Evaluating the potential of molecular dietary analysis of predators for the detection of emerging plant pests

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Abstract

Monitoring plant pests is crucial for maximising yields across agricultural and forest production systems, but also for the mitigation of invasive species spread. Traditional monitoring methods, such as mass trapping and direct observation, scale poorly and introduce latency between collection, detection and response. Since many plant pests are frequently consumed by predators, molecular dietary analyses like dietary DNA metabarcoding could provide effective, scalable and passive monitoring of emerging and established pests.

To understand the efficacy of molecular dietary analysis for pest monitoring, how it can be implemented and the likely obstacles to its success, we conducted a systematic review of the literature to extract information from studies focused on the detection of

pests using molecular dietary analysis of predators. Of an initial 667 potential studies, data were extracted from 45. Across eight broad predator groups, these studies demonstrate the efficacy of dietary DNA metabarcoding for detecting a range of potential pest groups, alongside the methodological variation in current applications. Insectivorous bats, passerine and near-passerine birds and web-building spiders were the focal predators of most studies, with critical gaps across broader taxonomic groups. We exemplify the application of dietary metabarcoding to detecting pests in these three predator groups through case studies. Dietary metabarcoding of predators presents a viable and efficacious monitoring tool for plant pests and could become an integral component of the wider biomonitoring toolbox. For a fully robust monitoring approach using this method, we must, however, streamline implementation, circumvent methodological pitfalls and expedite the generation of usable monitoring datasets.

Keywords: biomonitoring, ecological interactions, metabarcoding, molecular diagnostics, network ecology, trophic interactions

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, forestry, and horticulture, and the increasingly frequent introduction of non-native pests to those systems (Montgomery et al., 2023; 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 pest species is paramount, particularly 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 (Hawthorne et al., 2024; Petsopoulos et al., 2024), or rely on invasive methods which involve destructive sampling of host plants (e.g., tree felling). This introduces significant latency to detections, hindering eradication efforts. New technologies and techniques, 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 various origins (Taberlet et al., 2012). By rapidly analysing large mixed samples, metabarcoding enables rapid identification of organisms from large volumes of biological material that would otherwise require laborious morphological examination by highly trained expert taxonomists (Hawthorne et al., 2024; Yu et al., 2012). Whilst the application of DNA metabarcoding to existing monitoring schemes holds great promise (Cuff 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 (Hawthorne et al., 2024), dietary metabarcoding

leverages predators as biological samplers (Cuff, Tercel, et al., 2024; Melcher et al., 2024), 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, Windsor, 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 (Chaves et al., 2026; 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 (Cuff, Gajski, et al., 2024). Exploiting the targeted foraging ecology of predators seeking their prey can provide continuous, passive surveillance across large areas reducing the aforementioned limitations of bulk sample metabarcoding (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 monitor emerging and established pests. Through a systematic review of the literature, we identify existing examples of pest detection using dietary metabarcoding and assess the technical, logistical and conceptual benefits, variations and challenges therein. Using this as a foundation, we highlight three key case studies that could effectively integrate this approach and discuss decisions that may influence its efficacy. 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 established and emerging pests.

Systematic review methods

A systematic review of the literature was conducted in accordance with PRISMA guidelines. Pre-defined search terms were queried against recognised literature databases to identify literature concerning the application of molecular methods to the analysis of pests in the diets of predators. 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, 612 were removed when screening titles and abstracts for article relevance and a further 10 were removed due to irrelevance during data extraction, leaving 45 studies for the final synthesis (Figure 1). Each study was screened for predator species, geographic location and habitat context, pest species detected, capture and sampling methods, molecular methods (DNA extraction, PCR primers, sequencing platform) and reference databases used.

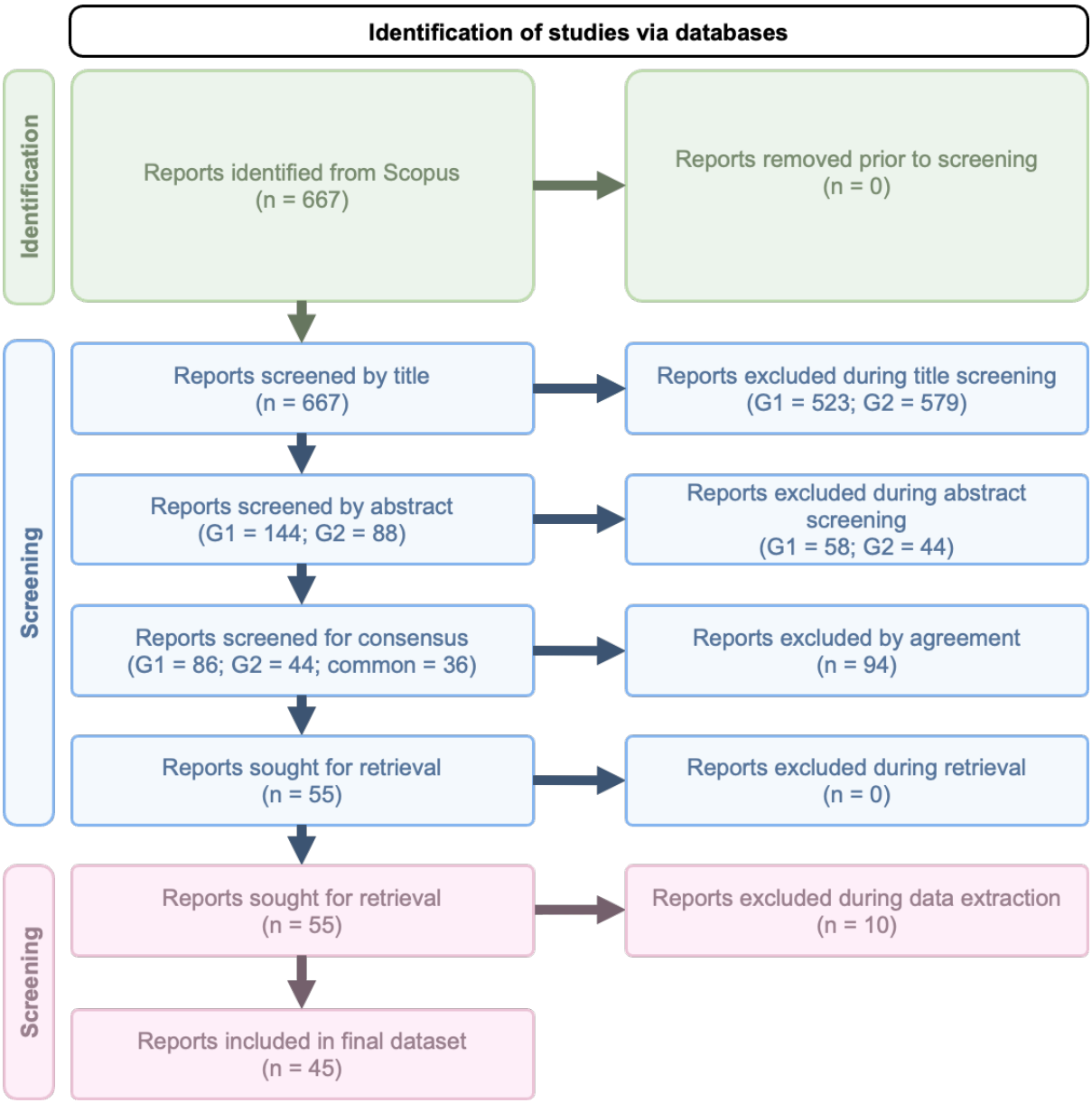

Figure 1: PRISMA workflow of the systematic review indicating the number of articles included and excluded at each stage of the review. Each stage was conducted independently by two teams of balanced expertise and scope, and articles and data extraction confirmed by consensus.

Synthesis of findings

The 45 articles from which data were extracted included studies from 26 countries across 6 continents (Figure 2). The studies were conducted in forest (31 studies; 68.9

%), agricultural (6, 13.3 %), mixed forest and agricultural (6, 13.3 %), urban park (1, 2.2 %) and various unspecified (1, 2.2 %) habitats.

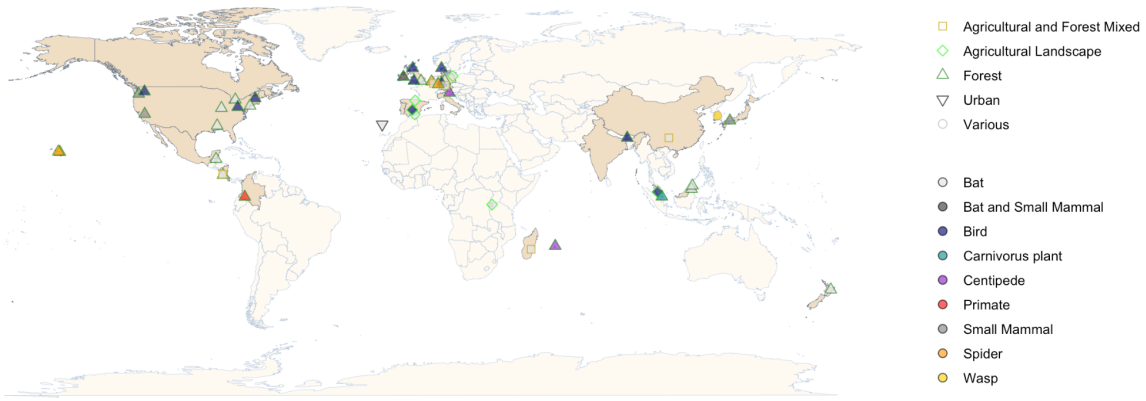

Figure 2: Map of study locations for the 45 papers included in the review, with points filled based on the focal predator of the study and outlined based on the focal habitat type.

Predators studied

Eight coarse groups of consumers comprising 167 species were retained in the final data extraction, including bats (Chiroptera; 88 species), birds (Passeriformes, Piciformes; 58), spiders (Araneae; 7), small mammals (Rodentia, Carnivora and Eulipotyphla; 5), centipedes (Geophilomorpha, Lithobiomorpha, Scolopendromorpha; 4), carnivorous plants (Caryophyllales; 3), wasps (Hymenoptera; 1) and primates (Primates; 1; Figure 3; Table A1). Bats were the most common focal predator group of the studies (23 studies; 51.1 %), followed by birds (10; 22.2 %), spiders (4; 8.9 %) and small mammals (4; 8.9 %). 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 notably low arthropod predator diversity within articles retained for data extraction is 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.

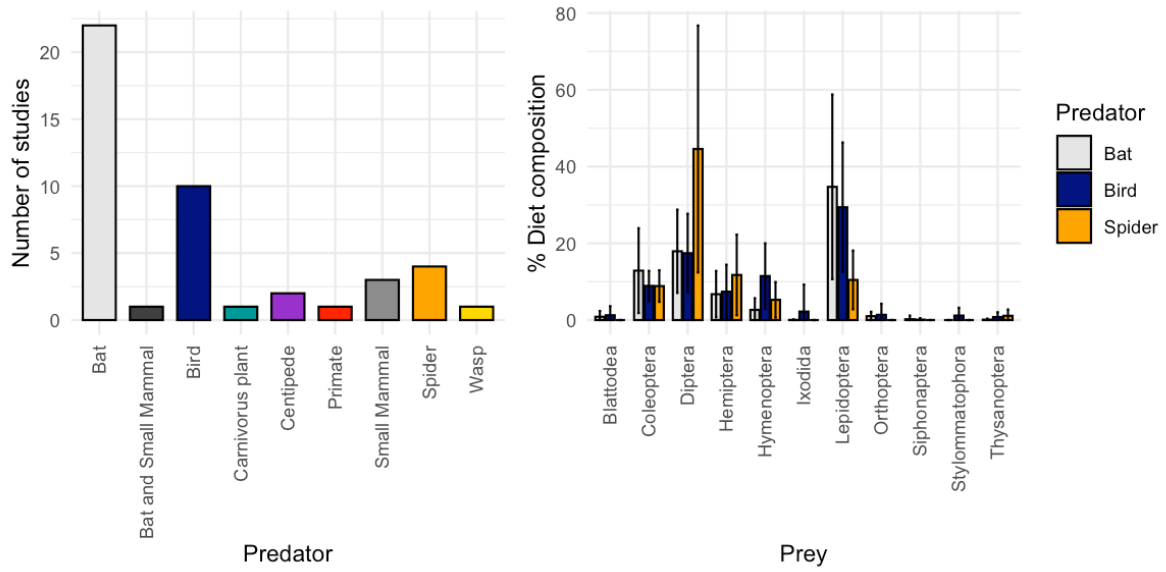

Figure 3: Left: The number of articles that included each of seven coarse predator groups, including the parallel analysis of bat and small mammal diets in one study. Right: The diet composition of three focal predator groups from the systematic review as percent richness of invertebrate orders containing common plant pests. One study was excluded from this plot due to the absence of invertebrate detection data. The same data with the addition of other predator groups can be viewed in Figure A1.

Pest detections

The richness of prey detected was likely influenced by the diversity of prey available and the variable ecologies of those species but can provide some guidance for the selection of suitable predators for monitoring of particular pest groups. Detection of prey orders varied markedly across predator groups (Figure 3; 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 guide monitoring programme design, but more taxonomically comprehensive analyses across a broader range of contexts may be required to explore the potential of different predators for pest monitoring programmes fully.

Sampling methods

Eleven distinct sampling 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 (35.5 %), reflecting the prevalence of aerial predator-focused studies.

Of the predator groups, bat samples were collected using the greatest diversity of methods, with mist nets being most common (52.3 % of bat-focused studies), followed by guano traps (34.8 %) and harp traps (17.4 %). The prevalent use of guano traps, which are typically a plastic sheet laid under or near roosts, indicates the widespread use of non-invasive sampling methods. Bats are often legally protected, necessitating non-lethal methods, but even non-invasive guano collection methods may require

licensing and compliance with protective legislation. The extensive requirements of licensing (particularly for mist-netting and harp trapping) could significantly contract the pool of appropriately qualified personnel for sample collection. To minimise or mitigate this licensing barrier, monitoring could draw upon existing pools of licenced bat surveyors to collect guano.

Bird samples were collected primarily using mist nets (40 %) and nest box monitoring (40 %), with minimal use of guano traps (10 %). The use of nest boxes suggests a focus on breeding ecology, where nest box access facilitates repeated sampling from known individuals or set locations. As is the case with bats, wild birds are often legally protected, and the licensing requirements associated with mist netting could similarly restrict the pool of qualified personnel available for guano sample collection. To minimise or mitigate this licensing barrier, monitoring schemes could draw upon a pool of appropriately licenced bird ringers which may have established sites for target species and/or established nest box networks, which are surveyed or trapped regularly throughout the breeding season. Nest boxes may be the most streamlined source of guano given the reduced risk to birds, suitability for continuous monitoring and the existence of established nest box sites and transects globally.

Across the systematic review, small mammals were often live trapped (50 %), or their faeces were hand collected (25 %) or located by detection dogs (25 %). Small mammals are often protected by wildlife legislation, requiring licenses for trapping and sample collection. Where the target species is not protected and trapping is part of pest control activities (e.g., rats), fewer restrictions may apply, although consultation with current legislation is still advised. For invasive species, such as the white-toothed shrew, *Crocidura russula* (Hermann, 1780), included in one reviewed study (Browett et al., 2021), additional legislative restrictions may apply which could prohibit the

release or transport of invasive animals, and legal powers or licenses may exist for the management and control of the species. Such cases usually require specific trapping methods, but could be an accessible means for sampling small mammals.

Ground-dwelling arthropod predators, including spiders and centipedes, were exclusively hand collected in the reviewed studies, as were carnivorous plants. Unless protected as, for example, red-listed species, there are few legislative barriers to the 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.

Molecular methods

Across the 45 studies reviewed, 15 DNA extraction methods were used, often reflecting the focal sample types, including faeces, partial predator bodies and plant material. Faecal and soil extraction protocols, used in 30 cases (66.7 %), were most prevalent likely due to the frequency of bat guano studies since extraction kits designed for soil or faeces usually reduce the prevalence of PCR inhibitors otherwise abundant in faeces. Together, these represented 73.9 % (17 of 23) of bat, 100 % (10) of bird and 50 % (2 of 4) small mammal dietary studies, respectively. Blood and/or tissue extraction kits were used in ten studies (22.2 %), including all of the arthropod-focused studies, 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 the use of professional services, which may reflect

cost-benefit considerations of developing in-house capability or technical expertise requirements, although often at the expense of transparent detailed reporting of the methods used.

All but three of the studies that reported their methods used spin-column-based DNA extractions, which are user-friendly, compatible with standard equipment and cost-effective at smaller scales. The time and labour associated with such kits can limit the scalability and throughput of analysis though, unless using user-prepared buffers and third party plasticware. Magnetic bead-based protocols are increasingly commonplace (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.

Of the gene regions used, cytochrome c oxidase subunit I (COI) was most prevalent (47 of the 72 PCR primer pair applications; 65.3 %), followed by 16S (5, 6.9 %), 18S (4, 5.6 %) and 28S (1, 1.4 %), alongside primers not targeting arthropod prey (e.g., trnL and rbcL for plant metabarcoding). PCR primers targeting other taxa (e.g., plants, vertebrates) are not discussed further given their irrelevance to plant pest monitoring. Given the highly populated reference databases available for COI, it has been used in most animal barcoding and metabarcoding studies since the method's inception (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).

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). Predator exclusion primers were used in 25 of 72 cases (34.7 %).

The most commonly used primer pair was ZBJ-ArtF1c/ZBJ-ArtR2c, which, alongside slightly modified variants and use of one or the other primer in combination with another primer, accounted for 19 of 72 primer pairs used (26.4 %; 40.4 % of COI uses). It is noteworthy that ZBJ-ArtF1c and 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 most of the reviewed studies conducted bat dietary analysis, but it is also notable that these primers were applied to 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 without the benefit of predator exclusion when applied to non-bat predators, potentially resulting in minimal yet biased prey data.

Multi-marker and multi-locus metabarcoding, the use of multiple PCR primer pairs within or across genetic loci for the same samples, were conducted in 18 studies (40 %). Multi-marker metabarcoding can be beneficial for cross-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).

Illumina sequencing was used across most of the reviewed studies (41 studies; 91.1 %), the only exceptions being older Ion Torrent systems (4; 8.9 %). Sequencing depths were, however, very inconsistently reported and highly variable, with potential implications for recovery of prey data. Variation in bioinformatic processes was, however, high, with many studies using existing bioinformatics pipelines such as Obitools (Boyer et al., 2016), DADA2 (Callahan et al., 2016) and QIIME2 (Bolyen et al., 2019). Most studies used NCBI's GenBank or BOLD for reference databases to assign taxonomy to sequences (40 studies; 88.9 %), with only one using a custom database constructed from samples collected during the study. Only three studies (7.5 %) used curated reference databases, which can reduce errors during taxonomic assignment caused by incorrectly identified or otherwise false records. Similarly, the filtering of data to remove false positives varied from stringent % read count thresholds

(Drake et al., 2022) and use of experimental controls to no filtering due to concerns about false negatives (Littleford-Colquhoun et al., 2022) nor any reported controls.

Altogether, the variation in methods 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 due to different data standards. 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.

Exemplary case studies

Three predator groups serve as suitable examples of how molecular dietary analysis can be applied to pest monitoring across our review: insectivorous bats, passerine and near-passerine birds and web-building spiders (Figure 4). These examples offer important insights into how pest detection and monitoring through trophic interactions might function in practice, forming the foundation for further applications. A basic understanding of the trophic ecology of species within these groups, informed by empirical research, natural history and pilot studies, could be used to guide monitoring of emerging and established pests through their dietary analysis.

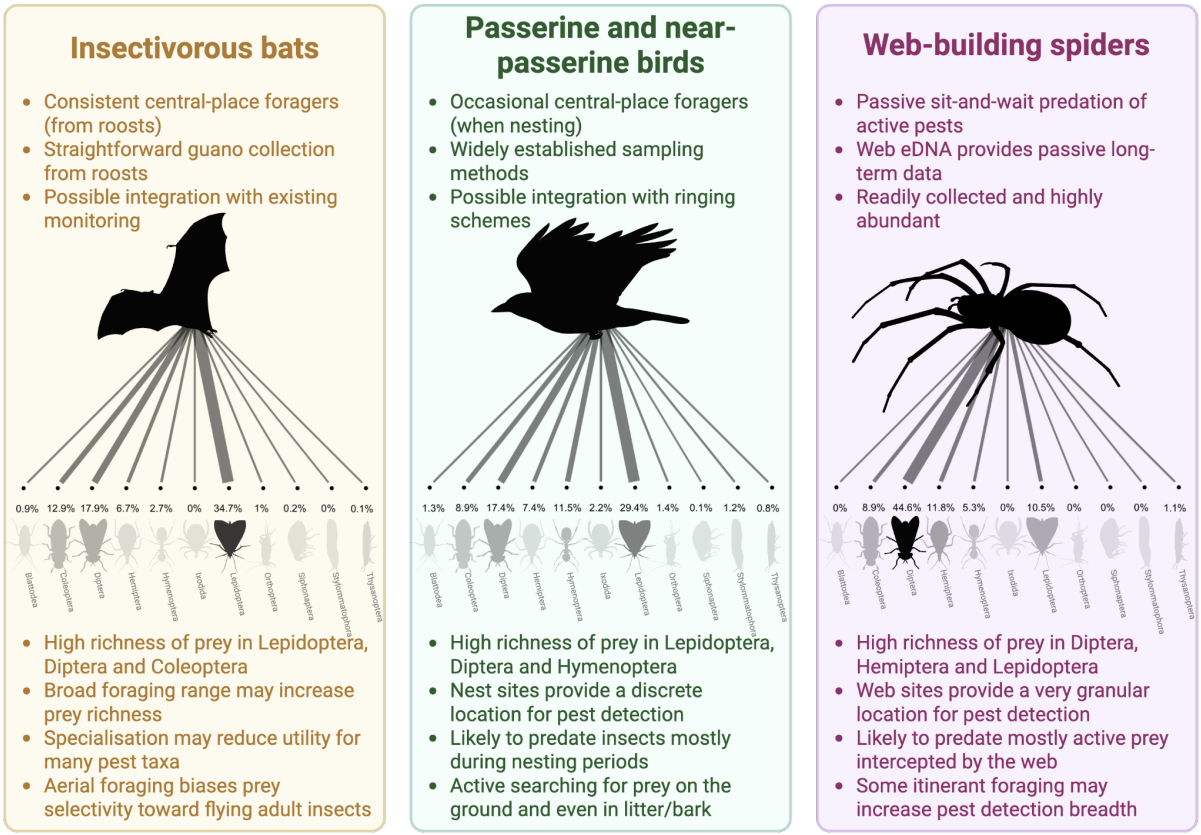

Figure 4: The three highlighted case study groups, some considerations for their use and the relative richness of prey orders according to articles included in the systematic review. One bat study was excluded from the prey richness visualisation due to the absence of prey detection data. Created in BioRender. Cuff, J. (2026) https://BioRender.com/f2txmsi

Insectivorous bats

The use of bats for pest monitoring offers potential to target a range of larger Lepidopteran pests by leveraging existing bat roost/box networks. Roost collection of guano could be integrated into existing monitoring and, as central-place foragers with ranges often exceeding a kilometre (Entwistle et al., 1996; Racey & Swift, 1985), detections can be localised but represent large areas, streamlining monitoring efforts. Leisler's bat (*Nyctalus leisleri*) and the brown long-eared bat (*Plecotus auritus*; BLEB)

are examples of insectivorous bats highlighted by this review as potentially viable pest monitoring targets. Leisler's bat predates prominent pests including the spongy moth (*Lymantria dispar*; Bourlat et al., 2023), although their roosts are relatively cryptic, constraining their identification and long-term monitoring (Boston et al., 2020; Ruczyński & Bogdanowicz, 2005). BLEBs, however, roost in deciduous and mixed woodlands, using natural cavities, wood crevices, nest-boxes and bat boxes with high annual fidelity (Boye & Dietz, 2005; Entwistle et al., 1997; Meschede et al., 2002), whilst consuming many Lepidoptera and Diptera (Hollyfield, 1993; Razgour et al., 2011; Robinson, 1990). Although many bats are generalists, larger moths (e.g., Noctuidae and Geometridae) are commonly consumed (Andreas et al., 2012; Hurpy et al., 2025; Razgour et al., 2011). This generalism may result in inconsistent detection of emerging pests, but may reduce the likelihood of neophobia toward emerging pests (Crane and Ferrari, 2017).

Passerine and near-passerine birds

As central-place foragers when nesting, many passerines and near-passerines forage within 50 m of nest sites (Naef-Daenzer & Keller, 1999; Stauss et al., 2005), which can localise detections with great accuracy. Beyond breeding seasons, nesting birds 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. The reviewed literature suggests that blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*), both abundant and widespread woodland birds (Musgrove et al., 2013; Balmer et al., 2013), are effective targets for pest monitoring. 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 commonly predate Tortricidae, Geometridae and Noctuidae moths (Höhn et al., 2024; Nour et al., 1998; Shutt et al., 2020), including prominent pests such as pine processionary moth, *Thaumetopoea pityocampa* (Gonzalez Cano, 1981; Pimentel & Nilsson, 2007), and oak processionary moth, *Thaumetopoea processionea* (LIFE Oak Processionary, 2020). As secondary cavity-nesters, they readily colonise artificial nest boxes (Minot & Perrins, 1986; Perrins, 1980), which can facilitate sampling. If linearly distributed, nest box transects could also provide an opportunity to track pest emergence.

Web-building spiders

Web-building spiders often passively intercept aerial prey, facilitating predation of a broad 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 many spiders can strongly reflect the prey available to them (Cuff, Tercel, et al., 2024; Melcher et al., 2024). Molecular dietary analysis of web-building spiders is therefore well-placed to monitor pests, especially since these spiders are widespread across a broad range of habitats on almost every land mass (Hesselberg, and Gálvez, 2023). In the reviewed literature, the cross spider (*Araneus diadematus*) predated pests such as the oak pinhole borer (*Platypus cylindrus*) and elm zigzag sawfly (*Aproceros leucopoda*; Van Schrojenstein Lantman et al., 2021), with their wider diet including small Diptera, Hemiptera (e.g., aphids), Coleoptera and Hymenoptera (Nyffeler & Bonte, 2020; Van Schrojenstein Lantman et al., 2021). Hand collection of spiders could be easily integrated with collection of other samples given the relative ease of finding and collecting them and the lack of legislative barriers to

their collection. This system is likely the most cost-effective to establish of those highlighted, especially if paired with existing established networks for logistical streamlining.

Implementation of predator molecular dietary analysis for pest detection

Monitoring design decisions

Implementing the above case studies requires careful consideration of the suitability of this approach (Figure 5) and the optimal predator group to target, asthe accuracy of pest detection could impact policy and practice. If the target pest is readily collected in targeted traps (e.g., pheromone traps), the cost of molecular analysis may be unnecessary, though this relies on relevant expertise being readily accessible. Where this is not the case, investment in molecular infrastructure for monitoring may be beneficial. For large scale monitoring (e.g., hundreds or thousands of collection events), molecular methods may be more cost effective and rapid. If taxonomic expertise is available, non-targeted bulk sampling methods may capture target pests, and can also be integrated with molecular analyses like DNA metabarcoding to expedite accurate taxonomic identification (Hawthorne et al., 2024), which may be more appropriate if predation of a particular pest is rare.

Prey-specific diagnostic assays, which use PCR or qPCR to detect specific target species, could be more effective and cheaper than metabarcoding, especially when monitoring one or few known pests (Rennstam Rubbmark et al., 2019). They eliminate sequencing costs and provide straightforward presence/absence data without bioinformatic analysis. Metabarcoding is, however, applicable when data are required for broad communities of potentially unexpected species, making it ideally suited to

continuous passive monitoring for emerging pests. Optimal PCR primers will depend on the focal predator and target pests, but should ideally be validated *in silico* and *in vitro* (Elbrecht et al., 2019; Elbrecht & Leese, 2017; Piñol et al., 2019). Amplification of the predator itself, as discussed above, could reduce the accuracy of monitoring (Cuff, Kitson, et al., 2023), circumvented in some of the reviewed bat studies by using bat-exclusion primers (e.g., ZBJ-ArtF1c/ZBJ-ArtR2c; Zeale et al., 2011). Misapplication of exclusion primers can, however, exacerbate the predator amplification problem and introduce biases (Cuff et al., 2021; Krehenwinkel et al., 2019).

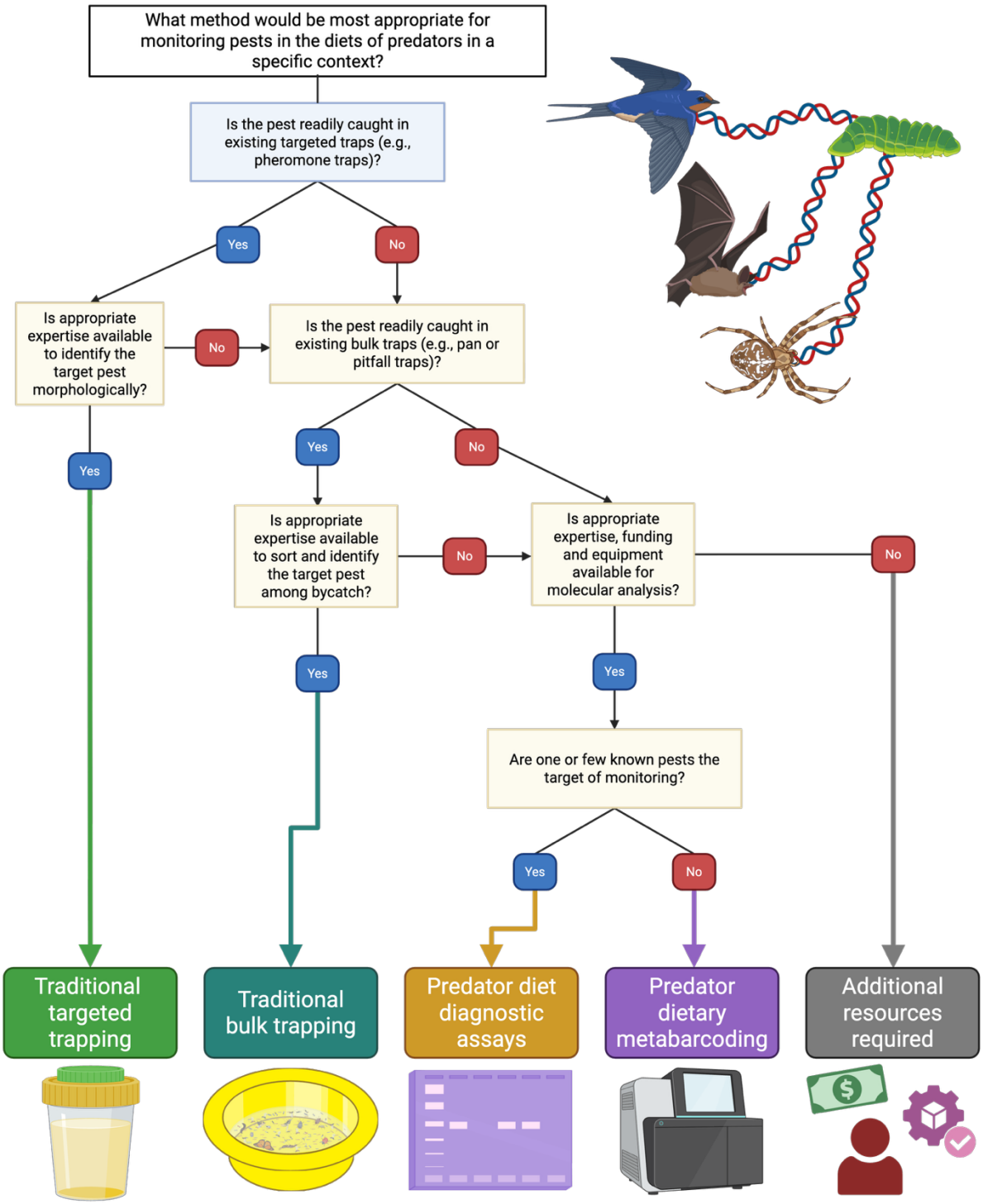

Figure 5: A flow diagram to inform selection of an appropriate method for monitoring plant pests based on information synthesised in this review. Created in BioRender. Cuff, J. (2026) https://BioRender.com/f2txmsi

Selecting a focal predator for monitoring will largely depend on the target pests, the relevant life stage of the pests and the life stage of the predators. For pest 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). For pest orders primarily predated by birds, in non-nesting periods, when birds may feed more frequently on plant material, bats and spiders may be more viable. Spiders are often well-placed to consume smaller 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. Importantly, monitoring need not rely on a single predator group; for example, Chaves et al. (2026) demonstrated that birds and bats exhibit a high degree of pest prey complementarity, which would translate to complementarity for monitoring purposes. Whilst understanding the trophic niches of these predators can guide monitoring programme design, interactions change across space, time and in response to biotic and abiotic changes. It is therefore crucial that predator selection is based on a sound fundamental understanding of the drivers of interactions under different conditions, rather than the identities of interacting partners alone. This requires development of our understanding of fundamental foraging ecology, which, in turn, requires wider research related to the dynamic mechanisms driving interactions.

Economic implications

Pest monitoring through the molecular analysis of predator diets may be constrained by access to equipment, expertise and funding. Some or all of the molecular analysis can be outsourced, but this often increases costs. 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. Sequencing is often the most expensive component of molecular-based monitoring, but smaller units of sequencing are usually sufficient to analyse many samples, making the costs highly scalable. Collection of samples for molecular analysis using DNA- and DNase-free materials may incur additional costs, and even preserving, storing and archiving DNA for prolonged periods incurs energy and infrastructure costs. The costs of molecular analysis ultimately vary with access to equipment, expertise and competitive pricing of consumables and external services. Costs will, however, increase for research labs that are not set up or experienced in implementing these protocols, especially for validation and optimisation of processes. 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 increased sequencing depth. Similarly, multi-marker metabarcoding can multiply costs, but can also mitigate some of the taxonomic biases imposed by PCR primers.

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 can vary massively in cost, but sequencers are likely the most expensive equipment required. Nanopore sequencers, however, require much smaller capital investment, although commercial provision of Nanopore sequencing may require the provider to invest in larger sequencers such as the GridIon or Promethion. For less experienced end-users, hiring of appropriate expertise or external services will also incur costs. Analysis of sequencing data can also be computationally expensive, often performing best on high-performance computing clusters or high-specification computers that cost significantly more than standard

computers. To circumvent these costs, species-specific diagnostic assays, discussed above, are significantly cheaper per sample, making them cost-effective for routine monitoring of known pest species at scale. Such assays still require validation or development of novel assays, making them most economical when monitoring pests for which such monitoring is already established.

Conceptual challenges and limitations

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, 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, 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 (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, will obscure estimates of pest frequencies, obscuring stochastic appearances and widespread emergences. The 'predator problem', whereby DNA of the focal predator is amplified by the PCR primers used, results in the loss of sequencing data (Cuff, Kitson, et al., 2023), impacting the likelihood of detecting pests. Poor access to curated and sufficiently comprehensive reference databases for accurate taxonomic assignment of sequences results in error-prone, inconsistently accurate and rarely validated reference data, introducing potential detection gaps and errors. 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).

Conclusions

Previous successes in the molecular analysis of predator diets for detecting plant pests using DNA metabarcoding demonstrate the efficacy of this approach, particularly for insectivorous bats, passerine and near passerine birds, and web-building spiders.

The studies reviewed varied in their methods and practices, with notable variation in DNA extraction methods and PCR primers, largely attributed to differences in sample types and contexts. Wider implementation of this approach in future monitoring programmes may need to harmonise and standardise approaches to ensure comparability across applications. Whilst the methodological feasibility is evidenced across the literature, there are complex logistical and methodological challenges that may complicate wider implementation, and validation within a specific monitoring context is currently lacking. Dietary metabarcoding of predators does, nevertheless, present a viable and efficacious monitoring tool for plant pests and could become an integral component of the wider biomonitoring toolbox.

Author contributions

Kyle A. Miller: Conceptualization; data curation; funding acquisition; project administration; writing – review and editing; writing – original draft. Molly Davidson: Conceptualization; data curation; funding acquisition; writing – review and editing; writing – original draft. Christopher Hirst: Conceptualization; data curation; funding acquisition; visualisation; writing – review and editing; writing – original draft. Alice Walker: Conceptualization; data curation; funding acquisition; project administration; writing – review and editing. James J.N. Kitson: Conceptualization; data curation; funding acquisition; writing – review and editing; writing – original draft. Jordan P. Cuff: Conceptualization; data curation; funding acquisition; visualisation; writing – review and editing; writing – original draft.

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Data availability

All data are openly available via Zenodo: https://doi.org/10.5281/zenodo.20432027

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Evaluating the potential of molecular dietary analysis of predators for the detection of emerging plant pests - Supplementary materials

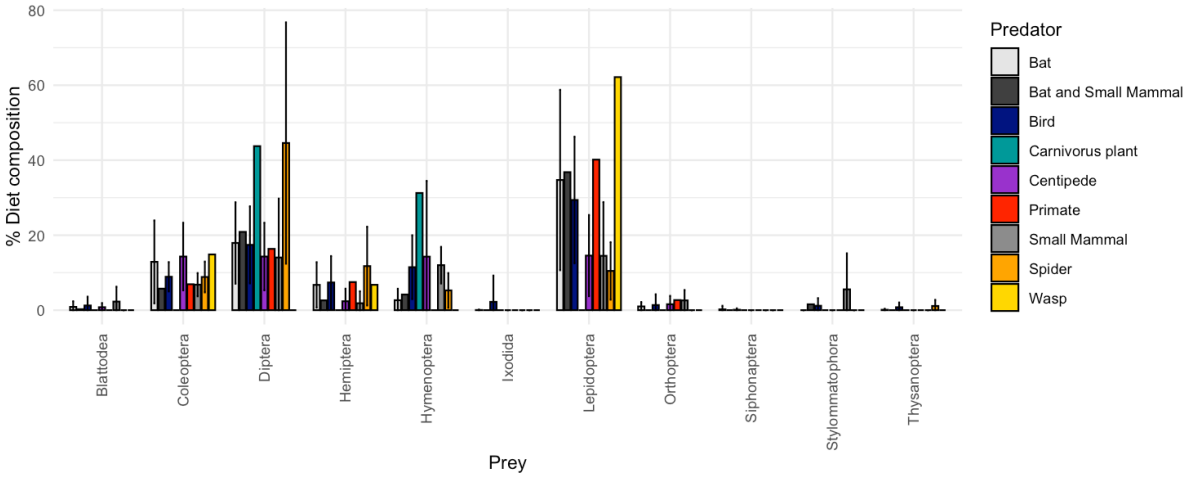

Figure A1: The diet composition of all coarse predator groups included in the systematic review as percent richness of invertebrate orders containing common plant pests.