNA metabarcoding

DNA was extracted and amplified from all the collected samples. The libraries generated ca. 8 million raw sequence reads, which were reduced to 2038.749 reads during the bioinformatic processing and to 109 OTUs. Non-target amplification from diverse sources was observed in samples, extractions, and PCR negative controls representing 50.35 %

Table 1

Diet of free-ranging cats (*Felis catus*) on the Ecological Park of Funchal as revealed by molecular and morphological analyses. N – Number of occurrences of a particular prey taxon; FO – frequency of occurrence in total (%); S – frequency of occurrence in summer (%); A – frequency of occurrence in autumn (%).

	Molecular analyses ($n = 58$)				Morphological analyses ($n = 57$)			
	N	FO	S	A	N	FO	S	A
Mammalia	130	98,28	41,38	56,90	69	85,96	33,33	52,63
Rodentia	100	94,83	41,38	53,45	63	80,70	29,82	50,88
Rattus sp.	–	–	–	–	30	52,63	14,04	38,60
Rattus sp.2	6	10,34	0	10,34	–	–	–	–
Rattus rattus	42	72,41	25,86	46,55	–	–	–	–
Rattus norvegicus	7	12,07	10,34	1,72	–	–	–	–
Mus musculus	45	77,59	34,48	43,10	33	57,89	24,56	33,33
Lagomorpha	15	25,86	15,52	10,34	6	10,53	3,51	7,02
Oryctolagus cuniculus	15	25,86	15,52	10,34	6	10,53	3,51	7,02
Artiodactyla	15	24,14	13,79	10,34	–	–	–	–
Bos sp.1	1	1,72	1,72	0	–	–	–	–
Sus scrofa	14	24,14	13,79	10,34	–	–	–	–
Aves	55	56,90	31,03	25,86	21	33,33	17,54	15,79
Passeriformes	29	37,93	18,97	18,97	18	31,58	15,79	15,79
Sylvia sp.1	4	6,90	6,90	0	–	–	–	–
Sylvia atricapilla	14	24,14	12,07	12,07	–	–	–	–
Erithacus rubecula	11	18,97	10,34	8,62	1	1,75	0	1,75
Non Passeriformes	–	–	–	–	2	3,51	3,51	0
Galliformes	24	32,76	22,41	10,34	–	–	–	–
Phasianidae 1	1	1,72	0	1,72	–	–	–	–
Alectoris rufa	13	22,41	17,24	5,17	–	–	–	–
Gallus gallus	10	17,24	8,62	8,62	–	–	–	–
Anseriformes	2	3,45	0	3,45	–	–	–	–
Cairina moschata	2	3,45	0	3,45	–	–	–	–
Reptilia	21	34,48	18,97	15,52	26	45,61	28,07	17,54
Squamata	10	17,24	10,34	6,90	26	45,61	28,07	17,54
Teira dugesii	9	15,52	10,34	5,17	26	45,61	28,07	17,54
Tarentola mauritanica	1	1,72	0	1,72	–	–	–	–
Testudines	11	18,97	8,62	10,34	–	–	–	–
Pelusios castaneus	11	18,97	8,62	10,34	–	–	–	–
Actinopteri	5	8,62	0	8,62	–	–	–	–
Scombriformes	5	8,62	0	8,62	–	–	–	–
Thunnus sp.	5	8,62	0	8,62	–	–	–	–
Insecta	–	–	–	–	21	36,84	12,28	15,79
Orthoptera	–	–	–	–	21	36,84	12,28	15,79
Total	211				137			

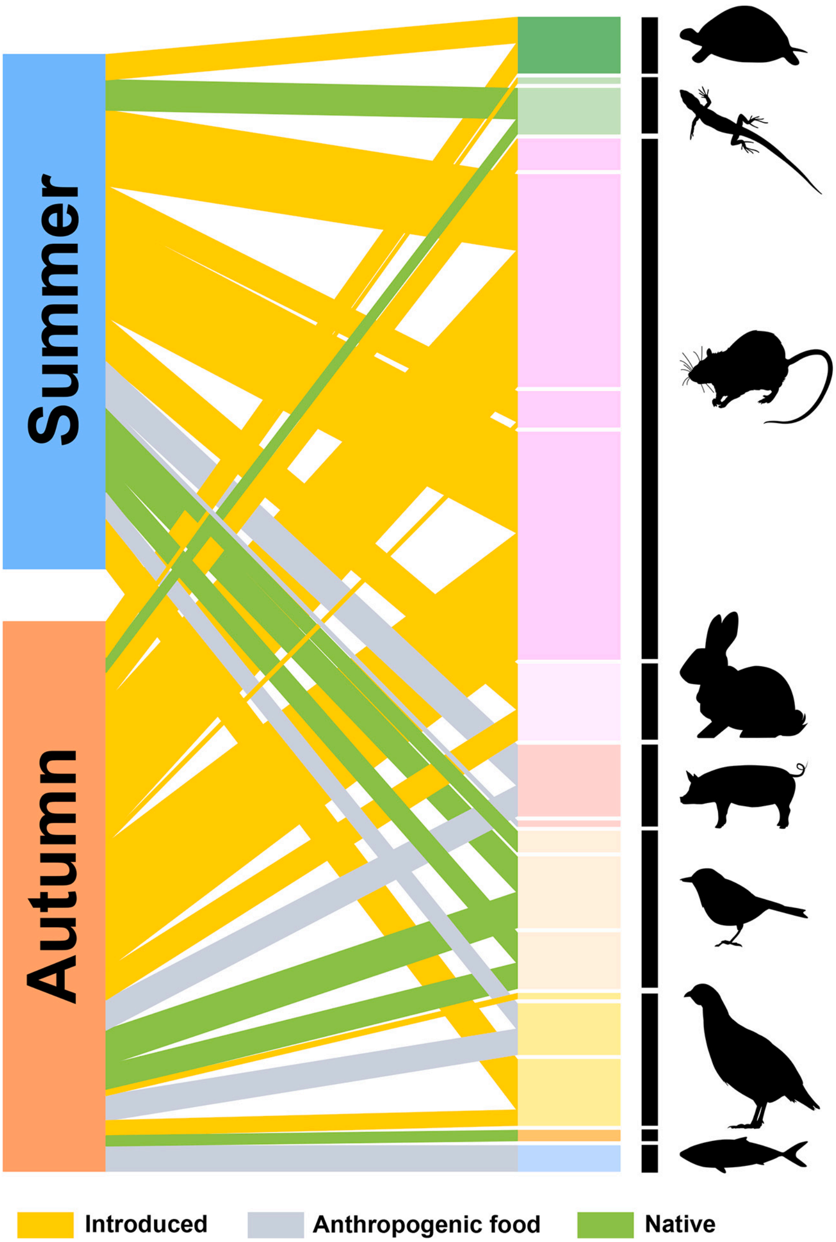

Fig. 2. Trophic network based on DNA metabarcoding, displaying the Operational Taxonomic Units (OTUs) consumed by free-ranging cats (*Felis catus*) in the Ecological Park of Funchal, Madeira Island, Portugal. Link width between seasons (summer, above; autumn, below) and their prey detection is proportional to its frequency of occurrence in scats samples. White lines separate the different OTUs, and various colours indicate different orders. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

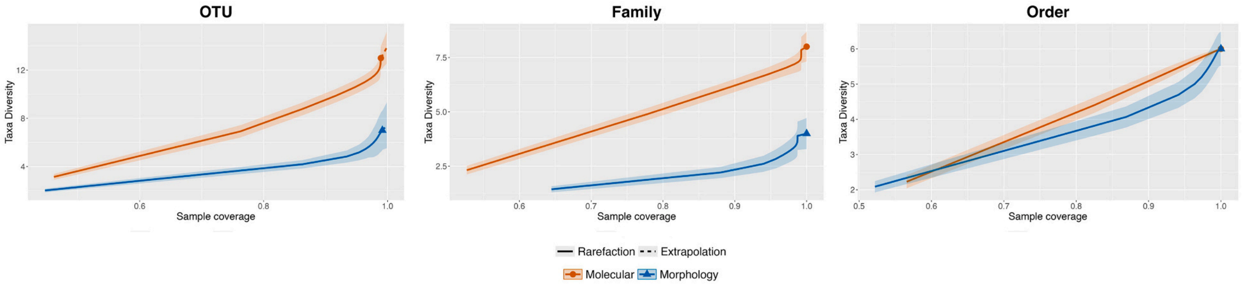

Fig. 3. Rarefactions curves for the two methods at different taxonomic levels. Showing the observed (full line) and estimated (dashed line) richness, and respective 84 % confidence interval by sample coverage.

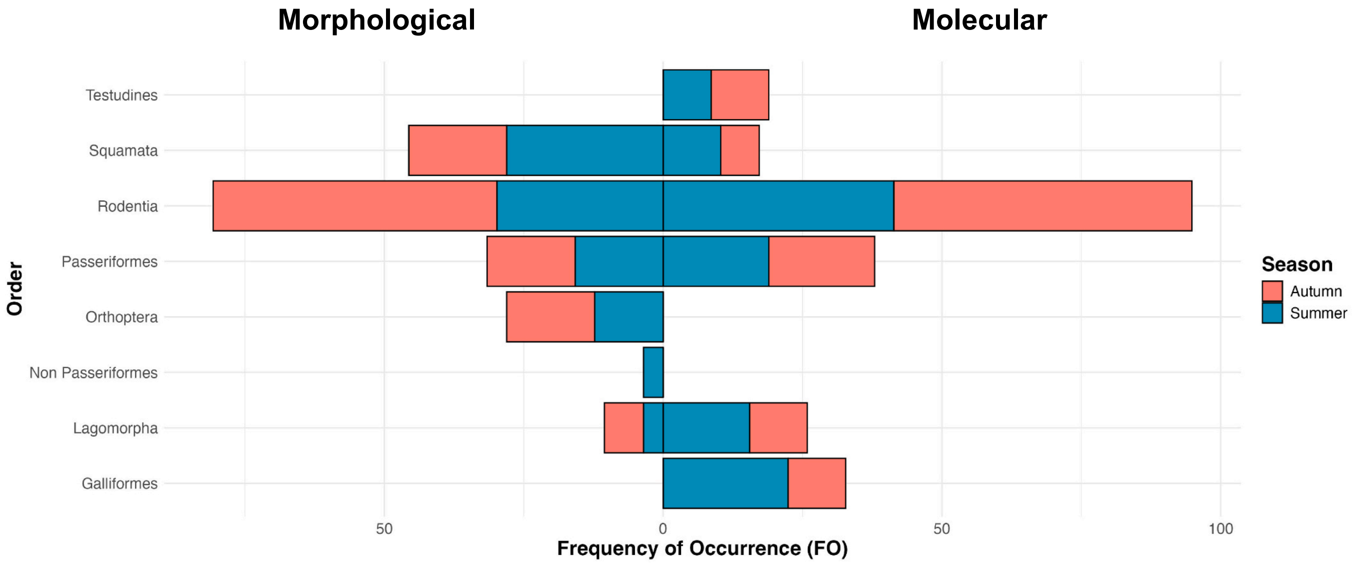

Fig. 4. Comparison of the frequencies of occurrence of the different Orders identified using morphological (left) and molecular methods (right), divided by Season. For exact frequency of occurrence values, see Table 1.

of the total reads, which were removed from the final diet dataset. Bacteria represented most of the non-target OTU diversity (44 %). A significant portion of cat reads was also detected, with a FO of 26.67 %. Additionally, 9.87 % of the reads belonged to humans. After applying negative controls, removing singletons, replicates, and filtering taxa, the final dataset of cat diet consisted of 910,517 reads and 18 OTUs. The 18-vertebrate prey OTUs identified belong to four classes, nine orders, 12 families, 14 genera, and 13 species (Table 1). Rodentia (94.83 %) was the most prevalent order, followed by Passeriformes (37.93 %), Galliformes (32.76 %), Lagomorpha (25.86 %), Artiodactyla (24.14 %), Testudines (18.97 %), Squamata (17.24 %), Scombriformes (8.62 %), and Anseriformes (3.45 %). Anthropogenic food was present in 37.93 % of the scats, while wild prey in 98.28 %, with only one sample not containing wild prey. Excluding taxa derived from anthropogenic sources reduces the total number of OTUs to 13. Native prey species were present in 43.86 % of the samples, whereas non-native species were identified in all the samples. No invertebrates were detected in this analysis (12SV5 primers are specific for vertebrate DNA).

Prey richness was statistically higher within wild prey in comparison to anthropogenic food, at all taxonomic levels (order: $p < 0.05$; family: p < 0.05; OTU: $p < 0.01$; Fig. S1). However, we found no relationship between the diversity of consumed wild food and anthropogenic trophic items ($R^2 = 0.05$, $p = 0.08$).

3.3. Diet characterisation using morphological scatology

Of 58 examined faecal samples, prey remains were detected in 57. We identified 8 OTUs (Tables 1, S2) from four classes, six orders, four families, five genera, and four species. Rodentia was the most frequently detected order (80.70 %), followed by Squamata (45.61 %), Orthoptera (36.84 %), Passeriformes (31.58 %), Lagomorpha (10.53 %) and non-Passeriformes (3.51 %). At the genus level, we could discern only five taxa, with *Mus* being the most predominant, followed by *Rattus*, *Teira*, *Oryctolagus* and *Erithacus*. Native and non-native prey were identified in 77.19 % and 85.96 % of the scats, respectively. Some samples contained multiple non-native or native taxa, resulting in 71 occurrences of non-native prey and 65 occurrences of native prey.

3.4. Method-associated differences in the richness and composition of cat diet

DNA metabarcoding revealed greater prey diversity compared to morphological scatology, especially at the OTU and family levels. However, the two approaches revealed similar niche breadths at the order level (Fig. 3). Detection method significantly affected diet composition at the order level ($p < 0.01$) and the Simper analysis revealed that composition differences were due to five orders (Fig. 4; Table 1): Squamata was more frequently detected by morphological than by molecular methods ($p < 0.05$; 45.61 % vs. 17.24 %), and, as anticipated due to the use of vertebrate-specific primers, Orthoptera was detected only via morphological scatology ($p < 0.001$). Conversely, Rodentia was more frequently detected by molecular analyses ($p < 0.05$; 94.83 % vs 80.70 %), while Testudines ($p < 0.01$) and Galliformes ($p < 0.001$) were detected only by DNA metabarcoding.

3.5. Seasonal variation in the richness and composition of cat diet

Molecular and morphological analyses revealed higher prey diversity during summer than autumn, but differences were not statistically different (molecular - order: $p = 0.098$; family: $p = 0.053$; OTU: $p = 0.154$; morphological - order: $p = 0.592$; family: $p = 0.829$; OTU: $p = 0.898$; Fig. S2). Indeed, rarefaction curves showed overlapping niche breadths between seasons (Fig. 5), with high sample coverage (~100 %) indicating extensive prey identification for both seasons.

Seasonal effects on diet composition were significant only at the OTU level in molecular analyses (OTU: $p < 0.05$; family: $p = 0.11$; order: $p = 0.14$), though the homogeneity of multivariate dispersion test indicated this was due to a lack of homogeneity in group variances ($p < 0.05$). Simper analysis revealed higher consumption of Galliformes during summer compared to autumn ($p < 0.01$; Table 1; Fig. 4). At the OTU level, differences between seasons were attributed to four OTUs: *Rattus rattus* ($p < 0.05$) were more frequently detected in autumn, *Alectoris rufa* ($p < 0.01$) and *Rattus norvegicus* ($p < 0.05$) were more consumed during summer, and *Sylvia* sp.1 was only detected in summer ($p < 0.05$).

Morphological analyses revealed significant seasonal effects across OTU, family, and order (PERMANOVA results: OTU: $p < 0.05$; family: $p < 0.01$; order: $p < 0.05$). Simper analysis indicated higher consumption of Squamata in summer ($p < 0.01$; Fig. 4; Table 1) and increased Lacertidae frequency in summer ($p < 0.01$). Conversely, Muridae prevalence rose in autumn ($p < 0.01$). At the OTU level, *Teira dugesii* was more consumed in summer ($p < 0.01$), while *Rattus* sp. was more frequent in autumn ($p < 0.05$).

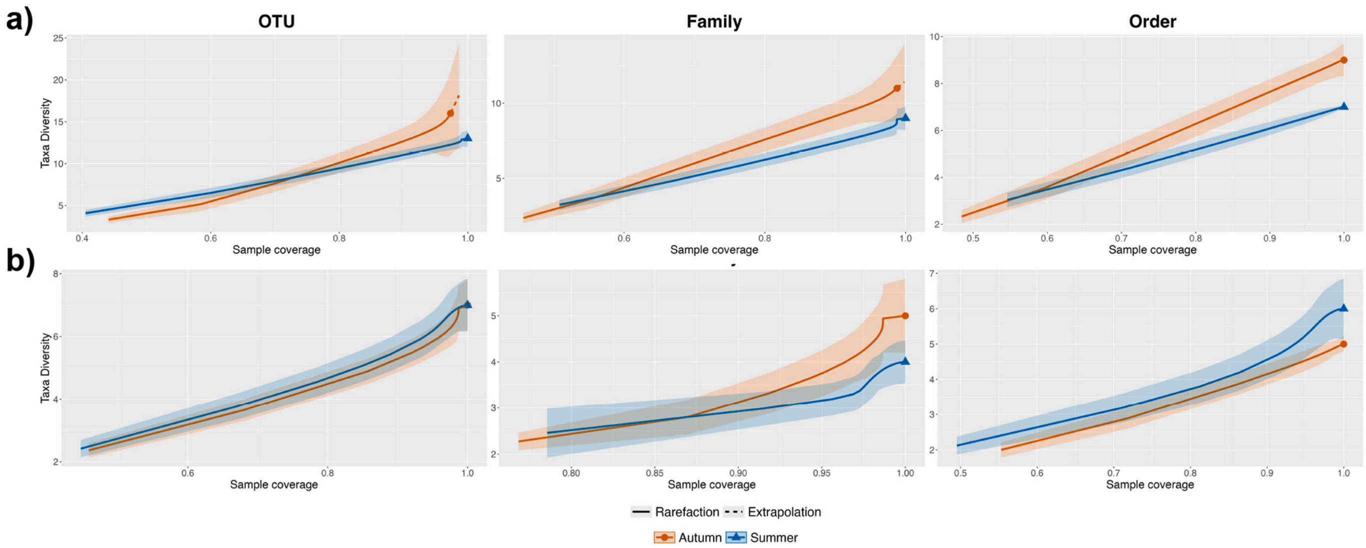

Fig. 5. Rarefactions curves for seasons at different taxonomic levels in (a) molecular and (b) morphological analyses. Showing the observed (solid line) and estimated (dashed line) richness, and respective 84 % confidence interval by sample coverage.

3.6. *Annual prey intake by free-ranging cats*

Each cat at the Ecological Park of Funchal is estimated to prey around 607 vertebrates annually (Table 2), including 380 mammals, 134 birds, and 93 reptiles. The average total consumption by the free-ranging cat population is estimated to be ~21,885 vertebrates annually (15,198 and 33,435 specimens, considering the 95 % confidence interval for cat densities). These correspond to a mean of 13,687 (9505–20,911) mammals, 4830 (3354–7380) birds, and 3367 (2338–5143) reptiles.

4. Discussion

Free-ranging cats are among the most harmful and widespread mammalian predators. Although a thorough understanding of their diet is crucial for a comprehensive assessment of their impacts, this is only the second study to use DNA metabarcoding to investigate their dietary habits, and the first to do so in an insular ecosystem.

4.1. *DNA metabarcoding vs. morphological scatology*

In line with previous research (Gil et al., 2020; Massey et al., 2021; Zarzoso-Lacoste et al., 2016), DNA metabarcoding yielded a higher number of prey taxa at both the species and genus levels, compared to morphological scatology. This enabled the identification of multiple prey taxa (e.g., *Erithaculus rubecula* and *Sylvia atricapilla*) not previously detected in the diet of cats in Madeira (Medina et al., 2010) and missed by our morphological analysis. Yet, the sole endemic lacertid to Madeira, *Teira dugesii*, was more frequently detected in morphological analyses than through DNA metabarcoding, suggesting that relying solely on the latter would underestimate its presence in the cats' diet. Since DNA metabarcoding is poorly suited for quantitative assessments (Lamb et al., 2019), morphological analysis provides a more accurate reflection of predation rates. The higher detection of lizards through morphological methods further indicates that a single cat can consume multiple reptiles per meal - consistent with previous findings of >8 adult lizards identified in a single cat regurgitation (Soto et al., 2023). On the other hand, the non-native gecko *Tarentola mauritanica*, whose population in Madeira has been expanding since the mid-1980s (Silva-Rocha et al., 2022), was only identified through DNA metabarcoding. Hence, these results highlight the benefits associated with using both methods (Gil et al., 2020; Plimpton et al., 2021).

4.2. *Cat diet in the Ecological Park of Funchal*

Our findings suggest that the free-ranging cats using this peri-urban protected area feed primarily on wild prey, but nonetheless have access to anthropogenic food (pet food or human food leftovers). Yet, we found no correlation between the incidence of anthropogenic food and the diversity of wild prey consumed. This is in line with previous studies (e.g., Hernandez et al., 2018; Plimpton et al., 2021) and suggests that the consumption of pet food or leftovers does not deter cats from hunting wild animals. Indeed, camera-trap data indicates that throughout the protected area cat activity was higher away from locations associated with anthropogenic food (e.g., picnic areas and rental accommodations; Soto et al., 2023; Fig. S3).

Non-native mammals were the most frequently detected group in our samples (94.83 %), followed by birds and reptiles (56.90 % and 34.48 %, respectively). This is consistent with findings by Medina et al. (2010) in mountainous areas of Madeira, as well as other studies conducted in the Canary Islands and Azores (e.g., Flores Ravelo and Rando Reyes, 2021; Hervías et al., 2014; Nogales and Medina, 2009). Nonetheless, our estimations indicate that a single cat can consume >90 *Teira dugesii* lizards per year, and that the entire cat population consumes >3000 reptiles annually (ca. 400 lizards per km^2/year). If combined with the >4000 passerines consumed per year (Table 2), and accounting that the protected area has only 7.5 km^2, this means that the cat population of the park consumes >1000 native vertebrates per km^2/year. However, this assumes one individual of each species per faecal sample. This is a significant underestimation, as demonstrated by a cat's regurgitation containing at least eight adult *Teira dugesii* and one mouse (Fig. S3). Using a similar method, but accounting for the minimum number of individuals per species in each sample based on repeated anatomical structures, Gómez-Alceste and Rando (2024) estimated that each cat in Teide National Park, Tenerife (Canary Islands, Spain), likely consumes >800 reptiles per year.

As in Medina et al. (2010), we did not find any evidence of seabird predation. Nonetheless, several studies have documented cat predation on seabirds in Madeira and other oceanic islands (including in Macaronesia), highlighting the significant negative impact of free-ranging

Table 2
Annual estimates of vertebrate consumption by individual cats and by the overall cat population based on the results of molecular and morphological dietary analyses and on the minimum, mean, and maximum cat population estimates for the Ecological Park of Funchal, Madeira Island, Portugal. These estimates assume that each cat consumes approximately 170 g of prey per day.

Class	Order	Family	OTU	Sample count	Mass of each prey (g)	Total mass (g)	%W	Intake per year/cat	Min. cat pop. (25)	Mean cat pop. (36)	Max. cat pop. (55)
Aves	Galliformes	Phasianidae	*Alectoris rufa*	13	527.86	6862.18	22.47	26.43	660.78	951.52	1453.71
			Phasianidae 1	1	812.86	812.86	2.66	2.03	50.83	73.19	111.82
	Non-Passeriformes		Non-Passeriformes	2	613.23	1226.47	4.02	4.07	101.66	146.39	223.65
	Passeriformes		Passeriformes	20	36.51	730.18	2.39	40.66	1016.58	1463.87	2236.47
		Muscicapidae	*Erithacus rubecula*	12	17.70	212.40	0.70	24.40	609.95	878.32	1341.88
		Sylviidae	*Sylvia atricapilla*	14	16.70	233.80	0.77	28.46	711.60	1024.71	1565.53
			Sylvia sp.1	4	13.70	54.80	0.18	8.13	203.32	292.77	447.29
Total birds				66	2038.57	10,132.69	33.18	134.19	3354.71	4830.78	7380.35
Mammalia	Lagomorpha	Leporidae	*Oryctolagus cuniculus*	21	170.00	3570.00	11.69	42.70	1067.41	1537.07	2348.29
	Rodentia	Muridae	*Mus musculus*	80	59.86	4788.96	15.68	162.65	4066.31	5855.49	8945.88
			Rattus norvegicus	7	283.00	1981.00	6.49	14.23	355.80	512.36	782.76
			Rattus rattus	42	75.57	3173.89	10.39	85.39	2134.81	3074.13	4696.59
			Rattus sp.	37	179.28	6633.52	21.72	75.23	1880.67	2708.16	4137.47
Total mammals				187	767.72	20,147.38	65.97	380.20	9505.00	13,687.20	20,911.00
Reptilia	Squamata	Lacertidae	*Teira dugesii*	45	5.63	253.17	0.83	91.49	2287.30	3293.71	5032.06
		Phyllodactylidae	*Tarentola mauritanica*	1	6.73	6.73	0.02	2.03	50.83	73.19	111.82
Total reptiles				46	12.36	259.90	0.85	93.53	2338.13	3366.90	5143.88
Total						30,539.97	100	607.91	15,197.83	21,884.88	33,435.24

%W is the percentage of biomass contributed by each prey item.

cats on seabird populations (Cook and Yalden, 1980; Hervías et al., 2013; Lamelas-Lopez et al., 2021; Medina et al., 2011). In our study, the absence of seabirds as prey, specifically the Manx shearwater (*Puffinus puffinus*), can be justified by the fact that sample collection did not occur during the nesting season or the time of fledging of this species (Nunes et al., 2010). This, combined with the reduced estimated population size of this species in Madeira (Rodríguez et al., 2020), might explain their absence in our samples. According to Bonnaud et al. (2011), seabirds can become the preferred prey item of insular cats during their breeding season. Indeed, the current low population of *P. puffinus* in Madeira might reflect the impacts of non-native mammals, including cats, which are likely to have been associated with island-level extirpation of *Puffinus* species (e.g., *Puffinus lherminieri* from Santa Luzia, Cabo Verde; Alho et al., 2022).

An intriguing finding from our molecular analysis was the detection of *Pelusios castaneus* in multiple samples (Table 1). This terrapin is not known to have any established populations in Madeira (Borges et al., 2008). However, the species has multiple non-native populations in oceanic islands (Guedes et al., 2023) and it is kept as a pet in Madeira, with at least one individual already found in the wild (Rocha, R.; pers. comm).

4.3. Seasonal dietary differences

Although we found no differences in niche breadth between summer and autumn, our results revealed differences in diet composition between seasons (e.g., *Rattus rattus* and *Mus musculus* were more frequent in autumn, while *Teira dugesii* was more prevalent during summer). This dietary pattern can be attributed to the opportunistic and generalist nature of cats, whose prey composition is generally proportional to local prey availability (e.g., Herrera et al., 2022b; Hervías et al., 2014; Krauze-Gryz et al., 2017; Plimpton et al., 2021; Seymour et al., 2020; Széles et al., 2018; van Heezik et al., 2010). Indeed, cats seem to alter their prey preference from small mammals to birds or lizards during periods of reduced availability of mammals (Peck et al., 2008; Széles et al., 2018). This behaviour can explain the prevalence of the native lizard *Teira dugesii* in the summer samples, as it matches the period of peak activity of *Teira dugesii* in Madeira (Pacheco, 2008). Additionally, the higher incidence of non-native mammals in autumn, and reptiles in summer months aligns with previous studies suggesting that the presence of non-native species adapted to cat predation (with high population growth and effective anti-predator strategies) can promote an increase in cat numbers and exacerbate their impact on native prey species through hyperpredation (Fig. S3; Courchamp et al., 1999; Ringler et al., 2015).

5. Conclusion

Our study underscores the advantages of integrating molecular and morphological methods in cat diet analysis, emphasising that while free-ranging cats prey on considerable numbers of non-native mammals, they also exert substantial pressure on native insular species. This highlights the critical need to assess the conservation impacts of free-ranging cats on endangered and geographically restricted species, especially considering emerging laws that are likely to augment their global impact (Carrete et al., 2022; Li et al., 2021).

CRediT authorship contribution statement

Alexandra Galão: Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation. **Elena J. Soto:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation. **João Nunes:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Nuno M. Pedroso:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Ricardo Rocha:**

Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Catarina Rato:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Funding

This project was funded by a National Geographic Society grant EC-64368R-20 to RR, Câmara Municipal do Funchal, and *PuffinusLife4Best*. CR is supported by a postdoctoral contract from Fundação para a Ciência e Tecnologia (FCT), Portugal (DL57/2016/CP1440/CT0005). Lab work was supported via national funds through FCT and EU funds European Regional Development Fund (ERDF) under the project PTDC/BIA-EVL/27958/2017—POCI-01-0145-FEDER-027958 (to CR). This work was further supported by National Funds through FCT under the Project UIDB/05183/2020 (to NP).

Declaration of competing interest

The authors confirm that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Acknowledgements

The authors would like to thank the Ecological Park of Funchal staff and Funchal's Natural History Museum for general support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2025.111089.

Data availability

Diet raw data was deposited on FigShare (https://figshare.com/s/909edc2e665392ab83b4).

References

Alho, M., Granadeiro, J.P., Rando, J.C., Geraldes, P., Catry, P., 2022. Characterization of an extinct seabird colony on the island of Santa Luzia (Cabo Verde) and its potential for future recolonizations. J. Ornithol. 163, 301–313.

Alves, J.C., dos Santos, Y., Guedes, P., Rocha, R., 2024. Reassessment of the conservation status of *Crocidura fingui*, a shrew endemic to Príncipe Island, Gulf of Guinea. Oryx 1–4.

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62, 245–253.

Blancher, P., 2013. Estimated number of birds killed by house cats (*Felis catus*) in Canada. Avian Conservation and Ecology 8.

Bonnaud, E., Medina, F.M., Vidal, E., Nogales, M., Tershy, B., Zavaleta, E., Donlan, C.J., Keitt, B., Le Corre, M., Horwath, S.V., 2011. The diet of feral cats on islands: a review and a call for more studies. Biol. Invasions 13, 581–603.

Borges, P.A.V., et al., 2008. A List of the Terrestrial Fungi, Flora and Fauna of Madeira and Selvagens Archipelagos. Secretaria Regional do Ambiente e dos Recursos Naturais do Governo Regional da Madeira, Funchal, Madeira.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., Coissac, E., 2016. obitools: a unix-inspired software package for DNA metabarcoding. Mol. Ecol. Resour. 16, 176–182.

Carrete, M., Clavero, M., Arrondo, E., Traveset, A., Bernardo-Madrid, R., Vilà, M., Blas, J., Nogales, M., Delibes, M., García-Rodríguez, A., 2022. Emerging laws must not protect stray cats and their impacts. Conservation Science and Practice 4, e12706.

Carrión, P.L., Valle, C.A., 2018. The diet of introduced cats on San Cristobal Island, Galapagos: cat feces as a proxy for cat predation. Mamm. Biol. 90, 74–77.

Chao, A., Jost, L., 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. Ecology 93, 2533–2547.

Cook, L., Yalden, D., 1980. A note on the diet of feral cats on Deserta Grande. Bocagiana 52, 1–4.

Courchamp, F., Langlais, M., Sugihara, G., 1999. Control of rabbits to protect island birds from cat predation. Biol. Conserv. 89, 219–225.

Courchamp, F., Chapuis, J.-L., Pascal, M., 2003. Mammal invaders on islands: impact, control and control impact. Biol. Rev. 78, 347–383.

Crowley, S.L., Cecchetti, M., McDonald, R.A., 2020. Our wild companions: domestic cats in the Anthropocene. Trends Ecol. Evol. 35, 477–483.

de Sousa, L.L., Silva, S.M., Xavier, R., 2019. DNA metabarcoding in diet studies: unveiling ecological aspects in aquatic and terrestrial ecosystems. Environ. DNA 1, 199–214.

Drummond, A.J., et al., 2010. Geneious [computer software]. Retrieved from. http://www.geneious.com/.

Evans, H.K., Bunch, A.J., Schmitt, J.D., Hoogakker, F.J., Carlson, K.B., 2021. High-throughput sequencing outperforms traditional morphological methods in Blue Catfish diet analysis and reveals novel insights into diet ecology. Ecol. Evol. 11, 5584–5597.

Fardell, L.L., Pavey, C.R., Dickman, C.R., 2023. Influences of roaming domestic cats on wildlife activity in patchy urban environments. Front. Ecol. Evol. 11.

Fitzgerald, A.M., Karl, B.J., 1979. Foods of feral house cats (*Felis catus* L.) in forest of the Orongorongo Valley, Wellington. New Zealand Journal of Zoology 6, 107–126.

Flores Ravelo, A.J., Rando Reyes, J.C., 2021. Trophic Ecology of Cats (*Felis catus*) in Montaña de Guaza: Implications for the Conservation of the Critically Endangered Giant Lizard of Tenerife (*Gallotia intermedia*).

Gil, V., Pinho, C.J., Aguiar, C.A., Jardim, C., Rebelo, R., Vasconcelos, R., 2020. Questioning the proverb 'more haste, less speed': classic versus metabarcoding approaches for the diet study of a remote island endemic gecko. PeerJ 8, e8084.

Gómez-Alceste, M., Rando, J.C., 2024. Shifts in the trophic ecology of feral cats in the alpine ecosystem of an oceanic island: implications for the conservation of native biodiversity. Mammal Research 69, 1–8.

Guedes, P., dos Santos, Y., Matilde, E., Alves, J., Rato, C., Rocha, R., 2023. Phylogenetic relationships of the West African mud turtle (*Pelusios castaneus*) on the islands of São Tomé and Príncipe, West Central Africa. Amphibia-Reptilia 44, 391–397.

Hernández, P., Rando, J., 2024. The number of pet cats (*Felis catus*) on a densely-populated oceanic island (Gran Canaria; Canary Archipelago) and its impact on wild fauna. J. Nat. Conserv. 79, 126587.

Hernandez, S.M., Loyd, K.A.T., Newton, A.N., Carswell, B.L., Abernathy, K.J., 2018. The use of point-of-view cameras (Kittycams) to quantify predation by colony cats (*Felis catus*) on wildlife. Wildl. Res. 45, 357–365.

Herrera, D.J., Cove, M.V., McShea, W.J., Decker, S., Flockhart, D.T.T., Moore, S.M., Gallo, T., 2022a. Spatial and temporal overlap of domestic cats (*Felis catus*) and native urban wildlife. Front. Ecol. Evol. 10.

Herrera, D., Cove, M., McShea, W., Flockhart, D., Decker, S., Moore, S., Gallo, T., 2022b. Prey selection and predation behavior of free-roaming domestic cats (*Felis catus*) in an urban ecosystem: implications for urban cat management. Biol. Conserv. 268, 109503.

Hervías, S., Henriques, A., Oliveira, N., Pipa, T., Cowen, H., Ramos, J.A., Nogales, M., Geraldes, P., Silva, C., de Ybáñez, R.R., Oppel, S., 2013. Studying the effects of multiple invasive mammals on Cory's shearwater nest survival. Biol. Invasions 15, 143–155.

Hervías, S., Oppel, S., Medina, F.M., Pipa, T., Díez, A., Ramos, J.A., Ruiz de Ybáñez, R., Nogales, M., 2014. Assessing the impact of introduced cats on island biodiversity by combining dietary and movement analysis. J. Zool. 292, 39–47.

Jones, K.E., Bielby, J., Cardillo, M., Fritz, S.A., O'Dell, J., Orme, C.D.L., Safi, K., Sechrest, W., Boakes, E.H., Carbone, C., 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology 90, 2648.

Krauze-Gryz, D., Żmihorski, M., Gryz, J., 2017. Annual variation in prey composition of domestic cats in rural and urban environment. Urban Ecosyst. 20, 945–952.

Lamb, P.D., Hunter, E., Pinnegar, J.K., Creer, S., Davies, R.G., Taylor, M.I., 2019. How quantitative is metabarcoding: a meta-analytical approach. Mol. Ecol. 28, 420–430.

Lamelas-Lopez, L., Pietrzak, M., Ferreira, M., Neves, V.C., 2021. Threats and conservation status of Common and Roseate terns *Sterna hirundo/S. dougallii* in the Azores: a case study for Terceira Island. Mar. Ornithol. 49, 301–309.

Lepczyk, C.A., Fantle-Lepczyk, J.E., Dunham, K.D., Bonnaud, E., Lindner, J., Doherty, T. S., Woinarski, J.C., 2023. A global synthesis and assessment of free-ranging domestic cat diet. Nat. Commun. 14, 7809.

Li, Y., Wan, Y., Shen, H., Loss, S.R., Marra, P.P., Li, Z., 2021. Estimates of wildlife killed by free-ranging cats in China. Biol. Conserv. 253, 108929.

Lino, S., Rossa, M., Fernandes, J.M., Barros, T., Lino, A., Hipólito, D., Ferreira, E., Aliácar, S.C., Cadete, D., Fonseca, C., Torres, R.T., Rosalino, L.M., Carvalho, J., 2023. Dog in sheep's clothing: livestock depredation by free-ranging dogs may pose new challenges to wolf conservation. Eur. J. Wildl. Res. 69, 107.

Loss, S.R., Will, T., Marra, P.P., 2013. The impact of free-ranging domestic cats on wildlife of the United States. Nat. Commun. 4, 1396.

Loss, S.R., Boughton, B., Cady, S.M., Londe, D.W., McKinney, C., O'Connell, T.J., Riggs, G.J., Robertson, E.P., 2022. Review and synthesis of the global literature on domestic cat impacts on wildlife. J. Anim. Ecol. 91, 1361–1372.

MacGregor-Fors, I., Payton, M.E., 2013. Contrasting diversity values: statistical inferences based on overlapping confidence intervals. PloS One 8, e56794.

Magle, S.B., Crowther, M.S., 2023. Ecological impacts of domestic cat activity on wildlife. Front. Ecol. Evol. 11.

Martins, B., Silva-Rocha, I., Rocha, R., Mata, V.A., Gonçalves, Y., Rato, C., 2022. Trophic interactions of an invasive gecko in an endemic-rich oceanic island: insights using DNA metabarcoding. Front. Ecol. Evol. 10, 1044230.

Massey, A.L., Roffler, G.H., Vermeul, T., Allen, J.M., Levi, T., 2021. Comparison of mechanical sorting and DNA metabarcoding for diet analysis with fresh and degraded wolf scats. Ecosphere 12, e03557.

Mata, V.A., Amorim, F., Corley, M.F.V., McCracken, G.F., Rebelo, H., Beja, P., 2016. Female dietary bias towards large migratory moths in the European free-tailed bat (*Tadarida teniotis*). Biol. Lett. 12, 20150988.

McLennan, E.A., Wise, P., Lee, A.V., Grueber, C.E., Belov, K., Hogg, C.J., 2022. DNA metabarcoding reveals a broad dietary range for Tasmanian devils introduced to a naive ecosystem. Ecol. Evol. 12, e8936.

Medina, F.M., Nogales, M., 2009. A review on the impacts of feral cats (*Felis silvestris catus*) in the Canary Islands: implications for the conservation of its endangered fauna. Biodivers. Conserv. 18, 829–846.

Medina, F.M., Oliveira, P., Menezes, D., Teixeira, S., García, R., Nogales, M., 2010. Trophic habits of feral cats in the high mountain shrublands of the Macaronesian islands (NW Africa, Atlantic Ocean). Acta Theriol. 55, 241–250.

Medina, F.M., Bonnaud, E., Vidal, E., Tershy, B.R., Zavaleta, E.S., Josh Donlan, C., Keitt, B.S., Le Corre, M., Horwath, S.V., Nogales, M., 2011. A global review of the impacts of invasive cats on island endangered vertebrates. Glob. Chang. Biol. 17, 3503–3510.

Medina, F.M., Melo, T., Oliveira, P., Nogales, M., Geraldes, P., 2021. Trophic ecology of an introduced top predator (*Felis catus*) on a small African oceanic islet (Santa Luzia, Cabo Verde Islands). Afr. J. Ecol. 59, 88–98.

Moseby, K., Peacock, D., Read, J., 2015. Catastrophic cat predation: a call for predator profiling in wildlife protection programs. Biol. Conserv. 191, 331–340.

Nogales, M., Medina, F.M., 2009. Trophic ecology of feral cats (*Felis silvestris* f. *catus*) in the main environments of an oceanic archipelago (Canary Islands): an updated approach. Mammalian Biology 74, 169–181.

Nunes, J., Nunes, M., Fagundes, A.I., Valkenburg, T., 2010. Contributo para a conservaçao do Fura-bucho-do-atlântico *Puffinus puffinus*, uma espécie ameaçada na ilha da Madeira. Airo 20, 12–21.

Pacheco, C.S.D.C.P., 2008. Densidade populacional de *Teira dugesii* e escolha de substratos nas principais séries de vegetação na Ilha da Madeira. Universidade da Madeira, Portugal.

Peck, D., Faulquier, L., Pinet, P., Jaquemet, S., Le Corre, M., 2008. Feral cat diet and impact on sooty terns at Juan de Nova Island, Mozambique Channel. Anim. Conserv. 11, 65–74.

Plimpton, L.D., Henger, C.S., Munshi-South, J., Tufts, D., Kross, S., Diuk-Wasser, M., 2021. Use of molecular scatology to assess the diet of feral cats living in urban colonies. J. Urban Ecol. 7.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rato, C., Dellinger, T., Carretero, M.A., 2022. Dietary variation is driven by landscape heterogeneity in an insular omnivorous endemic lizard, revealed by DNA metabarcoding. Diversity 14. https://doi.org/10.3390/d14121078.

Ringler, D., Russell, J.C., Le Corre, M., 2015. Trophic roles of black rats and seabird impacts on tropical islands: mesopredator release or hyperpredation? Biol. Conserv. 185, 75–84.

Rocha, R., 2015. Look what the cat dragged in: *Felis silvestris catus* as predators of insular bats and instance of predation on the endangered *Pipistrellus maderensis*. Basbastella 8 (1), 8–11.

Rodríguez, A., Rodríguez, B., Montelongo, T., Garcia-Porta, J., Pipa, T., Carty, M., Danielsen, J., Nunes, J., Silva, C., Geraldes, P., Medina, F.M., Illera, J.C., 2020. Cryptic differentiation in the Manx shearwater hinders the identification of a new endemic subspecies. J. Avian Biol. 51.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4.

Russell, J.C., Kueffer, C., 2019. Island biodiversity in the Anthropocene. Annu. Rev. Env. Resour. 44, 31–60.

Russell, J.C., Meyer, J.-Y., Holmes, N.D., Pagad, S., 2017. Invasive alien species on islands: impacts, distribution, interactions and management. Environ. Conserv. 44, 359–370.

Russell, J.C., Abrahão, C.R., Silva, J.C., Dias, R.A., 2018. Management of cats and rodents on inhabited islands: an overview and case study of Fernando de Noronha, Brazil. Perspectives in Ecology and Conservation 16, 193–200.

Schenk, J., Kleinbölting, N., Traunspurger, W., 2020. Comparison of morphological, DNA barcoding, and metabarcoding characterizations of freshwater nematode communities. Ecol. Evol. 10, 2885–2899.

Seymour, C.L., Simmons, R.E., Morling, F., George, S.T., Peters, K., O'riain, M.J., 2020. Caught on camera: the impacts of urban domestic cats on wild prey in an African city and neighbouring protected areas. Global Ecology and Conservation 23, e01198.

Silva-Rocha, I., Santos, J.M., Rocha, R., Rato, C., 2022. Bioclimatic and local drivers modulating the expansion of an introduced temperate reptile in a subtropical island. Global Ecology and Conservation 37, e02164.

Soto, E.J., Nunes, J., Nóbrega, E., Palmeirim, A.F., Rocha, R., 2023. Density and ecological drivers of free-ranging cat abundance and activity in Madeira Island, Macaronesia. Conservation Science and Practice 5, e13040.

Soto, E.J., Nóbrega, E., Nascimento, P., Abrahams, E., Powell, K., Bellmunt Ribas, A., Silva, I., Gomes, M., Rodrigues, J.M., Nunes, J., 2024. Widespread population of invasive ferrets *Mustela furo* (Carnivora: Mustelidae) on the island of Madeira, Macaronesia. Mammalia 88, 311–315.

Széles, G.L., Purger, J.J., Molnár, T., Lanszki, J., 2018. Comparative analysis of the diet of feral and house cats and wildcat in Europe. Mammal Research 63, 43–53.

Szentivanyi, T., Oedin, M., Rocha, R., 2024. Cat–wildlife interactions and zoonotic disease risk: a call for more and better community science data. Mammal Rev. 54, 93–104.

Teerink, B., 2003. Hair of West European Mammals: Atlas and Identification Key. Cambridge University Press.

Tobias, J.A., et al., 2022. AVONET: morphological, ecological and geographical data for all birds. Ecol. Lett. 25, 581–597.

Trouwborst, A., McCormack, P.C., Martínez Camacho, E., 2020. Domestic cats and their impacts on biodiversity: a blind spot in the application of nature conservation law. People and Nature 2, 235–250.

van Heezik, Y., Smyth, A., Adams, A., Gordon, J., 2010. Do domestic cats impose an unsustainable harvest on urban bird populations? Biol. Conserv. 143, 121–130.

Waits, L.P., Paetkau, D., 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J. Wildl. Manag. 69, 1419–1433.

Woinarski, J., Murphy, B.P., Legge, S., Garnett, S.T., Lawes, M., Comer, S., Dickman, C. R., Doherty, T.S., Edwards, G., Nankivell, A., 2017. How many birds are killed by cats in Australia? Biol. Conserv. 214, 76–87.

Woinarski, J.C.Z., Murphy, B.P., Palmer, R., Legge, S.M., Dickman, C.R., Doherty, T.S., Edwards, G., Nankivell, A., Read, J.L., Stokeld, D., 2018. How many reptiles are killed by cats in Australia? Wildl. Res. 45, 247–266.

Woo, C., Kumari, P., Eo, K.Y., Lee, W.-S., Kimura, J., Yamamoto, N., 2023. Combining vertebrate mitochondrial 12S rRNA gene sequencing and shotgun metagenomic sequencing to investigate the diet of the leopard cat (*Prionailurus bengalensis*) in Korea. PloS One 18, e0281245.

Zarzoso-Lacoste, D., Bonnaud, E., Corse, E., Gilles, A., Meglecz, E., Costedoat, C., Gouni, A., Vidal, E., 2016. Improving morphological diet studies with molecular ecology: an application for invasive mammal predation on island birds. Biol. Conserv. 193, 134–142.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2013. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30, 614–620.