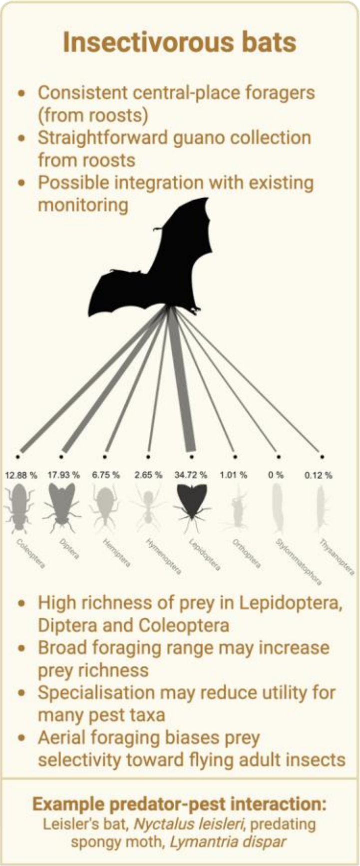
Evaluating the potential of predator diet analysis as a monitoring tool for plant pests of concern

Project Final Report

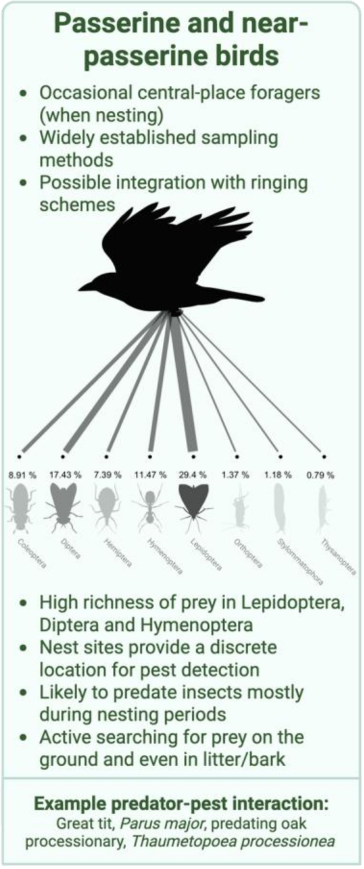


Insectivorous bats

- Consistent central-place foragers (from roosts)
- Straightforward guano collection from roosts
- Possible integration with existing monitoring

- High richness of prey in Lepidoptera, Diptera and Coleoptera
- Broad foraging range may increase prey richness
- Specialisation may reduce utility for many pest taxa
- Aerial foraging biases prey selectivity toward flying adult insects

Example predator-pest interaction:
Leisler's bat, *Nyctalus leisleri*, predating spongy moth, *Lymantria dispar*

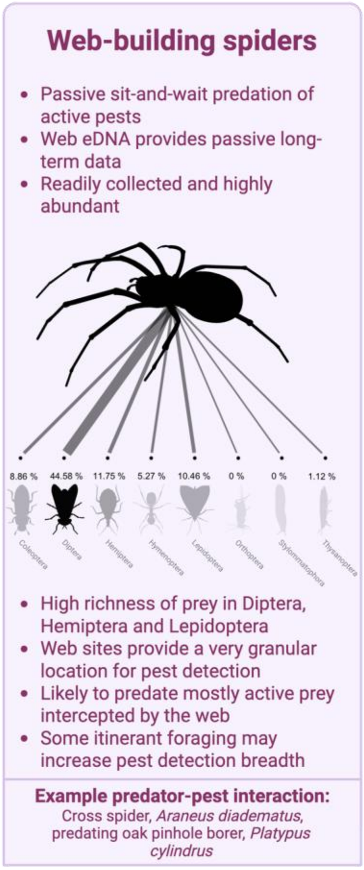


Passerine and near-passerine birds

- Occasional central-place foragers (when nesting)
- Widely established sampling methods
- Possible integration with ringing schemes

- High richness of prey in Lepidoptera, Diptera and Hymenoptera
- Nest sites provide a discrete location for pest detection
- Likely to predate insects mostly during nesting periods
- Active searching for prey on the ground and even in litter/bark

Example predator-pest interaction:
Great tit, *Parus major*, predating oak processionary, *Thaumetopoea processionea*



Web-building spiders

- Passive sit-and-wait predation of active pests
- Web eDNA provides passive long-term data
- Readily collected and highly abundant

- High richness of prey in Diptera, Hemiptera and Lepidoptera
- Web sites provide a very granular location for pest detection
- Likely to predate mostly active prey intercepted by the web
- Some itinerant foraging may increase pest detection breadth

Example predator-pest interaction:
Cross spider, *Araneus diadematus*, predating oak pinhole borer, *Platypus cylindrus*

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Content

Executive Summary

Background and Objectives

This review assesses the feasibility of using predator molecular dietary analysis to monitor the spread and emergence of plant pests. Traditional monitoring methods for plant pests scale poorly and introduce latency to detections and responses. Molecular analyses like DNA metabarcoding of bulk invertebrate traps offer highly resolved and scalable biomonitoring, but direct sample collection and processing of target pests is hindered by bycatch and poor capture efficiency for target pests. An alternative application of metabarcoding – analysing predator diets – may circumvent these collection challenges by monitoring natural interactions between native predators and emerging pests. To understand whether this approach could be effective, how it could be implemented and the likely obstacles to its success, we have conducted a systematic review of the literature.

Key Research Questions

This review had the following objectives:

1. Systematically review current applications of predator molecular dietary analysis for the detection of pests.
2. Develop guidance for both targeted and generalised pest detection.
3. Establish broadly applicable case studies to exemplify potential deployment.
4. Evaluate alignment of this approach with existing policy and regulations.

Research

A systematic literature review was conducted in accordance with PRISMA (Preferred Reporting Items for Systemic reviews and Meta-Analyses) guidelines. Pre-defined search terms were developed based on preliminary searches, reference to existing literature and the study team's expertise. Studies concerning the application of molecular methods to the detection of pests in the diets of predators were sought, for which the search strategy targeted three key concept areas: molecular analytical methods, predator-prey interactions and forest ecosystems. The search terms were queried against an online literature database, Scopus, to identify the relevant literature available, following which search terms were refined and finalised.

An initial search identified 667 potential studies which were filtered in successive steps based on title, abstract and the availability of relevant data. To minimise selection bias and ensure systematic evaluation, the project team was divided into two independent review teams, each containing expertise in molecular ecology, trophic ecology and biomonitoring. At each stage of the review, the two moderation teams met and agreed a consensus list of articles for the next stage.

From the initial 667 studies, 523 were removed during screening for article relevance. From the remaining articles, a further 107 were removed by consensus due to irrelevance or during data extraction due to a lack of available information, which left 40 studies that were considered relevant to the key research questions.

Main Findings

The studies reviewed demonstrate the efficacy of dietary DNA metabarcoding for identifying predator-pest interactions, and the limited range of wild predators for which molecular methods have been applied for dietary analysis. Insectivorous bats, passerine and near-passerine birds and web-building spiders were the focus of most studies included in the review, with critical gaps across broader taxonomic groups that are likely to predate pest species. Whilst the UK was one of best-represented countries in the reviewed studies, the limited overall number of relevant studies means that more evidence from UK and Scottish contexts would demonstrate more directly how effective this approach would be and could identify other locally appropriate predator taxa to monitor.

Several key predator-pest interactions were identified from the reviewed literature, including some interactions between UK native predators and UK plant health risk register pest species, although none in UK systems. The reviewed studies provide some generalisable findings that will guide implementation of monitoring; for example, bats are likely to be the most effective group to target for monitoring adult Lepidoptera, whereas spiders may be optimal for monitoring Diptera and Hemiptera. This has guided the development of three case studies exploring the use of bats, birds and spiders for monitoring pests in a Scottish forestry context.

The reviewed studies varied greatly in their methodological approach to predator dietary analysis. Whilst standardisation is vital to safeguard data standards and adherence to best practice, this goal is complex given the methodological requirements across different contexts. For instance, the methods involved in dietary analysis of bat guano will necessarily differ from dietary analysis of whole spider bodies given the different sample compositions, target taxa and predator-prey phylogenetic similarity. The PCR primers used for metabarcoding in the reviewed studies, for example, were often suboptimal and large amounts of data were lost to amplifying DNA of the focal predator instead of its prey. The taxonomic biases of many PCR primers may also distort monitoring outcomes, highlighting the need for selection of appropriate primers. There is an opportunity to standardise generalisable steps of dietary analysis workflows across contexts, which will be crucial for wider implementation. Development of best practice standards, documentation and protocols for wider use may be vital for the widespread and uniform adoption of this monitoring approach.

Several opportunities for integration with ongoing monitoring schemes were highlighted including coordination with bird ringing groups, bat conservation groups, and nest box schemes. However, these species are protected under varying licenses and would require consultation to ensure that any additional activities were permissible under current legislation.

Recommendations

This review concludes that detecting plant pests via metabarcoding of predator diets is feasible, efficacious and ready for wider implementation. However, to reach a fully realised monitoring system that uses predator dietary metabarcoding to detect plant pests, there are several key preceding requirements that need to be considered:

- Refine operational and logistical implementation for streamlined and cost-effective delivery.
- Mitigate conceptual and methodological pitfalls by trialling, developing and adhering to best practice.
- Streamline laboratory and computational workflows to generate accurate results with sufficient speed to facilitate rapid intervention.

To achieve the infrastructure development, methodological refinement and validation required to robustly monitor for plant pests through dietary metabarcoding we suggest the following:

1. Pilot studies validating the detection of priority plant pests in the diets of bats, birds and spiders through comparison of results against parallel conventional monitoring programmes such as established trapping sites to confirm the validity of the technique in a Scottish context.
2. A follow-on study, based on the outcomes of the pilot studies, focused on determining the distribution and spread of a pest solely using predator molecular dietary analysis. The pilot studies should identify an optimal predator to focus this on, and a pest the predator is found to interact with which is established and spreading.
3. A final study that establishes whether it is possible to use multiple predator sampling points across a forest or landscape to triangulate likely locations of pests.

The proposed monitoring scheme would likely require collaboration between existing groups such as government employees, landowners, foresters, bird ringing groups, consultancies, and conservation trusts, whilst also including molecular ecologists and bioinformaticians with experience using these methods. This scale of collaboration could be logistically challenging, but necessary to ensure adherence with best practices across domains. We would therefore recommend consultation among these groups to understand where current capacity for collaboration already exists and where it is likely to be required in the future. This may identify opportunities for capacity building both within and beyond the Scottish Government.

Ultimately, the selection of suitable predators for application of the proposed monitoring approach to specific contexts remains one of the most critical steps. Whilst this review has identified past interactions of some predator groups across a broad range of contexts, these interactions will dynamically change over space, time and both biotic and abiotic gradients (e.g., climate change). Critical gaps in our understanding of trophic ecology in Scottish ecosystems could be addressed by enhancing our fundamental understanding of how predators select prey, particularly how predators might exploit novel prey sources such as newly established plant pests. This information would enhance our ability to predict optimal predators for monitoring those pests using broader foraging ecological insight without needing to continually conduct in depth dietary analysis across a large range of predator species.

1 Introduction

As anthropogenic climate change and globalisation intensify, the Anthropocene presents several distinct challenges for managing our natural resources and ecosystems (Britton et al., 2009; Mantyka-pringle et al., 2012). Among these are the spread of existing pests across production systems like agriculture and forestry, and the increasingly frequent introduction of non-native pests to those systems (Montgomery et al., 2023; C. Wang et al., 2021). Given the increasing demand for resources from those production systems (Hemathilake & Gunathilake, 2022; Nepal et al., 2021), monitoring and managing the spread of these species is paramount. Monitoring these pests is crucial for predicting and managing outbreaks, and adhering to national and industry regulations (Brockerhoff et al., 2023). Traditional pest surveillance methods are expensive, time-intensive and require highly skilled diagnostic personnel, or rely on invasive methods such as tree felling. This introduces significant latency to detections, hindering eradication efforts. New technologies and techniques do, however, present opportunities to upscale monitoring and enhance its breadth, sensitivity and speed (Cuff & Watt, 2025).

Advances in high throughput sequencing have significantly expanded access to molecular approaches like DNA metabarcoding, the parallel sequencing of many diagnostic fragments of DNA from mixed samples of any origin (Taberlet et al., 2012). By rapidly analysing large mixed samples, metabarcoding enables rapid identification of organisms from large volumes of biological samples that would otherwise require laborious morphological examination by highly trained expert taxonomists (Hawthorne et al., 2024; Yu et al., 2012). These samples may include bulk invertebrate samples, or gut contents from predators for dietary analysis. Whilst the application of DNA metabarcoding to existing monitoring schemes holds great promise (Cuff, Deivarajan Suresh, et al., 2023; Petsopoulos et al., 2024), the collection of target organisms still presents a significant challenge, especially for emerging pests that may not interact with existing trapping techniques or otherwise require continuous passive sampling (Roe et al., 2024).

Dietary metabarcoding, the analysis of DNA from an animal's gut contents or faeces, can identify the trophic interactions of animals in natural systems (Pompanon et al., 2012; Symondson, 2002). Unlike bulk sampling approaches that require direct collection of target pest species, dietary metabarcoding leverages predators as biological samplers, circumventing the challenges of trapping rare or cryptic pests directly. This can generate new data on dynamic ecological processes and interactions that are impossible to detect otherwise, including rare or ecologically cryptic interactions (Cuff et al., 2022; Evans et al., 2016). By applying these techniques across different systems, various predator-pest interactions have been identified, providing valuable insights into the suppression of those pests (Cuff, Tercel, et al., 2022; Mata et al., 2021; Saqib et al., 2022). Beyond biological control, these interactions also provide a valuable opportunity to monitor pests within natural or semi-natural systems, exploiting the targeted foraging ecology of predators seeking their prey to provide continuous, passive surveillance across large areas reducing the aforementioned limitations of bulk sample metabarcoding (Cuff, Gajski, et al., 2024; Melcher et al., 2024). Our understanding of this is, however, based on distinct and contextually disparate examples which have not been appraised together. The identification of suitable target predators for the detection of plant pests is crucial, but the necessary ecological information is limited for many taxa.

This review addresses the potential for DNA dietary analysis, particularly DNA metabarcoding, to identify emerging and established pests. Through a systematic review, we identify existing examples of pest detection using dietary metabarcoding and assess the technical, logistical and conceptual benefits and challenges therein. Using this as a foundation, we propose three key case studies that could effectively integrate this approach, and we provide a framework to aid decisions surrounding their adoption. Through this review, we hope to enable wider use of molecular dietary analysis for pest monitoring to upscale our capacity for detecting and mitigating the spread of emerging pests.

2 Methods

2.1 Systematic Review Protocol

A systematic literature review was conducted in accordance with PRISMA guidelines. Pre-defined search terms were queried against recognised literature databases to identify the available relevant literature concerning the application of molecular methods to the analysis of pests in the diets of predators. The search strategy targeted three key concept areas: molecular analytical methods, predator-prey relationships, and forest ecosystems.

The following search string was queried against Scopus in April 2025: (metabarcod* OR metagenomi* OR "molecular diagnosti*" OR lamp OR ddpcr OR "molecular diet*" OR "diagnostic PCR") AND (predato* OR predation OR biocontrol OR "biological control" OR omnivor* OR prey OR invert* OR insect*) AND (tree OR fores* OR woodland OR *forest). This search returned 667 articles.

To minimise selection bias and ensure systematic evaluation, the project team was divided into two independent review teams, each containing expertise in molecular ecology, trophic ecology, and biomonitoring. Both teams conducted parallel screening at each stage of review, with consensus meetings held to resolve discrepancies before advancing to the next stage.

Of the initial 667 articles, 523 were removed when screening titles and abstracts for article relevance against the aforementioned topics. From the remaining articles, a further 107 were removed by consensus or during data extraction, leaving 40 studies for final analysis (Figure 1).

Each included study was screened for the following data: predator taxonomic groups and species, geographic location and habitat context, pest species detected, capture and sampling methods, molecular protocols (DNA extraction methods, PCR primers, sequencing platforms), and reference databases used. Particular attention was paid to identifying predators present in Scotland and pests listed on the UK Plant Health Risk Register (UKPHRR) to assess relevance for Scottish forest surveillance applications.

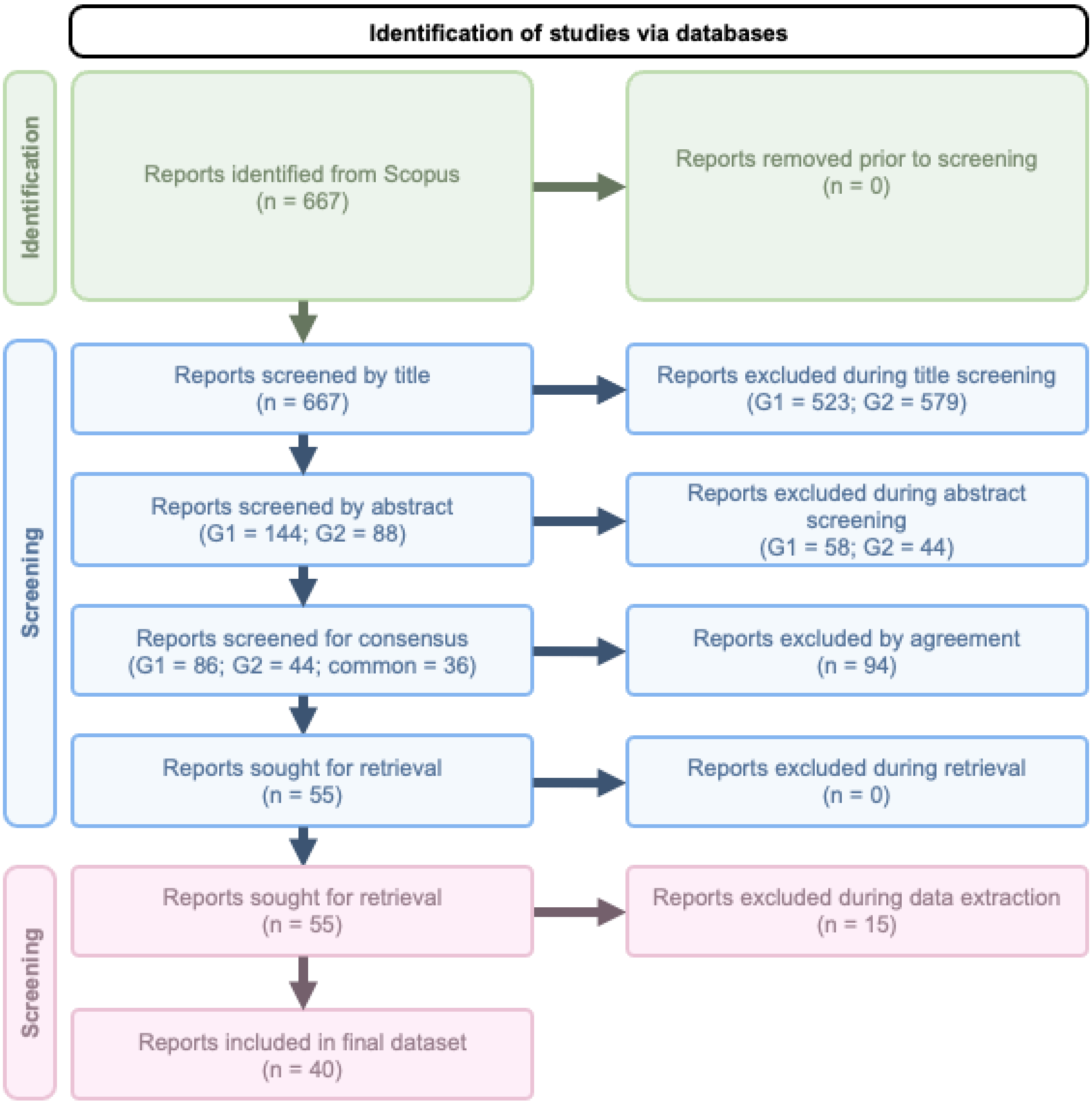


Figure 1: PRISMA workflow of the systematic review indicating the number of articles included and excluded at each stage of the review. Each stage was conducted independently by two teams of balanced expertise and scope, and articles and data extraction confirmed by consensus.

3 Synthesis of findings

The 40 articles from which data were extracted included data from 21 countries across 5 continents (Figure 2). The studies were conducted in forest (28 studies; 70 %), agricultural (6, 15 %), mixed forest and agricultural (5, 12.5 %) and urban park (1, 2.5 %) habitats. The review's predominant inclusion of forest systems is consistent with the forest-focused search terms used. Notably, despite the forest-biased search terms, more than a quarter of studies (27.5 %) were conducted in agricultural or mixed agricultural systems, broadening the relevance of the outcomes of the review. The studies focused on forest systems included research across six coarse predator groups, with bats representing 41.4 % of those studies, and birds 27.6 %. Studies in agricultural systems were similarly mostly related to bats (66.7 %) and birds (33.3 %), whereas mixed agricultural-forest systems were exclusively studied in relation to bats.

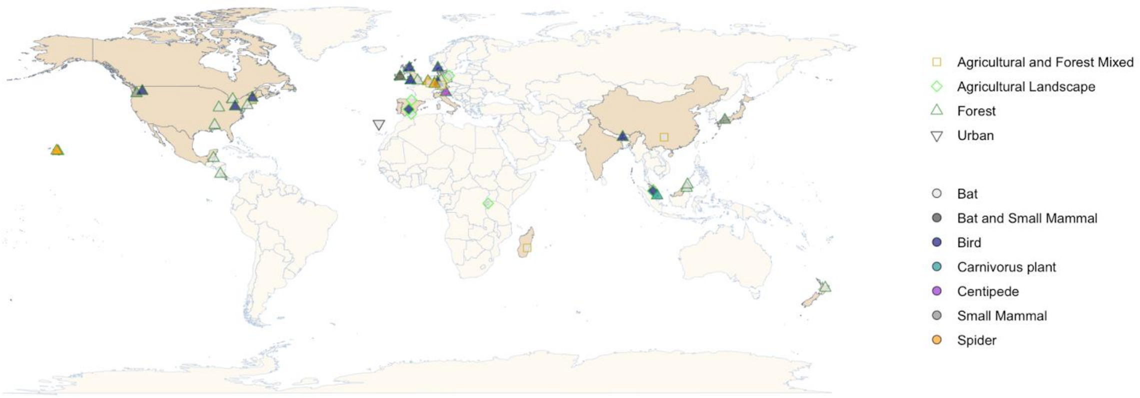


Figure 2: Map of study locations for the 40 papers included in the review, with points filled based on the focal predator of the study and outlined based on the focal habitat type.

3.1 Ecological data

3.1.1 Predators studied

Six coarse groups of consumers comprising 162 species were retained in the final data extraction, including bats (Chiroptera; 87 species), birds (Passeriformes, Piciformes; 58), spiders (Araneae; 7), small mammals (Rodentia, Carnivora and Eulipotyphla; 4), centipedes (Chilopoda; 3) and carnivorous plants (Caryophyllales; 3; Figure 3; Table A1). One article included both bats and small mammals studied together. Bats were the most common focal predator group of the studies (22 studies; 55 %), followed by birds (10; 25 %), spiders (4; 10 %) and small mammals (3; 7.5 %). Regardless of the plant pest focus, bats, birds and spiders have been regular focal subjects of molecular dietary analysis since the inception of these methods given their cryptic ecologies (Oehm et al., 2011; Piñol et al., 2014; Zeale et al., 2011) and, in the case of spiders, fluid feeding (Lafage et al., 2020; Macías-Hernández et al., 2018). The high prevalence of aerial insectivore dietary studies retained in the review reflects their high consumption rates of arthropod prey and their potential value for pest monitoring systems. One dietary study was retained for each of centipedes and carnivorous plants, indicating limited exploration of these alternative consumer groups, and likely many more. The notably low arthropod predator diversity within articles retained for data extraction is particularly surprising given the many molecular dietary analyses of invertebrate predators including beetles (Ammann et al., 2020), dragonflies (Kaunisto et al., 2017) and ants (Tercel et al., 2025); this may either reflect mismatched search engine optimisation for these studies or a lack of reports of pest predation.

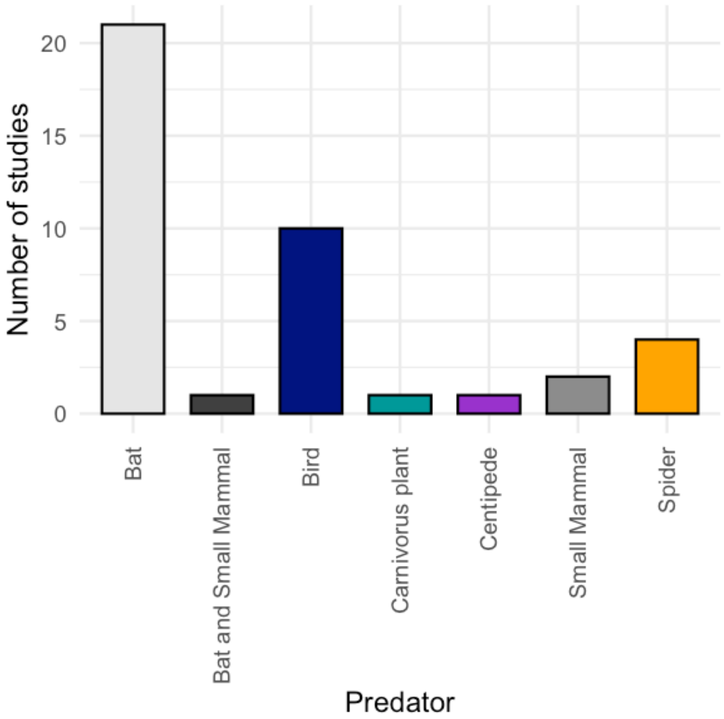


Figure 3: The number of articles that included each of seven coarse predator groups, including the parallel analysis of bat and small mammal diets in one study. The full list of predator species can be found in Table A1.

3.1.2 Pests detected and wider predator trophic niches

Of the 40 studies, 17 reported detections of one or more UKPHRR species, totalling 45 species, of which 23 were relevant to forestry (Table A2). Given the disparate geographical contexts of the studies, their relevance to Scottish forests requires some generalisation to represent potential emerging pests in Scottish forests. We therefore assessed the richness of prey across eight taxonomic orders in each study to determine the coverage of these groups by different predators. These findings will be influenced by the diversity of prey available in each of the orders, and how variable the ecologies of those species are, but can provide some guidance for the selection of suitable predators for monitoring of particular pest groups. Representation of prey orders varied across the three most common predator groups (bats, birds and spiders) (Figure 4; Figure A1). Lepidoptera, for example, was better represented in the diets of bats, closely followed by birds. Diptera, however, was better represented in the diets of spiders. These findings can help to guide monitoring programme design (discussed below in 5.1 Monitoring design decisions).

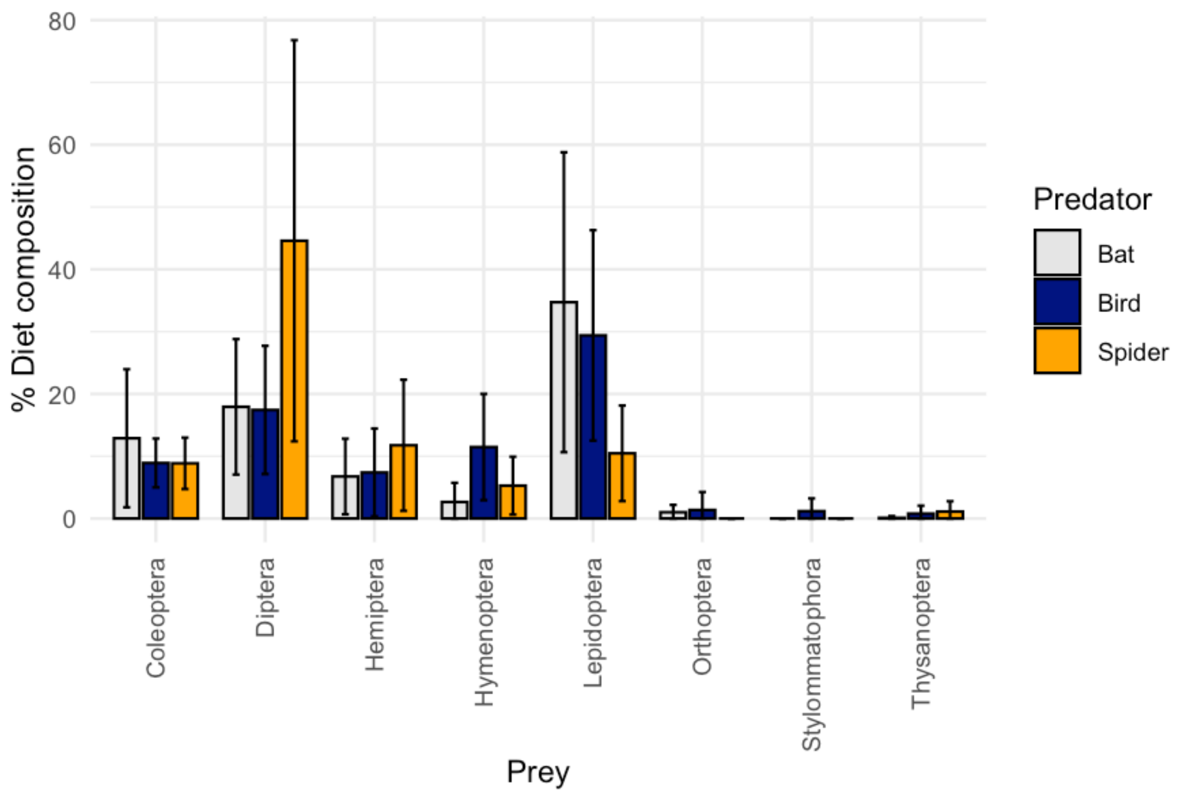


Figure 4: The diet composition of three focal predator groups from the systematic review as percent richness of invertebrate orders containing common plant pests. Bars represent the mean for each predator and prey combination, and error bars represent standard deviation. The same data with the addition of other predator groups can be viewed in Figure A1.

3.1.3 Sample collection methods and licensing

Ten distinct capture methods were used across the reviewed studies, including net-based live capture techniques (mist nets, harp traps, hand nets, sweep nets, whoosh nets), live traps for ground-dwelling species, passive collection methods (guano traps, nest boxes), hand collection, opportunistic sampling, and carcass examination. Mist nets were the dominant technique (31.2 %), reflecting the prevalence of aerial predator-focused studies.

3.1.3.1 Bat sample collection

Of the predator groups, bat samples were collected using the greatest diversity of methods, with mist nets being most common (42.3 % of bat-focused studies), followed by guano traps (23.1 %) and harp traps (15.4 %). The prevalent use of guano traps - typically implemented as a temporary plastic sheet laid under or near roosts - indicates the widespread use of non-invasive sampling methods for protected species. All bat species found in Scotland and their roosts are legally safeguarded under the Conservation (Natural Habitats, &c.) Regulations 1994 (as amended). It should be noted that, for implementation in Scotland, even non-invasive guano capture methods will likely require licensing from the statutory nature conservation organisation, NatureScot. Advice and consultation regarding the requirement of a licence to carry out sampling should therefore be sought from NatureScot at an early stage of monitoring programme design.

The bat sampling methods identified in the systematic review range in their risk of potentially committing an offence via disturbance of bats, with mist netting deemed high risk, alongside sampling within roosts. Licensing would therefore require detail on the objectives, methods, site, timeline, evidence of experience and qualification, and justification of the requirement for disturbing bats and their roosts provided to NatureScot. It should also be noted that the application for a research licence for bat disturbance is subject to European protected species tests, requiring: licensable purpose, no satisfactory alternative and no detriment to species conservation status (NatureScot, 2025a). In reality, the extensive requirements of licensing (particularly for mist netting and harp trapping) would significantly contract the pool of appropriately qualified personnel for sample collection. To minimise or mitigate this licensing barrier, pilot studies could draw upon a pool of licenced Bat Workers to collect guano, as part of volunteer bat groups (of which there are 14 regional groups in Scotland) or regular monitoring. NatureScot and bat groups could also play a key role in identifying initial suitable sites for monitoring, minimising scoping effort. For bat predator systems, deploying guano traps outside of roosts is likely to be optimal for initial investigation, which, if approved by NatureScot, minimally disturbs bat populations and may require less qualified personnel.

3.1.3.2 Bird sample collection

Bird samples were collected using a balance of mist nets (36.4 %) and nest box monitoring (36.4 %), with minimal use of guano traps (9.1 %). The use of nest boxes suggests a focus on breeding ecology, where nest box access facilitates repeated sampling from known individuals or set locations. All wild birds in Scotland are protected under the Wildlife and Countryside Act 1981 (as amended), creating regulatory barriers for sample collection. For implementation in Scotland, both mist netting and nest box monitoring would require licensing from NatureScot or a permit issued by the authorised body British Trust for Ornithology (BTO), which operates under NatureScot's authority (BTO, 2024). Advice and consultation regarding the requirement of a licence to carry out sampling should be sought from NatureScot or the BTO at an early stage of monitoring programme design.

As with bats, there is a range in risk of bird guano capture methods, with mist netting and whoosh nets considered highest risk, followed by nest box sampling (BTO, 2025). Sampling from active nest boxes (i.e. containing nesting birds or broods) requires a scientific research licence from NatureScot or the BTO, with similar application requirements as outlined above (NatureScot, 2025b). Similarly, mist netting requires a licence issued by NatureScot or alternatively, a mist net endorsed ringing permit issued by the BTO (BTO, 2024). In reality, due to the extensive requirements of licensing (particularly for the use of mist nets) this would significantly narrow the pool of appropriately qualified personnel to sample guano. Utilisation of the BTO Constant Effort Sites (CES) program may be an appropriate avenue for full active period monitoring of pests by qualified ringing personnel.

To minimise or mitigate this licensing barrier pilot studies could draw upon a pool of appropriately licenced (i.e. cavity-nesting passerine and near passerine pullus endorsement, passerine and near-passerine mist net endorsement) ringers, part of the 13 regional Ringing Groups in Scotland. Many of these groups will have established sites for target species and/or established nest box networks, which are surveyed or trapped regularly throughout the breeding season. Previous Scottish studies have utilised birds roosting in nest boxes prior to nesting as a source of faeces as there less risk associated with disturbance, etc (Shutt et al., 2020), but this method does not provide a reasonable length of surveillance across pest activity peaks. For bird predator systems, utilising nest boxes to capture guano is the likely best avenue to explore for pilot studies, as this method is less high risk, more suitable for continuous monitoring and there are at least two established nest box transects across Scotland that are continuously monitored (see section 4.2).

3.1.3.3 Small mammal sample collection

Across the systematic review, small mammals were primarily live trapped (75 %) or their faeces were hand collected (25 %). Of the mammal species included in the systematic review, two are found within the UK further: the pygmy shrew, *Sorex minutus*, and the greater white-toothed shrew, *Crocidura russula*, the latter of which is an invasive species. The pygmy shrew, along with several other small mammals (e.g., water voles, hedgehogs, some shrews), is protected under the Wildlife and Countryside Act, 1981 (as amended). For such species, licenses would be required for trapping and sample collection, which would require coordination and consultation with NatureScot (NatureScot, 2025a; NatureScot, 2025b). Advice and consultation regarding the requirement of a licence to carry out sampling should be sought at an early stage of monitoring programme design.

Where the target species is not protected and trapping is part of pest control activities (e.g., for rats), fewer restrictions may apply, although consultation with up-to-date legislation is still advised. For invasive species, such as the white-toothed shrew above, additional restrictions may apply under legislation such as the Invasive Alien Species (IAS) Regulation, the Invasive Alien Species (Enforcement and Permitting) Order 2019. This includes prohibition of the release/transport of invasive animals, and legal powers and/or licenses for the management and control of the species (e.g., general licenses exist for controlling stoats to protect wild birds or livestock). Such cases usually require specific trapping methods, but could be an accessible means for sampling small mammal diets within invaded sites. All small mammals, including pests and invasive species, must, however, be trapped, handled and otherwise interacted with in accordance with the Animal Welfare Act 2006.

3.1.3.4 Arthropod and other sample collection

Ground-dwelling arthropod predators, including spiders and centipedes, were exclusively hand collected, as were carnivorous plants. Unless protected as, for example, red-listed species, there are few legislative barriers preventing collection of arthropods. There is, however, an increasing call for consideration of the ethics underpinning insect monitoring and collection (Barrett & Fischer, 2024). The welfare of insects has seen particular focus given the arguments for insect sentience, resulting in evolving guidelines, the methods discussed in this report may align with this goal by reducing non-target harms and death.

3.2 Technical considerations

3.2.1 DNA extraction methods

Across the 40 studies reviewed, 15 DNA extraction kits were used. Some of this diversity reflects the sample types used, including faeces, partial predator bodies and plant material, which vary in their inhibitor prevalence. Three studies outsourced DNA extractions and did not report the methodology used (Ling et al., 2025; Salinas-Ramos et al., 2015; Q. Wang et al., 2024). Additionally, two studies used multiple DNA extraction methods (Stillman et al., 2022; Tosa et al., 2023) due to the use of several dietary source materials, and one study focused on more than one predator order (Browett et al., 2021), resulting in 43 different approaches across the 40 reviewed studies. The Qiagen QIAamp DNA Stool Mini Kit was the most commonly used extraction method (18 uses; 41.9 %), followed by the Qiagen DNeasy PowerSoil Kit (5 uses; 11.6 %) and the Zymo Quick-DNA Fecal/Soil Microbe Kit (3 uses; 7.0 %). Most of the extraction kits used were Qiagen (28 of 43 applications; 65.1 %), followed by Zymo Research (3 applications; 7.0 %) and Macherey-Nagel (2 applications; 4.7 %).

Faecal/soil-specialised extraction protocols, used in 28 of the studies (70.0 %), were most prevalent likely due to the high frequency of bat-focused studies. The non-invasive nature of faecal analysis is well aligned with guano collection from roosts and is particularly advantageous when studying protected species, which includes many bats and other vertebrates. Extraction kits designed for faeces usually include reagents or steps that reduce the prevalence of PCR inhibitors otherwise abundant in faeces, whilst also sometimes limiting further fragmentation of the already degraded template DNA. Soil extraction kits, being similarly effective at removing inhibitors, are sometimes used in lieu of faecal kits. Together, these represented 72.7 % (16 of 22) of bat, 100 % (10) of bird and 66.7 % (2 of 3) of small mammal dietary studies. Some bird faecal studies reported slight modifications to manufacturer protocols (Magalhães De Oliveira et al., 2020; Stenhouse et al., 2023). Blood and/or tissue extraction kits were used in 6 studies (15.0 %), including all but one of the arthropod-focused studies (which used a broad-spectrum magnetic bead-based approach), likely because arthropod dietary analysis typically uses whole or partial body DNA extractions to access diverticulated or otherwise cryptic guts (Cuff, Kitson, et al., 2023; Macías-Hernández et al., 2018).

The notable outsourcing of DNA extraction in three studies indicates that some users are leveraging specialised facilities rather than developing in-house capabilities, which may reflect cost-benefit considerations or technical expertise requirements. Aside from those studies, all but three used spin-column-based DNA extractions. This method is relatively cost-effective at smaller scales, usually available in user-friendly kits with pre-prepared buffers and easily deployed in laboratories containing standard equipment. The cost and manual handling required for these kits, unless combined with user-prepared buffers and third party plasticware, and integrated with robotic liquid handling systems, can limit the scalability and throughput of analysis. Magnetic bead-based protocols are increasingly commonplace in this field (Oberacker et al., 2019), although poorly represented in this review possibly due to the latency of publishing. For larger scale deployments, the integration of such approaches with robotics-based analysis platforms, like the Kingfisher system used in one study (Aldasoro et al., 2025), may optimise cost and time. The diversity of extraction methods used suggests that standardisation remains limited. Whilst this may reflect adaptation to specific research questions or sample types, it can complicate cross-study comparisons, meta-analyses and the uniformity of data standards.

3.2.2 PCR primer usage

Of the gene regions used for metabarcoding of arthropod prey, cytochrome c oxidase subunit I (COI) was most prevalent (40 of the 47 PCR primer pairs used for arthropod detections; 85.1 %), followed by 16S (4, 8.5 %), 18S (2, 4.3 %) and 28S (1, 2.1 %) markers alongside PCR primers targeting other taxa (e.g., plants, vertebrates) which are not discussed further given their irrelevance to plant pest monitoring. Given the highly populated reference databases available for COI, most animal barcoding and metabarcoding studies have used it since the inception of DNA barcoding (Hebert et al., 2003). The conserved regions necessary for primer annealing are, however, often suboptimal in COI, driving compromises in taxonomic breadth, biases and specificity (Deagle et al., 2014). As reference databases continue to be populated for non-standard barcoding markers, more suitable primers in alternative gene regions may become more commonplace, perhaps exemplified in this case by Holmquist et al. (2023), who used 16S, 18S and 28S primers for spider dietary analysis (Krehenwinkel et al., 2019).

Two COI primer pairs were markedly more frequently used across the assessed studies than the others. The most commonly used primer pair was ZBJ-ArtF1c/ZBJ-ArtR2c, which, alongside its variants and use of one or the other primer in combination with another primer, accounted for 19 of 47 primer uses (40.4 %; 47.5 % of COI uses). The ANML primers accounted for 10 uses (21.3 %; 25 % of COI uses). The forward primer ZBJ-ArtF1c was used in 16 studies, while LCO1490 (the forward primer commonly applied for standard animal COI barcoding since 1994; (Folmer et al., 1994)) appeared in 15 studies. For reverse primers, ZBJ-ArtR2c and its variants dominated with 18 uses, followed by CO1-CFMRa (of the ANML set) with 10 uses. It is noteworthy that ZBJ-ArtF1c/ZBJ-ArtR2c were designed for bat dietary analysis with the exclusion of bat DNA central to their design (Zeale et al., 2011). Their dominance is therefore intuitive given that bat dietary studies represented the majority of studies included in the review, but it is also notable that they were applied to other non-bat taxa (Browett et al., 2021; Kennedy et al., 2019; Sato et al., 2022; Supriya et al., 2020; Trevelline et al., 2018; Van Schrojenstein Lantman et al., 2021). The bias of these primers toward Lepidoptera and against Hemiptera, Hymenoptera and Thysanoptera (Cuff et al., 2021; Krehenwinkel et al., 2019) may inflate the relative frequency of Lepidoptera detections across all studies using these primers.

Amplification of DNA of the focal predator was widely reported and is a well-characterised problem for predator-prey dietary analyses which results in the loss of potential prey data to identification of the predator itself (Cuff, Kitson, et al., 2023). To reduce amplification of predator DNA, several studies used primers that did not amplify the focal predator (henceforth 'exclusion primers') or predator-specific blocking probes that interfere with amplification of the predator using more general PCR primers. These are variably successful but usually beneficially bias amplification against the predator, resulting in better data yields for prey detections (Lafage et al., 2020; Vestheim & Jarman, 2008). Blocking probes were used in two studies: once for blocking mammal amplification (Ingala et al., 2021) and once for blocking spider amplification (Van Schrojenstein Lantman et al., 2021). Blocking probes can be more stochastic in how they influence taxonomic biases given their variable success in annealing before the general primers (Cuff, Kitson, et al., 2023; Piñol et al., 2014). Exclusion primers were widely used, as mentioned above regarding the bat exclusion primers ZBJ-ArtF1c/ZBJ-ArtR2c, and were used in 23 of 48 cases (47.9 %).

Multi-marker and multi-locus metabarcoding, the use of multiple PCR primer pairs for the same samples, was conducted in ten studies (25 %). Multi-marker metabarcoding can be beneficial for validation of findings and mitigation of the taxonomic biases of a single PCR primer pair (Cuff, Windsor, et al., 2022; Da Silva et al., 2019), or for detecting multiple phylogenetically disparate taxa (De Barba et al., 2014; Tercel et al., 2021). This may have extended the taxonomic breadth of prey detections, increasing the likelihood of pest detection, especially when general and predator exclusion primers are paired, with each achieving breadth and depth of detections, respectively (Cuff, Kitson, et al., 2023).

3.2.3 High-throughput sequencing and bioinformatic analysis

Illumina sequencing was used across most of the reviewed studies (36 studies; 90 %), the only exceptions being older Ion Torrent systems (4; 10 %). Illumina MiSeq was the most commonly used platform (28, 70 %), followed by Ion Torrent (4; 10 %), Illumina NovaSeq (7.5 %) and HiSeq (7.5 %). The broad use of Illumina MiSeq likely reflects the suitability of its throughput for moderately large research projects of 100-500 samples. Studies with higher throughput requirements would benefit from platforms like NovaSeq. Sequencing depths were, however, very inconsistently reported and highly variable, with potential implications for recovery of prey data. From the sequencers used and the number of recovered reads reported, it seems clear that some studies shared sequencing runs with other projects, which may have reduced overall costs.

Although not formally extracted as part of the systematic review, variation in bioinformatic processes was high. Many studies used pre-built bioinformatics pipelines such as Obitools (Boyer et al., 2016), DADA2 (Callahan et al., 2016) and QIIME2 (Bolyen et al., 2019), whereas others used custom pipelines or a combination of different pipelines and packages. Most notably, this may result in drastic differences in the assignment of species and perceived diversity, especially given that many studies reported the diversity of operational taxonomic units (OTUs) rather than Linnean taxa. The use of amplicon sequence variants, which delineate all distinct sequences regardless of their phylogenetic placement, will, for example, result in much higher richness than using traditional OTUs, which may be clustered together based on sequence similarity. Similarly, the use of trained species classifiers can result in different outcomes to sequence similarity-based taxonomic assignment (Anderson & Dubnicka, 2014; Jeunen et al., 2023). Similarly, the filtering of data to remove false positives varied from stringent % read count thresholds (Drake et al., 2022) and use of experimental controls (which other studies did not report including at all) to no filtering at all due to concerns about false negatives (Littleford-Colquhoun et al., 2022).

Most studies used NCBI's GenBank or BOLD for reference databases to assign taxonomy to sequences (35 studies; 87.5 %), with only one using a custom database constructed from samples collected during the study. Only three studies (7.5 %) used curated reference databases. Reference database curation can reduce errors during taxonomic assignment generated by the inclusion of incorrectly identified or otherwise false records on public sequence repositories like GenBank. Altogether, the variation in bioinformatic and wider methodological processes is indicative of the low level of standardisation across metabarcoding studies. Without such standardisation, the comparability of monitoring efforts across time and space will be limited, and attempts to mitigate inaccuracies and errors may be inadequate. Best practice must, however, be context-dependent, which will first require validation of different methods across a broader range of contexts for the detection of pests in predator diets.

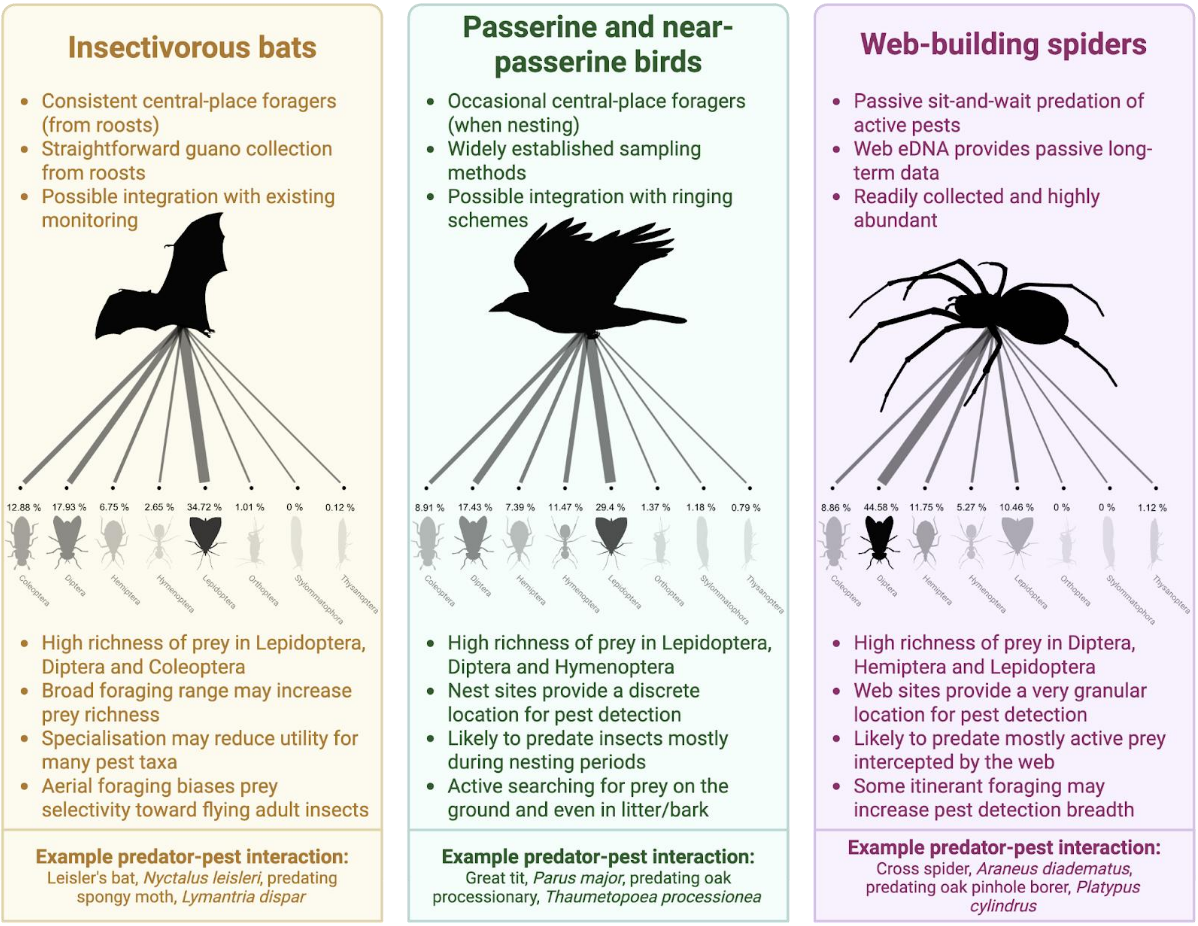
4 Case Studies

Based on the systematic review, we developed case studies demonstrating how predator molecular dietary analysis could be applied to pest detection in Scottish Forestry. As detailed above, insectivorous bats, passerine and near-passerine birds and web-building spiders were the most frequently studied groups across the review, and all frequently consume key forest pest orders, including Coleoptera, Diptera and Lepidoptera.

Despite its global spread, the systematic review highlighted only one study which documented predator-prey interactions between native Scottish predators and an established pest species, but in Madeira, Portugal (Gonçalves et al., 2024). Three examples of UK-native predators consuming wider UKPHRR pests were found through the review (Table 1). Only one study was conducted in Scotland itself (Shutt et al., 2020), one in both Wales and England (Stenhouse et al., 2023), one in England (Carr et al., 2020) and another in Ireland (Browett et al., 2021) otherwise providing the closest geographic context. Alongside Spain, the UK was the third best represented country in the reviewed literature, preceded only by the USA (9 studies) and Germany (6 studies), suggesting that, whilst a gap in evidence for Scottish forests exists, it is less severe than for many other countries. The reviewed studies therefore provide valuable proof-of-concept evidence that dietary metabarcoding can successfully detect pest species within predator diets in the context of Scottish forests. These examples offer important insights into how pest detection and monitoring through trophic interactions might function in practice, forming the foundation for Scottish case studies.

Table 1: Predator-pest interactions identified through the systematic review that pertain to predators and pests established in Scotland or in the UK with a likelihood of spreading to Scotland in the near future.

Predator order	**Predator species**	**Frequency in reviewed literature**	**Pests detected in diet**	**Reference**
Chiroptera	*Plecotus austriacus*	1	*Chrysodeixis chalcites*	(Gonçalves et al., 2024)
Chiroptera	*Nyctalus leisleri*	1	*Lymantria dispar*	(Bourlat et al., 2023)
Araneae	*Araneus diadematus*	1	*Platypus cylindrus*	(Van Schrojenstein Lantman et al., 2021)



Insectivorous bats

- Consistent central-place foragers (from roosts)
- Straightforward guano collection from roosts
- Possible integration with existing monitoring

- High richness of prey in Lepidoptera, Diptera and Coleoptera
- Broad foraging range may increase prey richness
- Specialisation may reduce utility for many pest taxa
- Aerial foraging biases prey selectivity toward flying adult insects

Example predator-pest interaction:
Leisler's bat, *Nyctalus leisleri*, predating spongy moth, *Lymantria dispar*

Passerine and near-passerine birds

- Occasional central-place foragers (when nesting)
- Widely established sampling methods
- Possible integration with ringing schemes

- High richness of prey in Lepidoptera, Diptera and Hymenoptera
- Nest sites provide a discrete location for pest detection
- Likely to predate insects mostly during nesting periods
- Active searching for prey on the ground and even in litter/bark

Example predator-pest interaction:
Great tit, *Parus major*, predating oak processionary, *Thaumetopoea processionea*

Web-building spiders

- Passive sit-and-wait predation of active pests
- Web eDNA provides passive long-term data
- Readily collected and highly abundant

- High richness of prey in Diptera, Hemiptera and Lepidoptera
- Web sites provide a very granular location for pest detection
- Likely to predate mostly active prey intercepted by the web
- Some itinerant foraging may increase pest detection breadth

Example predator-pest interaction:
Cross spider, *Araneus diadematus*, predating oak pinhole borer, *Platypus cylindrus*

Figure 5: The three focal case study groups, some considerations for their use and the relative richness of prey orders according to articles included in the systematic review.

4.1 *Case study 1: Insectivorous Bats*

Of the bat-prey interactions identified in the systematic review, one has notable potential in Scotland: the Leislers bat (*Nyctalus leisleri*) predating the spongy moth (*Lymantria dispar*; Bourlat et al., 2023). Though the current distribution of spongy moth is largely restricted to south-east England, several records of adult moths further north (NBN, 2025b) indicate a need to track its spread. Leisler's bat would be a suitable candidate for this, although its roosts are relatively cryptic, constraining their identification and long-term monitoring (Boston et al., 2020; Ruczyński & Bogdanowicz, 2005). The distribution of Leisler's bat in Scotland is also restricted to Dumfries and Galloway, and Stirlingshire (JNCC, 2019b). The brown long-eared bat (*Plecotus auritus*; BLEB) is, however, relatively widely distributed throughout Scotland (JNCC, 2019a) and could be a viable alternative. Brown long-eared bats roost in deciduous and/or mixed woodlands, using natural cavities, wood crevices, nest-boxes and bat boxes with high annual fidelity, though it should be noted that these summer roosts change every few days in maternity colonies (Boye & Dietz, 2005; Entwistle et al., 1997; Meschede et al., 2002). This species also exploits more urbanised environments, inhabiting areas that experience little disturbance such as barns or lofts statically throughout the summer (Entwistle et al., 1997; Meschede et al., 2002). This roosting fidelity would facilitate monitoring and guano collection using established networks of bat boxes and/or visible roosts in inhabited structures. Monitoring during hibernation would likely be less successful, as the species is thought to hibernate in less accessible locations such as bark crevices, caves or cellars (Horacek, 1975).

The BLEB is an insectivorous species which largely consumes Lepidoptera and Diptera (Hollyfield, 1993; Razgour et al., 2011; Robinson, 1990). Although the BLEB is a generalist, larger moths from Noctuidae and Geometridae are major components of its summer diet (Andreas et al., 2012; Hurpy et al., 2025; Razgour et al., 2011). Though there is limited evidence of the BLEB predating forestry pests within the UK and Europe, their frequent predation of Noctuidae (18 species on UKPHRR) and Geometridae (3 species on UKPHRR), suggests that they would be promising targets for molecular dietary analysis-based monitoring. Given their generalist diet, the detection of emerging pests may be inconsistent, but as the wide distribution of the BLEB may prove beneficial for tracking pest spread. The BLEB forages predominantly in deciduous and mixed woodland, with some occupancy of coniferous woodland and edge habitats (Boye & Dietz, 2005; Entwistle et al., 1996). As a central-place forager, the BLEB has been reported to forage 0.5 - 1.5km from its roost in summer (Entwistle et al., 1996; Racey & Swift, 1985), which limits detection to localised infestations, ensuring some confidence in pest location when they are detected.

The use of bats for pest monitoring offers potential to target a range of larger Lepidopteran pests across Scotland by leveraging existing bat roost/box networks, including those maintained by Forestry and Land Scotland, National Parks staff and individual regional bat groups. Roost collection of guano could be easily integrated into existing monitoring by Scottish Bat Workers, as specific methodologies are already integrated into guidance. This will not be easily integrated into current monitoring for regulated, quarantine Lepidopteran pests, given the current use of targeted visual surveys, though it could complement a newly established pheromone trapping network.

4.2 Case study 2: Near-passerines and passerine birds

Blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*) are among the UK's most abundant woodland birds (Musgrove et al., 2013), with widespread Scottish populations excluding the Hebrides and Northern Isles (Balmer et al., 2013). Their year-round abundance and high site fidelity make them reliable focal taxa for predator molecular dietary analysis-based pest monitoring. While predominantly using deciduous woodland for breeding, both species use a range of habitats including mixed and coniferous woodland (Blondel et al., 1992; Coomes et al., 2025; Grzędzicka, 2018; Mänd et al., 2005). As secondary cavity-nesters, they readily colonise artificial nest boxes (Minot & Perrins, 1986; Perrins, 1980), which can facilitate sampling, including through established Scottish transects operated by the University of Edinburgh (220 km eastern transect; Shutt et al., 2018) and University of Glasgow (56.3 km western transect; Roy & Dominoni, 2024).

During the breeding season, both species are insectivorous foliage-gleaners with nestling diets dominated by Lepidoptera larvae, supplemented by Araneae, Diptera and Hemiptera when caterpillars are scarce (Coomes et al., 2025; Perrins, 1991; Serrano-Davies & Sanz, 2017). They consistently target Tortricidae, Geometridae, and Noctuidae moths, which include key forest pests on the UKPHRR (Höhn et al., 2024; Nour et al., 1998; Shutt et al., 2020). Great tits predate pine processionary moth, *Thaumetopoea pityocampa*, across central Europe, consuming all larval instars including urticating caterpillars (Gonzalez Cano, 1981; Pimentel & Nilsson, 2007). Both tit species also predate oak processionary moth, *Thaumetopoea* *processionea*, with great tits consuming all life stages and blue tits consuming early instars (LIFE Oak Processionary, 2020). As central-place foragers when nesting, they typically forage within 30-50 m of nest sites depending on prey resource availability (Naef-Daenzer & Keller, 1999; Stauss et al., 2005), which may limit detection to localised infestations. Beyond breeding seasons, tits can predate fewer invertebrates and prioritise plant resources, especially when prey availability is poor (Coomes et al., 2025), which may reduce the year-round potential for monitoring emerging pests using this system.

Predator molecular dietary analysis of tits for pest detection has strong potential for Scottish pilot studies, especially if existing University of Edinburgh and University of Glasgow nest box transects could be leveraged. By aligning this with existing visual surveillance, direct comparison between dietary detection and conventional monitoring would enable validation of this approach. If linearly distributed, nest box transects could also provide an opportunity to track pest spread, potentially detecting pests before they reach established trapping locations. The combination of abundant, easily sampled predators with established infrastructure and strong ecological links to priority Lepidoptera pests makes this system a strong candidate for proof-of-concept studies. Alongside tits, great spotted woodpeckers, *Dendrocopos major*, could be a promising candidate for Scottish applications, especially for detecting bark boring beetles such as the great spruce bark beetle, *Dendroctonus micans*, although this case is more evidence deficient (Appendix 1).

4.3 Case study 3: Web-building Spiders

One arthropod predator-pest interaction was identified for potential pest monitoring in Scotland: the cross spider (*Araneus diadematus*) predated both the oak pinhole borer (*Platypus cylindrus*) and elm zigzag sawfly (*Aproceros leucopoda*; Van Schrojenstein Lantman et al., 2021) in the reviewed studies. The oak pinhole borer has a limited distribution in the UK, formerly restricted to Wales and southern England, with further spread into localised populations in central England. The elm zigzag sawfly is similarly distributed largely in the south-east of England, with records extending above York (NBN, 2025a). Both species are established in the UK and are considered a low relative mitigated risk (DEFRA, 2020, 2021). Molecular dietary analysis of the cross spider is well-placed to monitor pests such as these, given its widespread distribution across Scotland, albeit patchy on the west coast (British Arachnological Society, 2025), across a wide range of habitats, including woodland, scrub and hedgerows (British Arachnological Society, 2025). As a web-building spider, the cross spider passively intercepts mostly aerial prey, facilitating predation of a broad prey spectrum determined by web placement and structure (Cuff, Windsor, et al., 2023; Harwood et al., 2003; Van Schrojenstein Lantman et al., 2021). Consequently, the diet of the cross spider, as many other spiders, can strongly reflect the prey available to them (Cuff, Tercel, et al., 2024; Melcher et al., 2024). Their diet primarily includes small Diptera, Hemiptera (e.g., aphids), Coleoptera and Hymenoptera (Nyffeler & Bonte, 2020; Van Schrojenstein Lantman et al., 2021). As in the cases of bats and birds, this understanding of the cross spider's trophic ecology could be used to guide its deployment for the monitoring of emerging and established pests. This could also be easily aligned with existing monitoring programmes such as the Forest Trapping Network, which targets Scolytinae using multiple traps per site. Hand collection of spiders such as the cross spider could be easily integrated with collection of other samples given the relative ease of finding and collecting orb-weavers, the lack of legislative barriers to their collection and the opportunity it presents for comparison between trap catches and spider diets for validation. Despite limitations in potential pest detection, this system is likely the most cost-effective to establish, especially if paired with existing established networks for logistical streamlining.

5 Implementation of predator molecular dietary analysis for pest detection

5.1 Monitoring design decisions

Implementing the above case studies as real-world monitoring programmes requires careful consideration of 1) whether predator molecular dietary analysis is the most suitable approach; and 2) which predator group is best suited to the aims of the monitoring programme.

It is crucial that decision-making surrounding these considerations is robust, as the success and accuracy of pest detection could have severe short- and long-term economic ramifications for practitioners. The first consideration before applying predator molecular dietary analysis for pest monitoring should always be whether this is the most appropriate, cost-effective and accurate monitoring method available (Figure 6). If the target pest is already readily collected in targeted traps like pheromone traps, the costs of molecular analysis, especially if the initial capital costs are required, may be unnecessary. Equally, if this approach relies on unavailable taxonomic expertise, investment in molecular infrastructure may be beneficial. Similarly, if the scale of monitoring required is large, molecular methods may become more cost effective and rapid than morphological examination. If taxonomic expertise is available, non-targeted bulk sampling methods may be applicable if known to capture the target pest successfully, and if monitoring at a relatively small scale. Importantly, these can also be integrated with molecular analyses like DNA metabarcoding for scalable analysis of pest occurrences (Hawthorne et al., 2024), which may be more appropriate than dietary analyses if there is low confidence in predator-pest interactions occurring.

If traditional monitoring methods are not viable, access to the equipment, expertise and funding required for molecular analysis should be considered carefully. As in some of the studies included in the systematic review, it is possible to outsource some or all of the molecular analysis, but this often incurs additional costs. If this is not available, investment in these resources or alternative monitoring methods may be a priority. If predator molecular dietary analysis is the most viable monitoring method available, or at least seems promising, it is important to consider whether prey-specific diagnostic assays may be more effective than metabarcoding. These assays, which use PCR or qPCR to detect specific target species, offer several advantages over metabarcoding approaches. In many cases, especially when looking to detect one or few known species, diagnostic assays offer a cheaper, more scalable, more sensitive and more accurate approach (Rennstam Rubbmark et al., 2019). They eliminate sequencing costs and provide straightforward presence/absence data without complex bioinformatics. However, metabarcoding is most applicable when data are required for broad communities of potentially unexpected species, making it ideally suited to continuous passive monitoring for emerging pests through predator molecular dietary analysis, where the identify of novel threats may not be known in advance.

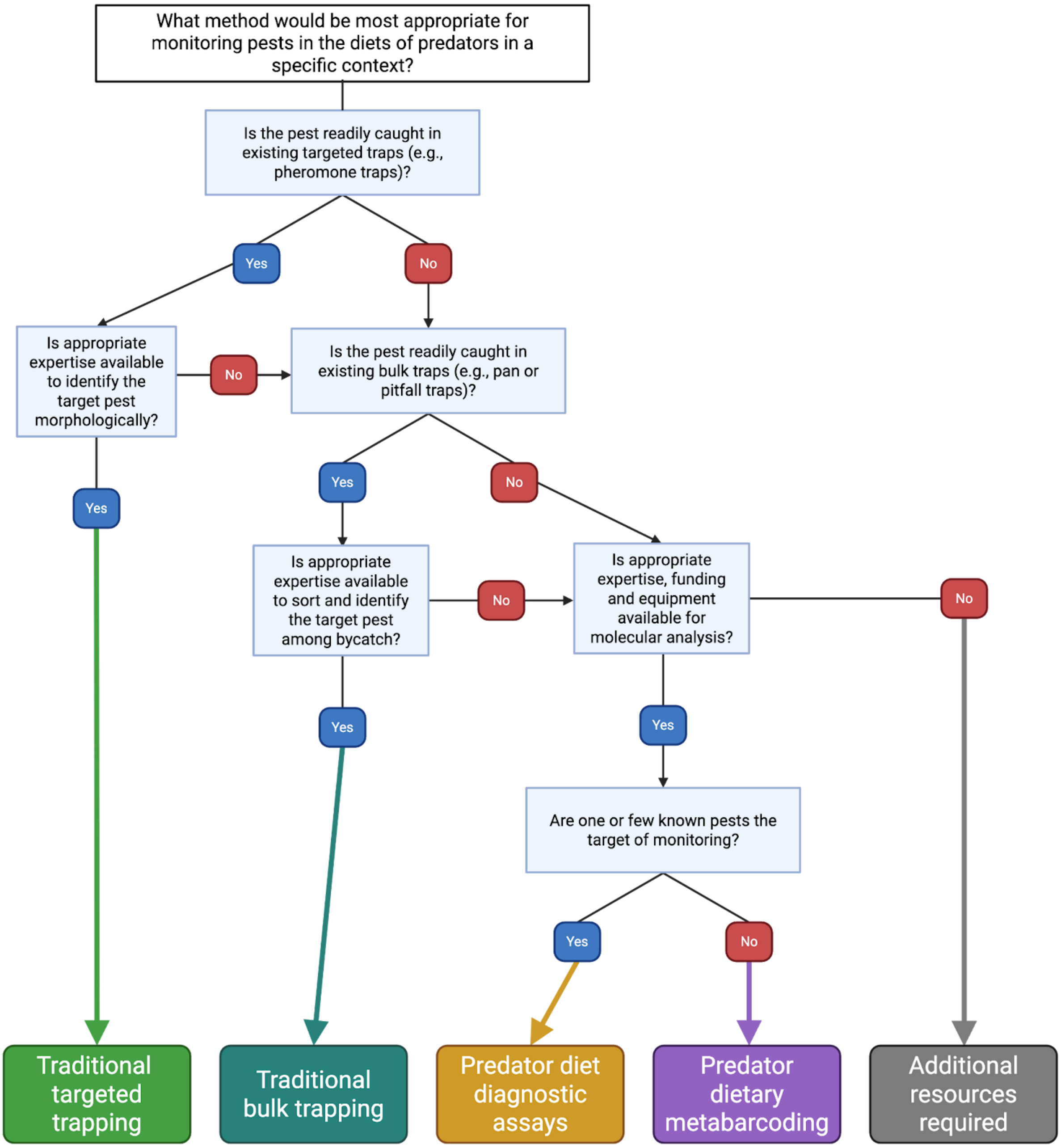


Figure 6: A flow diagram to inform selection of an appropriate method for monitoring plant pests based on information synthesised in this review.

Alongside selecting an appropriate method, choosing a focal predator is also an important decision that will largely depend on the target pests, the life stage of the pests and the life stage/activity of the predators (Figure 7). Based on the systematic review, we have identified which of three commonly used predator groups are best suited to each of eight common plant pest orders. For orders primarily predated by bats, birds and/or spiders often offer a suitable alternative predator group, especially when the pests are not in flight (i.e., when juvenile or larval), at which point spiders and/or birds may be more effective. For orders primarily predated by birds, in non-nesting periods, when birds will often feed more frequently on plant material, bats and spiders may be more viable. Spiders are often well-placed to consume active prey given that they usually sit and wait for prey on webs, so many juvenile or larval prey may be more successfully predated by birds. Whilst understanding the trophic niches of these predators can help to guide monitoring programme design, interactions change across space, time and in response to biotic and abiotic changes. It is therefore crucial that predator selection during the design of monitoring programmes is based on a sound fundamental understanding of the drivers of interactions under different conditions, rather than the identities of interacting partners across different contexts. This requires development of our understanding of fundamental foraging ecology, which requires wider research related to the dynamic mechanisms driving interactions.

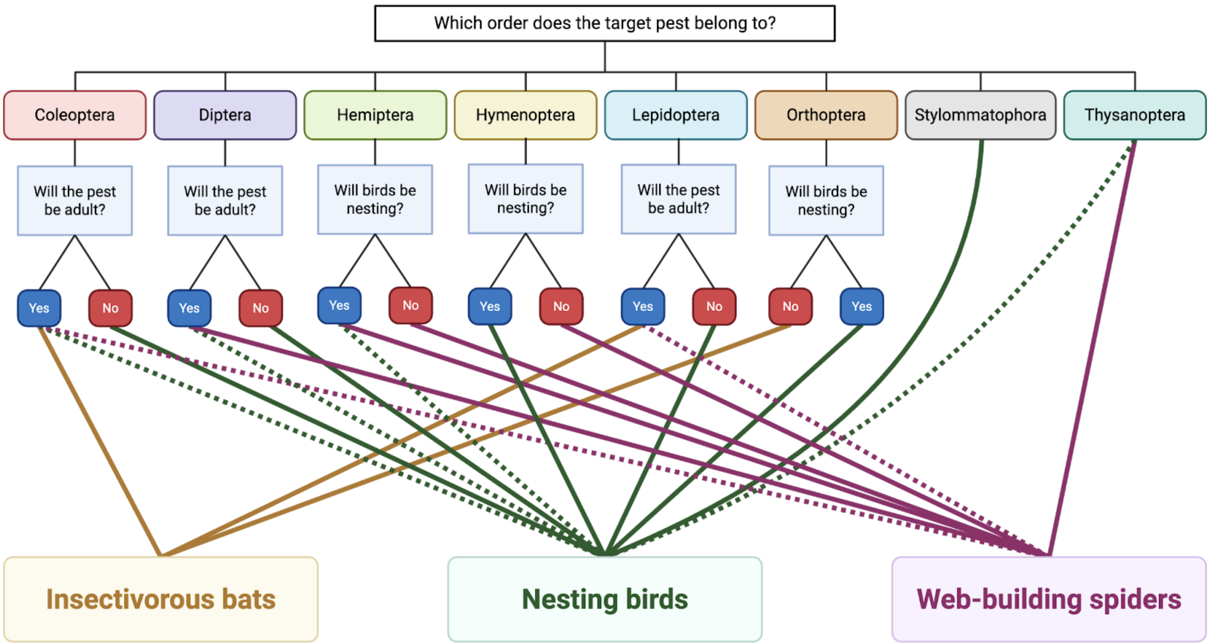


Figure 7: A flow diagram to inform selection of a broad predator group for monitoring of plant pests belonging to one of eight invertebrate orders. Solid lines indicate the primary recommendation, whereas dashed lines represent a viable (although often suboptimal) alternative.

Other decisions related to implementing predator molecular dietary analysis for pest monitoring may depend on the scale and context of the monitoring programme. The choice of DNA sequencing platform and kit/cartridge/flow cell will, for example, depend on the availability of equipment and the number of reads required (which will depend on the number of samples, technical replicates and PCR primer pairs). Optimal PCR primers will depend on the focal predator and target pests, but should ideally be informed by in silico and in vitro testing of the PCR primers to determine their biases ahead of wider application (Elbrecht et al., 2019; Elbrecht & Leese, 2017; Piñol et al., 2019). One of the most crucial considerations for primer selection in predator-prey dietary analyses is the amplification of the predator itself, which can inflate the number of sequencing reads required to make accurate conclusions about the trophic interactions occurring (Cuff, Kitson, et al., 2023). This makes primer selection, and even the design or modification of new primers, all the more crucial in these contexts. Given their frequency in the metabarcoding literature, bat studies using bat-exclusion primers (e.g., ZBJ-ArtF1c/ZBJ-ArtR2c; (Zeale et al., 2011) have provided a template for other studies to follow, unfortunately including the use of their primers for arthropod diet studies, which may even exacerbate the predator amplification problem (Cuff et al., 2021; Krehenwinkel et al., 2019). This highlights the need for careful design of monitoring activities through consultation with appropriate expertise and thorough testing prior to implementation. Beyond technical and logistical considerations, any future operational use of predator dietary data will require clear arrangements for data governance, sharing and communication, particularly where detections may contribute to regulatory or public-facing decisions.

5.2 Economic implications

The cost of traditional biomonitoring using, for example, bulk insect trapping and morphological identification, can be relatively low, especially at small scales, but typically multiplies linearly with increases in scale. Given that sequencing is often the most expensive component of molecular-based monitoring, and smaller units of sequencing are usually sufficient to analyse many samples in parallel, the costs are usually much more scalable for large volumes of samples (Table 2). Alongside the costs of the molecular analysis itself, the collection of samples for molecular analysis may also incur additional costs. Using sterile, DNA-, RNA- and nuclease-free collection equipment and materials will incur additional costs, but even storing and archiving samples (and their subsequently isolated DNA) at -20 °C for prolonged periods incurs energy and infrastructure costs. Collection itself will require time and labour, but accessing samples, especially from vertebrates, which are protected species, may also incur administrative fees for licensing, logistics and coordination with stakeholder groups like bird ringers or bat surveyors.

The costs of molecular analysis vary greatly depending on access to equipment, expertise and competitive pricing of consumables and external services. Whilst sequencing costs especially vary depending on sample size, many external companies can provide competitive pricing and the ability to scale sample numbers easily, especially for Illumina sequencing. These costs usually do not include sample collection and storage, although these can be kept minimal if completed in-house, and routine analysis may render automation of both lab and analysis work increasingly cost effective. Costs will, however, increase for research labs that are not set up or experienced in implementing these protocols, especially if PCR primer testing and validation need to be undertaken. The use of technical replicates (repeated molecular analysis of each sample) can increase the accuracy of molecular data and help with data filtering, but also incurs additional costs through reagents, plasticware and increases in the sequencing depth required. Similarly, multi-marker metabarcoding (discussed above) can multiply costs, but can also mitigate some of the taxonomic biases imposed by PCR primers. The sequencing kit/cartridge/flow cell used will be a significant determinant of final costs, but should be determined by the number of sequencing reads required to analyse all samples adequately.

One of the greatest cost determinants is access to equipment, the capital costs of which will greatly outweigh any capital costs associated with traditional monitoring approaches. Standard lab equipment, including pipettes, centrifuges, incubators, thermocyclers, gel electrophoresis equipment and the specialised equipment required for some protocols (e.g., magnetic racks) can vary massively in cost, but will likely exceed £15,000 for moderate quality equipment. Sequencers are among the most expensive equipment required, with Illumina sequencers costing between £50k (incl. VAT) for the lowest throughput MiSeq system and £240k (incl. VAT) for intermediate throughput NexSeq systems and £720k (incl. VAT) for NovaSeq 6000 systems. Nanopore sequencers, however, require much smaller capital investment when used for research purposes, with a MinION Mk1d sequencer costing £2,940 (incl. VAT). Commercial provision of Nanopore sequencing requires the provider to invest in larger sequencers such as the GridIon (£62k inc. VAT) and Promethion P2i (£93k inc. VAT), all listed costs are correct at time of writing. Additionally, while the cost of purchasing sequencers is high many companies offer competitive sequencing services which range in cost For less experienced end-users, consultation with or hiring of appropriate expertise will also incur costs, as would using external services (Table 2). Similarly, analysis of sequencing data can be computationally expensive, often performing best on high-performance computing clusters or high-specification computers that cost significantly more than standard computers.

Other options that could be explored are the species-specific diagnostic assays mentioned prior. These methods are often cheaper to run per sample as there is no need for expensive sequencing costs, making them cost-effective for routine monitoring of known pest species at scale (Baroja et al., 2022). However, unlike the wide variety of metabarcoding methodologies that are readily available, each species-specific diagnostic assay would require a development period with costs varying depending on the complexity of the assay and the initial available resources. This upfront investment in assay development means diagnostic approaches are most economical when monitoring established pest lists, whereas metabarcoding offers greater flexibility for detecting novel or unexpected threats without requiring prior knowledge of target species.

Table 2: Example costs for metabarcoding of dietary samples with varying numbers of samples, PCR primers and technical replication. The reagent costs are largely based on established protocols used in the Newcastle University Molecular Diagnostics Facility (Cuff et al., 2025), whereas the service costs are based on the additional overhead, staff and running costs estimated for civil service band three and four staff time. The costs presented roughly equate to those required for aliquots/subsets of bulk purchased reagents, which will be higher for purchasing smaller amounts for one-off uses. Costs for other institutions will vary depending on the scale of their operations, access to supplier discounts and other cost modifiers. These costs are based on 25 µL reaction volumes using magnetic bead-based DNA extractions with buffers prepared within the lab used where possible. A read count of 20,000 is expected for each sample, which is somewhat arbitrary and may be insufficient in cases with a high degree of predator DNA amplification. The values below are inclusive of controls (12 negative controls, 2 blank controls and 2 positive controls per plate, as per Drake et al. (2022), although some guides recommend even more frequent controls; Taberlet et al., 2018).

Sequencing platform	**Number of samples**	**Number of PCR primers**	**Number of technical replicates**	**Approximate read depth required**	**Sequencing kit/cartridge/flow cell**	**Estimated reagent cost**	**Estimated service cost**	**Cost per sample**
Illumina	10	1	1	>205,000	MiSeq Nano	£2,670	£8,135	£813.50
		2	1	>410,000	MiSeq Nano	£2,690	£8,164	£816.40
			3	>1,230,000	MiSeq V2	£3,380	£9,806	£980.60
	100	1	1	>2,050,000	MiSeq V2	£3,510	£11,830	£118.30
		2	1	>4,100,000	MiSeq V2	£3,780	£18,355	£183.55
			3	>12,300,000	MiSeq V3	£5,460	£21,990	£219.90
	1,000	1	1	>20,500,000	MiSeq V3	£7,140	£37,744	£37.74
		2	1	>41,000,000	2x MiSeq V3	£14,000	£48,838	£48.84
			3	>123,000,000	NovaSeq 6000 SP	£34,330	£93,278	£93.28
Nanopore	10	1	1	>205,000	SpotOn	£1,840	£7,305	£730.50
		2	1	>410,000	SpotOn	£1,860	£7,334	£733.40
			3	>1,230,000	SpotOn	£1,950	£8,376	£837.60
	100	1	1	>2,050,000	SpotOn	£2,080	£10,400	£104.00
		2	1	>4,100,000	SpotOn	£2,350	£16,925	£169.25
			3	>12,300,000	Promethion	£5,000	£21,530	£215.30
	1,000	1	1	>20,500,000	Promethion	£6,060	£36,664	£36.66
		2	1	>41,000,000	Promethion	£10,520	£45,358	£45.36
			3	>123,000,000	2 x Promethion	£29,780	£88,728	£88.73

5.3 Conceptual challenges and limitations

Alongside logistical barriers, dietary metabarcoding is subject to several key conceptual and technical limitations that could reduce its accuracy, sensitivity and reliability for detecting pests in the diets of predators. Most notably, the sensitivity of metabarcoding renders it highly vulnerable to false positive detections, including contaminants from the field or lab, and errors during sequencing or bioinformatics (Alberdi et al., 2019; Drake et al., 2022). These false positives can be removed using strict sample handling and lab procedures, stringent use of experimental controls and application of appropriate minimum sequence copy thresholds (Drake et al., 2022; González et al., 2023), but even this is not infallible (Littleford-Colquhoun et al., 2022; Petsopoulos et al., 2024). Accessing appropriate lab facilities that, for example, separate pre- and post-PCR workspaces to limit contamination of samples with highly concentrated amplified DNA, can be difficult for some researchers and practitioners. As well, the use of extensive stakeholder networks such as bird ringing groups and bat surveyors for sample collection, as described above, may require sample handling by those inexperienced with the sterile techniques required for molecular analyses. Even the application of strict data filtering thresholds can distort the accuracy of dietary data by increasing the rate of false negative detections (Littleford-Colquhoun et al., 2022), requiring nuanced approaches that may not always be practicable (Tercel & Cuff, 2022).

The accuracy of metabarcoding data can also be reduced by legitimate detections of indirect interactions and predator DNA, such as secondary detections, whereby the prey detected in a predator's guts may be the prey of its prey, rather than prey directly consumed by the predator (Sheppard et al., 2005; Tercel et al., 2021). For the detection of pests, this may be relatively inconsequential, but could misguide the design of monitoring programmes by focusing efforts on predators that only indirectly interact with target pests. Similarly, the lack of reliable quantitative data provided by metabarcoding (Deagle et al., 2019; Lamb et al., 2019), usually resulting in binary presence/absence records. This will obscure estimates of pest frequencies, making delineation of stochastic appearances and widespread emergences much harder, and the scale of response to detections may therefore be difficult to guide. The 'predator problem', whereby DNA of the focal predator is amplified by the PCR primers used, resulting in the loss of most of the sequencing data, is a problem specific to predator-prey molecular dietary analyses (Cuff, Kitson, et al., 2023), such as those discussed in this report. This is discussed above regarding both primer selection and economic implications, but it ultimately impacts the likelihood of detecting pests in the diet of individual predators given the associated data loss. Importantly, PCR primers that circumvent this data loss are only available for a relatively narrow range of predator taxa, demanding wider development, ideally within a standardised best practice framework.

The accessibility of appropriate expertise and equipment, as discussed in terms of economic implications, is a limitation likely to affect many end-users. Linked to this, access to curated and sufficiently comprehensive reference databases for accurate taxonomic assignment of sequences remains a challenge across all sectors. The availability of reference data has increased rapidly over recent years, but these data are error-prone, inconsistently accurate and rarely validated, introducing potential errors. Curated reference databases are available (Magoga et al., 2022; Richardson et al., 2018), but these are sporadically updated, if at all, resulting in the omissions of the rapidly growing body of new reference data available.

Software for building and curating reference databases are increasingly accessible (Jeunen et al., 2023), but these often still use unvalidated data. This represents one of the greatest challenges for metabarcoding-based monitoring, especially for emerging pests that may be poorly studied or not included in region-specific reference databases. Ultimately, standardisation of reference database use, and molecular-based biomonitoring more generally, is key to enhancing the accuracy and compatibility of adoption across contexts and regions (Iwaszkiewicz-Eggebrecht et al., 2024). These limitations are particularly pertinent where dietary detections may be used to inform policy or regulatory action, as misidentification or false positives could have disproportionate management, economic or reputational consequences.

6 Conclusion

Monitoring of plant pests is a significant challenge and requires a large amount of resourcing in its current format. Traditional monitoring methods scale poorly and are not sufficient to meet the level of monitoring required given the increased movement of species in the Anthropocene. In this review, we highlight previous successes in the molecular analysis of predator diets for detecting plant pests using DNA metabarcoding. Across the reviewed literature, three predator groups appeared most often: insectivorous bats, passerine and near passerine birds, and spiders. Across these predators, dietary metabarcoding was demonstrably effective for detecting prey. Despite few of the reviewed studies being conducted in the UK, many species from these three groups are distributed across the country, including several widely distributed across Scotland, making them suitable candidates for monitoring plant pests in Scottish forests.

The studies reviewed varied in their methods and practices, with notable variation in the DNA extraction methods used, but with some frequently used PCR primers that may be inappropriate for some of the predator groups they were used for. Illumina was the most commonly used sequencing platform but there was high variation in the downstream bioinformatics workflows. Some diversity in approaches can be attributed to different sample types and contexts, but any future monitoring programme based on this approach will need to harmonise and standardise approaches to reduce complexity for end users and ensure comparability across applications.

This review has demonstrated that monitoring plant pests through predator molecular dietary analysis is methodologically feasible and cost-effective. Additionally, we have highlighted three key predator orders that would be suitable for monitoring in Scotland. Despite this, there are still complex logistical, infrastructural and methodological challenges that need addressing before a fully-fledged monitoring scheme can be implemented. This will require validation of the approach in a Scottish forest context as well as enhanced collaboration between key stakeholder groups identified in this review.

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Appendix

Appendix 1: Great spotted woodpecker and conifer bark-boring beetles: an intuitive but complex case study

The systematic review identified one study that successfully demonstrated woodpecker-based pest detections in North American forests (Stillman et al., 2022). Researchers collected samples from nest boxes occupied by four woodpecker species (*Picoides arcticus*, *Dryobates villosus*, *Colaptes auratus*, *Dryobates albolarvatus*) across Washington and California, and detected multiple bark beetle pests including *Dendroctonus ponderosae* and *Dendroctonus valens*, which are closely related to European forest pests. This suggests that great spotted woodpeckers, *Dendrocopos major*, could be a promising candidate for Scottish pest monitoring. These birds are widely distributed across Scotland, with breeding populations in 682 out of 1,031 eligible 10 km squares (Balmer et al., 2013) and are currently expanding their range. They are a frequent natural enemy of the great spruce bark beetle, *Dendroctonus micans*, in Britain (King and Fielding, 1989), actively predating both adult bark beetles on bark surfaces and immature stages within breeding galleries. Their feeding signs serve as indicators for bark beetle presence in European spruce forests (Kautz et al., 2024), and they actively seek wood-boring invertebrates in coniferous trees during autumn-winter bark beetle activity peaks. Their foraging does, however, vary seasonally, with a focus on Lepidoptera larvae during breeding and conifer seeds when invertebrates become scarce during winter (Hogstad, 1971; Jiao et al., 2008; Stański et al., 2023; Török, 1990). This seasonal dietary variation introduces uncertainty surrounding the reliability of bark beetle detection from great spotted woodpecker diets.

Translating the success of Stillman et al. (2022) to a Scottish forestry context introduces several practical challenges. While woodpeckers use coniferous forests (Hebda et al., 2017), they can rely on deciduous woodland more frequently for nesting (Ćiković, 2014; Hanzelka et al., 2023; Pasinelli, 2007), with BTO surveys confirming deciduous woodland as their primary breeding habitat and coniferous forests ranking fourth by habitat use frequency (Massimino et al., 2025). This is confounded by their infrequent occupancy of nest boxes (Hanzelka et al., 2023), which, together with their primary use of deciduous forests, may constrain sampling and reduce their interaction with pests of commercial conifer plantations. Consequently, sample collection would necessitate non-selective capture methods such as mist netting within coniferous forests, which lack species specificity and introduce significant licensing requirements and logistical complexities. These practical limitations suggest that, despite their ecological importance as bark beetle predators, great spotted woodpeckers may be suboptimal for predator molecular dietary analysis-based pest monitoring.

Appendix 2: The diet composition of all coarse predator groups included in the systematic review as percent richness of invertebrate orders containing common plant pests.

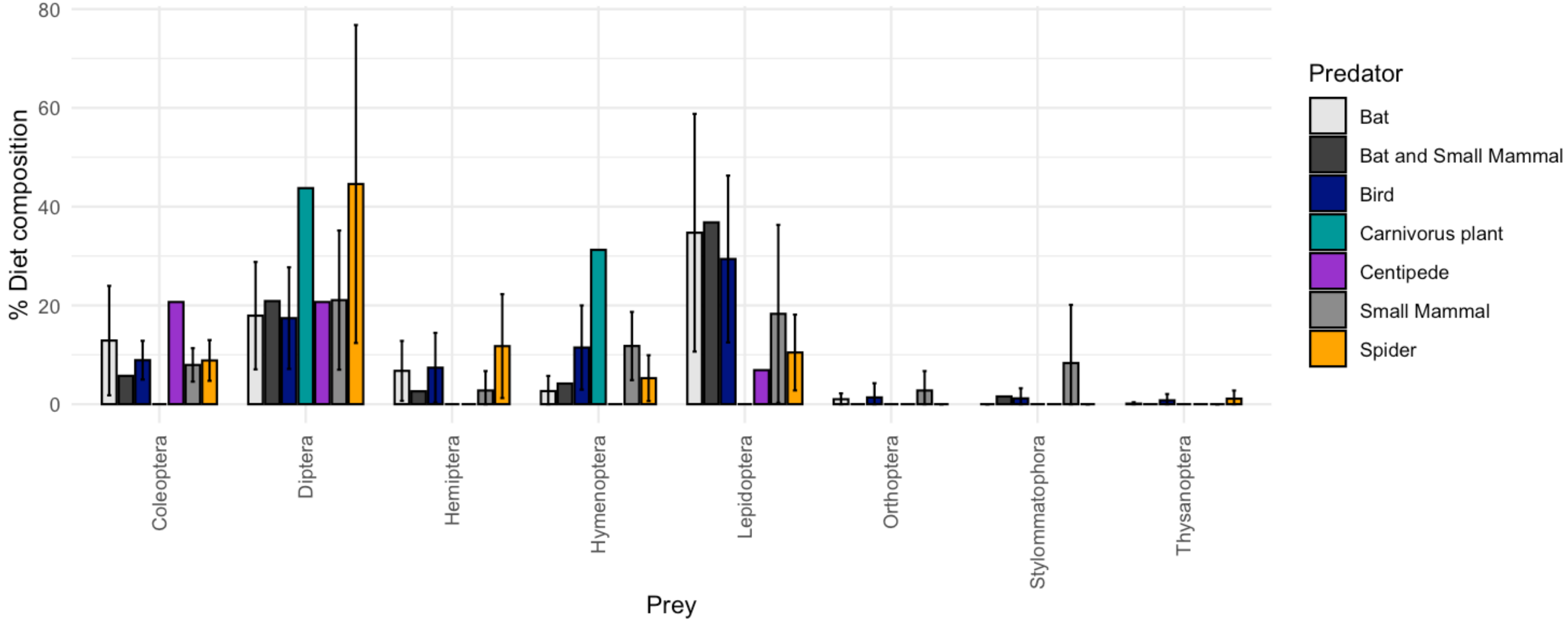

Figure A1: The diet composition of all coarse predator groups included in the systematic review as percent richness of invertebrate orders containing common plant pests. Bars represent the mean for each predator and prey combination, and error bars represent standard deviation.

Appendix 3: Predators studied across the 40 articles from which data were extracted for the systematic review

Table A1: Predators studied across the 40 articles from which data were extracted for the systematic review.

Predator order	Predator species
Bats (Chiroptera)	*Afronycteris nana*
Bats (Chiroptera)	*Artibeus intermedius*
Bats (Chiroptera)	*Artibeus jamaicensis*
Bats (Chiroptera)	*Artibeus lituratus*
Bats (Chiroptera)	*Bauerus dubiaquercus*
Bats (Chiroptera)	*Carollia perspicillata*
Bats (Chiroptera)	*Carollia sowelli*
Bats (Chiroptera)	*Chrotopterus auritus*
Bats (Chiroptera)	*Dasypterus intermedius*
Bats (Chiroptera)	*Dermanura phaeotis*
Bats (Chiroptera)	*Dermanura watsoni*
Bats (Chiroptera)	*Eptesicus furinalis*
Bats (Chiroptera)	*Eptesicus fuscus*
Bats (Chiroptera)	*Gardnernycteris keenani*
Bats (Chiroptera)	*Glossophaga soricine*
Bats (Chiroptera)	*Hipposideros diadema*
Bats (Chiroptera)	*Hipposideros dyacorum*

Bats (Chiroptera)	*Hipposideros ridleyi*
Bats (Chiroptera)	*Hipposideros ruber*
Bats (Chiroptera)	*Kerivoula hardwickii*
Bats (Chiroptera)	*Kerivoula intermedia*
Bats (Chiroptera)	*Kerivoula papillosa*
Bats (Chiroptera)	*Lasiurus borealis*
Bats (Chiroptera)	*Lasiurus cinereus*
Bats (Chiroptera)	*Lophostoma evotis*
Bats (Chiroptera)	*Mimon cozumelae*
Bats (Chiroptera)	*Miniopterus minor*
Bats (Chiroptera)	*Molossus nigricans*
Bats (Chiroptera)	*Mops condylurus*
Bats (Chiroptera)	*Mormoops megalophylla*
Bats (Chiroptera)	*Myotis austroriparius*
Bats (Chiroptera)	*Myotis bechsteinii*
Bats (Chiroptera)	*Myotis elegans*
Bats (Chiroptera)	*Myotis nattereri*
Bats (Chiroptera)	*Myotis pilosatibialis*
Bats (Chiroptera)	*Myotis sodalis*
Bats (Chiroptera)	*Natalus mexicanus*
Bats (Chiroptera)	*Nycteris spp.*

Bats (Chiroptera)	*Nycticeius humeralis*
Bats (Chiroptera)	*Otomops martiensseni*
Bats (Chiroptera)	*Perimyotis subflavus*
Bats (Chiroptera)	*Pteronotus mesoamericanus*
Bats (Chiroptera)	*Rhinolophus borneensis*
Bats (Chiroptera)	*Rhinolophus sedulus*
Bats (Chiroptera)	*Rhinolophus trifoliatus*
Bats (Chiroptera)	*Rhogeessa aeneus*
Bats (Chiroptera)	*Scotophilus dinganii*
Bats (Chiroptera)	*Sturnira parvidens*
Bats (Chiroptera)	*Trachops cirrhosis*
Bats (Chiroptera)	*Barbastella barbastellus*
Bats (Chiroptera)	*Chaerephon atsinanana*
Bats (Chiroptera)	*Chaerephon pumilus*
Bats (Chiroptera)	*Chalinolobus tuberculatus*
Bats (Chiroptera)	*Eptesicus fuscus*
Bats (Chiroptera)	*Hipposideros armiger*
Bats (Chiroptera)	*Hipposideros cervinus*
Bats (Chiroptera)	*Hipposideros larvatus*
Bats (Chiroptera)	*Ia io*
Bats (Chiroptera)	*Lasiurus seminolus*

Bats (Chiroptera)	*Miniopterus majori*
Bats (Chiroptera)	*Miniopterus manavi*
Bats (Chiroptera)	*Miniopterus schreibersii*
Bats (Chiroptera)	*Mops leucostigma*
Bats (Chiroptera)	*Mormopterus jugularis*
Bats (Chiroptera)	*Myotis altarium*
Bats (Chiroptera)	*Myotis chinensis*
Bats (Chiroptera)	*Myotis emarginatus*
Bats (Chiroptera)	*Myotis goudoti*
Bats (Chiroptera)	*Myotis laniger*
Bats (Chiroptera)	*Myotis lucifugus*
Bats (Chiroptera)	*Myotis pilosus*
Bats (Chiroptera)	*Myotis septentrionalis*
Bats (Chiroptera)	*Nyctalus leisleri*
Bats (Chiroptera)	*Nyctalus leisleri verrucosus*
Bats (Chiroptera)	*Nyctalus noctula*
Bats (Chiroptera)	*Pipistrellus madernesis*
Bats (Chiroptera)	*Pipistrellus pipistrellus*
Bats (Chiroptera)	*Plecotus austriacus*
Bats (Chiroptera)	*Pteronotus davyi*
Bats (Chiroptera)	*Pteronotus mesoamericanus*

Bats (Chiroptera)	*Pteronotus parnellii*
Bats (Chiroptera)	*Pteronotus personatus*
Bats (Chiroptera)	*Rhinolophus pearsoni*
Bats (Chiroptera)	*Rhinolophus rex*
Bats (Chiroptera)	*Rhinolophus sinicus*
Bats (Chiroptera)	*Rhynchonycteris naso*
Bats (Chiroptera)	*Rhinolophus hipposideros*
Birds (Passeriformes, Piciformes)	*Actinodura egertoni*
Birds (Passeriformes, Piciformes)	*Aerodramus sp.*
Birds (Passeriformes, Piciformes)	*Alcippe nipalensis*
Birds (Passeriformes, Piciformes)	*Alectoris rufa*
Birds (Passeriformes, Piciformes)	*Coccothraustes coccothraustes*
Birds (Passeriformes, Piciformes)	*Coturnix coturnix*
Birds (Passeriformes, Piciformes)	*Cyanistes caeruleus*
Birds (Passeriformes, Piciformes)	*Cyornis poliogenys*
Birds (Passeriformes, Piciformes)	*Dicrurus leucophaeus*
Birds (Passeriformes, Piciformes)	*Empidonax virescens*
Birds (Passeriformes, Piciformes)	*Enicurus immaculatus*
Birds (Passeriformes, Piciformes)	*Ficedula hypererythra*
Birds (Passeriformes, Piciformes)	*Ficedula strophiata*
Birds (Passeriformes, Piciformes)	*Fulvetta vinipectes*

Birds (Passeriformes, Piciformes)	*Garrulax albogularis*
Birds (Passeriformes, Piciformes)	*Garrulax erythrocephalus*
Birds (Passeriformes, Piciformes)	*Garrulax ocellatus*
Birds (Passeriformes, Piciformes)	*Garrulax subunicolor*
Birds (Passeriformes, Piciformes)	*Hirundo javanica*
Birds (Passeriformes, Piciformes)	*Hylocichla mustelina*
Birds (Passeriformes, Piciformes)	*Kittacincla malabarica*
Birds (Passeriformes, Piciformes)	*Leiothrix lutea*
Birds (Passeriformes, Piciformes)	*Macropygia unchall*
Birds (Passeriformes, Piciformes)	*Muscicapella hodgsonii*
Birds (Passeriformes, Piciformes)	*Niltava grandis*
Birds (Passeriformes, Piciformes)	*Niltava sundara*
Birds (Passeriformes, Piciformes)	*Orthotomus sutorius*
Birds (Passeriformes, Piciformes)	*Otis tarda*
Birds (Passeriformes, Piciformes)	*Parkesia motacilla*
Birds (Passeriformes, Piciformes)	*Parus major*
Birds (Passeriformes, Piciformes)	*Parus spilonotus/xanthogenys*
Birds (Passeriformes, Piciformes)	*Pellorneum ruficeps*
Birds (Passeriformes, Piciformes)	*Phoenicurus frontalis*
Birds (Passeriformes, Piciformes)	*Phylloscopus chloronotus*
Birds (Passeriformes, Piciformes)	*Phylloscopus maculipennis*

Birds (Passeriformes, Piciformes)	*Phylloscopus reguloides*
Birds (Passeriformes, Piciformes)	*Pseudominla castaneceps*
Birds (Passeriformes, Piciformes)	*Pseudominla cinerea*
Birds (Passeriformes, Piciformes)	*Pterocles alchata*
Birds (Passeriformes, Piciformes)	*Pterocles orientalis*
Birds (Passeriformes, Piciformes)	*Pycnonotus flaviventris*
Birds (Passeriformes, Piciformes)	*Pyrrhoplectes epauletta*
Birds (Passeriformes, Piciformes)	*Seicercus castaneceps*
Birds (Passeriformes, Piciformes)	*Seicercus whistleri*
Birds (Passeriformes, Piciformes)	*Setophaga caerulescens*
Birds (Passeriformes, Piciformes)	*Sitta frontalis*
Birds (Passeriformes, Piciformes)	*Stachyridopsis ruficeps*
Birds (Passeriformes, Piciformes)	*Suthara nipalensis*
Birds (Passeriformes, Piciformes)	*Tesia castaeacoronata*
Birds (Passeriformes, Piciformes)	*Tesia cyaniventer*
Birds (Passeriformes, Piciformes)	*Tetrax tetrax*
Birds (Passeriformes, Piciformes)	*Turdus boulboul*
Birds (Passeriformes, Piciformes)	*Yuhina bakeri*
Birds (Passeriformes, Piciformes)	*Yuhina flavicollis*
Birds (Passeriformes, Piciformes)	*Colaptes auratus*
Birds (Passeriformes, Piciformes)	*Dryobates albolarvatus*

Birds (Passeriformes, Piciformes)	*Dryobates villosus*
Birds (Passeriformes, Piciformes)	*Picoides arcticus*
Spiders (Araneae)	*Araneus diadematus*
Spiders (Araneae)	*Tetragnatha acuta*
Spiders (Araneae)	*Tetragnatha eurychasma*
Spiders (Araneae)	*Tetragnatha filiciphilia*
Spiders (Araneae)	*Tetragnatha montana*
Spiders (Araneae)	*Tetragnatha stelarobusta*
Spiders (Araneae)	*Pagiopalus sp.*
Centipedes (Chilopoda)	*Clinopodes flavidus*
Centipedes (Chilopoda)	*Eupolybothrus tridentinus*
Centipedes (Chilopoda)	*Lithobius validus*
Carnivorous plants (Caryophyllales)	*Nepenthes ampullaria*
Carnivorous plants (Caryophyllales)	*Nepenthes gracilis*
Carnivorous plants (Caryophyllales)	*Nepenthes rafflesiana*
Small mammals (Carnivora, Rodentia, Eulipotyphla)	*Spilogale gracilis*
Small mammals (Carnivora, Rodentia, Eulipotyphla)	*Apodemis speciosus*

Small mammals (Carnivora, Rodentia, Eulipotyphla)	*Crocidura russula*
Small mammals (Carnivora, Rodentia, Eulipotyphla)	*Sorex minutus*

Appendix 4: UK Plant Health Risk Register pest species identified in the diets of predators from studies included in the systematic review data extraction.

Table A2: UK Plant Health Risk Register pest species identified in the diets of predators from studies included in the systematic review data extraction.

Pest species	Forestry relevance?	Predator order
Acrogonia virescens	No	Passeriformes
Aproceros leucopoda	Yes	Araneae
Arrhenodes minutus	Yes	Chiroptera
Bactericera cockerelli	No	Passeriformes
Cameraria ohridella	Yes	Chiroptera
Choristoneura fumiferana	Yes	Chiroptera
Choristoneura rosaceana	Yes	Chiroptera
Chrysodeixis chalcites	No	Chiroptera
Conogethes punctiferalis	Yes	Chiroptera, Passeriformes
Creontiades pallidus	No	Chiroptera
Curculio elephas	Yes	Chiroptera
Dendroctonus ponderosae	Yes	Piciformes
Dendroctonus valens	Yes	Piciformes
Diabrotica undecimpunctata	No	Chiroptera
Drosophila suzukii	No	Passeriformes
Etiella zinckenella	No	Chiroptera
Grapholita molesta	No	Passeriformes
Grapholita packardi	No	Chiroptera
Halyomorpha halys	Yes	Chiroptera
Helicoverpa armigera	No	Chiroptera, Passeriformes
Helicoverpa assulta	No	Passeriformes
Herpetogramma licarsisalis	No	Chiroptera
Hylobius abietis	Yes	Chiroptera

Ips typographus	Yes	Chiroptera
Lobesia botrana	No	Chiroptera
Lygus lineolaris	Yes	Chiroptera
Lymantria dispar	Yes	Chiroptera, Rodentia
Malacosoma americanum	No	Chiroptera, Passeriformes
Malacosoma disstria	Yes	Chiroptera, Passeriformes
Monochamus notatus	Yes	Piciformes
Monochamus sartor	Yes	Passeriformes
Monochamus titillator	Yes	Passeriformes
Opogona sacchari	No	Chiroptera
Ostrinia nubilalis	No	Chiroptera
Platynota idaeusalis	Yes	Chiroptera
Platypus cylindrus	Yes	Araneae
Scirtothrips dorsalis	No	Passeriformes
Spodoptera eridania	No	Passeriformes
Spodoptera frugiperda	No	Chiroptera
Spoladea recurvalis	No	Chiroptera, Passeriformes
Tebenna micalis	No	Chiroptera
Thaumetopoea pinivora	Yes	Chiroptera
Thaumetopoea pityocampa	Yes	Chiroptera
Tuta absoluta	No	Chiroptera
Xylosandrus crassiusculus	Yes	Chiroptera, Passeriformes

Appendix 5: PCR primer pairs used in studies included in the systematic review and the predators that they were used for.

Table A3: PCR primer pairs used in studies included in the systematic review and the predators that they were used for.

Primer pair	Gene region	Predator group						Primer citation	Primer type
		Bats	Birds	Spiders	Small mammals	Centipedes	Carnivorous plants		
16S-MAVF/16S-MAVR with 16S-MAVBl	16S	1	0	0	0	0	0	https://doi.org/10.1111/1755-0998.12188	Mammal blocking
16S1F-deg/Ins16S_1R	16S	0	1	0	0	0	0	https://doi.org/10.1371/journal.pone.0000831, https://doi.org/10.1111/1755-0998.12265	General
Krehenwinkel_16S_F/Krehenwinkel_16S_R	16S	0	0	1	0	0	0	https://doi.org/10.1111/2041-210X.13183	Spider exclusion
Wilderlab-ZP-mt16S_F/Wilderlab-ZP-mt16S_R	16S	1	0	0	0	0	0	https://doi.org/10.1080/03014223.2023.2240711	General
Euk_1391f-EukBr	18S	0	0	0	0	0	1	https://doi.org/10.1371/journal.pone.0006372	General
Krehenwinkel_18S_F/Krehenwinkel_18S_R	18S	0	0	1	0	0	0	https://doi.org/10.1111/2041-210X.13183	Spider exclusion
Krehenwinkel_28S_F/Krehenwinkel_28S_R	28S	0	0	1	0	0	0	https://doi.org/10.1111/2041-210X.13183	Spider exclusion
ANML	COI	4	5	0	1	0	0	Folmer et al., 1994, https://doi.org/10.1111/1755-0998.12951	General

fwhF1/fwhR1	COI	1	0	0	0	0	0	https://doi.org/10.3897/mbmg.1.14625	General
fwhF2-fwhR2n	COI	1	0	0	0	0	0	https://doi.org/10.3897/mbmg.1.14625	General
LCO1490/HCO2198	COI	0	0	0	0	1	0	Folmer et al., 1994	General
LCO1490/NoAranR	COI	0	0	1	0	0	0	Folmer et al., 1994, https://dx.doi.org/10.1016/j.baae.2021.09.006	Spider exclusion
LCO1490/ZBJ-ArtR2c	COI	1	0	0	0	0	0	Folmer et al., 1994, https://doi.org/10.1111/j.1755-0998.2010.02920.x	Bat exclusion
LepF1/ZBJ-ArtR2c-deg	COI	0	1	0	0	0	0	https://doi.org/10.1073/pnas.0406166101, https://doi.org/10.1111/j.1755-0998.2010.02920.x	Bat exclusion
MG-LCO1490/MG-univR	COI	2	0	0	0	0	0	https://doi.org/10.1016/j.mambio.2015.08.002	General
mlCOIintF-XT/jgHCO2198	COI	1	0	0	0	0	0	https://doi.org/10.7717/peerj.4705, https://doi.org/10.1111/1755-0998.12138	General
mlCOIintF/C1-N-2191 (Nancy)	COI	0	2	0	0	0	0	https://doi.org/10.1186/1742-9994-10-34, https://doi.org/10.1093/aesa/87.6.651	Bird exclusion
mlCOIintF/dgHCO2198	COI	2	0	0	0	0	0	https://doi.org/10.1186/1742-9994-10-34, https://doi.org/10.1046/j.1095-8312.2003.00197.x	General

Wilderlab-CI-COI_F/Wilderlab-CI-COI_R	COI	1	0	0	0	0	0	https://doi.org/10.1080/03014223.2023.2240711	General
ZBJ-ArtF1c/ZBJ-ArtR2c	COI	11	2	1	2	0	0	https://doi.org/10.1111/j.1755-0998.2010.02920.x	Bat exclusion
ZBJ-ArtF1c/ZBJ-ArtR2c with Aradia-R-blk-C3	COI	0	0	1	0	0	0	https://doi.org/10.1111/j.1755-0998.2010.02920.x, https://doi.org/10.1002/ece3.7659	Spider blocking

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