

Comparing three types of dietary samples for prey DNA decay in an insect generalist predator

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Abstract

The rapidly growing field of molecular diet analysis is becoming increasingly popular among ecologists, especially when investigating methodologically challenging groups, such as invertebrate generalist predators. Prey DNA detection success is known to be affected by multiple factors; however, the type of dietary sample has rarely been considered. Here, we address this knowledge gap by comparing prey DNA detection success from three types of dietary samples. In a controlled feeding experiment, using the carabid beetle *Pterostichus melanarius* as a model predator, we collected regurgitates, faeces and whole consumers (including their gut contents) at different time points postfeeding. All dietary samples were analysed using multiplex PCR, targeting three different length DNA fragments (128, 332 and 612 bp). Our results show that both the type of dietary sample and the size of the DNA fragment contribute to a significant part of the variation found in the detectability of prey DNA. Specifically, we observed that in both regurgitates and whole consumers, prey DNA was detectable significantly longer for all fragment sizes than for faeces. Based on these observations, we conclude that prey DNA detected from regurgitates and whole consumers DNA extracts are comparable, whereas prey DNA detected from faeces, though still sufficiently reliable for ecological studies, will not be directly comparable to the former. Therefore, regurgitates and faeces constitute a useful, nonlethal source for dietary information that could be applied to field studies in situations when invertebrate predators should not be killed.

KEYWORDS

carabidae, diagnostic PCR, molecular diet analysis, prey detection, trophic interactions

1 | INTRODUCTION

DNA-based diet analysis is rapidly being employed as a widespread tool for empirically characterizing diet and trophic interactions in a broad range of vertebrates and invertebrates (Clare, 2015; Traugott, Kamenova, Ruess, Seeber, & Plantegenest, 2013). DNA-based methods for diet analysis typically rely on the detection of short fragments of prey DNA, recovered from predator's gut contents (e.g., Leray, Meyer, & Mills, 2015; Mollot et al., 2014) or other types of dietary samples such as faeces, regurgitates or whole consumers

(e.g., Ibanez et al., 2013; Kartzinel et al., 2015; Thalinge et al., 2016; Wallinger et al., 2015). The success of DNA-based approaches to analyse trophic interactions is mainly due to the fact that they allow the direct and accurate identification of trophic links from a minute amount of starting material, even for very small-sized organisms such as mites (Pérez-Sayas et al., 2015) or zooplankton (Durbin, Casas, & Rynearson, 2012). Furthermore, the rapid growth of public sequence databases and methodological improvements in detection sensitivity and high-throughput technology offer time- and cost-effective procedures applicable to a great variety of ecological

systems and to large sample sizes (e.g., Pompanon et al., 2012; Sint, Raso, Kaufmann, & Traugott, 2011; Valentini et al., 2009).

DNA-based diet analysis is particularly valuable for studying invertebrate generalist predators (Symondson, 2012). Indeed, DNA methods offer a sensitive and flexible alternative to traditional behavioural or dissecting techniques that often fail to detect prey that does not leave hard remains in these cryptic liquid feeders (Traugott et al., 2013). DNA techniques are, however, also subject to bias, and prey DNA detection success could be hampered by a variety of factors among which the type of dietary sample could play an important role (King, Read, Traugott, & Symondson, 2008; Pompanon et al., 2012; Traugott et al., 2013). In the case of arthropods, whole-body extracts are usually the most convenient source of dietary DNA that avoid laborious dissections. Besides the drawbacks of a lethal approach (e.g., sacrificing rare or endangered species, affecting population dynamics), whole-body extracts may pose additional challenges especially in the case of DNA metabarcoding diet analysis. As DNA metabarcoding combines general primers and high-throughput sequencing, the concomitant amplification of consumer DNA usually compromises the detection success of scarcer and degraded prey DNA (e.g., Piñol, San Andrés, Clare, Mir, & Symondson, 2014; Shehzad et al., 2012).

Waldner and Traugott (2012) demonstrated that regurgitates, a fluid mixture containing semidigested prey remains and digestive enzymes, obtained from predatory carabid beetles provided superior prey DNA detection rates compared to whole-body DNA extracts. Another prospective source of food DNA are faeces, although their use as a dietary source in invertebrates is still uncommon (e.g., Ibanez et al., 2013; Redd, Ling, Frusher, Jarman, & Johnson, 2014; Sint, Thurner, Kaufmann, & Traugott, 2015). Usually, both regurgitates and faeces seem to provide similar or better detection rates compared to whole-body extracts (Durbin et al., 2012; Egeter, Bishop, & Robertson, 2015; Unruh et al., 2016) and contain comparatively much less consumer DNA, making them putatively an ideal source for metabarcoding diet analysis. Nevertheless, to date, we lack a comparative and quantitative assessment of the respective efficiency in detection success between whole bodies, regurgitates and faeces as well as prospective interactions with other sources of nondietary variation such as the target DNA fragment size.

In this study, we address this knowledge gap by comparing the prey DNA detection rates for three types of dietary samples: whole consumers including their gut content, regurgitates and faeces. Using a controlled feeding experiment involving a widespread carabid predator, *Pterostichus melanarius* (Coleoptera: Carabidae), we test the following hypotheses: (i) postfeeding prey DNA detection success should be similar or better in regurgitates compared to whole beetles due to lesser degradation of prey DNA in the former; (ii) prey DNA detection success should be lower in faeces compared to regurgitates and whole bodies as faecal material represents the final stage of the digestion process; and (iii) prey DNA detection should decrease with increasing DNA fragment size and the time postfeeding for all types of samples.

2 | MATERIALS AND METHODS

2.1 | Sampling and maintenance of predators

Pterostichus melanarius individuals were collected by dry pitfall traps in two adjacent maize fields situated at the experimental site of INRA, Le Rheu (Ille-et-Vilaine, France; GPS coordinates: 48.10744282N; 1.78830482W). Regular 24-hr trapping sessions were conducted in July–August 2013 until a sufficient number of individuals had been collected ($N = 200$). All living beetles were brought to the laboratory where they were identified to species level and individually placed in plastic containers filled with loam. Beetles were stored at room temperature and continuously provided with water and food (field-collected earthworms and small pieces of apple).

2.2 | Feeding experiment

Prior to the feeding experiment, beetles were starved for 96 hr in fresh individual plastic Petri dishes (5 cm diameter) containing only a droplet of water. After the starvation period, all beetles were transferred to a new Petri dish and provided with one freshly freeze-killed mealworm (*Tenebrio molitor*, Coleoptera: Tenebrionidae) cut in half. Particular care was taken to ensure that the mealworms offered were of similar size. Carabids were allowed to feed for 1 hr in a dark climatic chamber at 20°C. All the beetles that had fully consumed the mealworm were transferred into fresh Petri dishes containing no food. Petri dishes with fed beetles were then blindly pulled off and alternately assigned to treatments until the required number of individuals per treatment was fulfilled. Beetles from all treatments were stored at room temperature and continuously provided with water during the experiment.

For the “whole beetle” treatment, batches of 10 carabids were frozen in 2-ml reaction tubes by immersion in liquid nitrogen at 0, 12, 24, 36, 48, 60, 72 and 96 hr postfeeding. Immersion in liquid nitrogen was necessary as previous tests showed that living beetles do not die immediately after placement at -20°C , leading them to regurgitate into the reaction tube. After immersion, all whole beetles were stored at -20°C . Thirteen starved beetles were never allowed to feed, and they were freeze-killed at 0 hr to be used as negative controls. For the “regurgitate” treatment, batches of 10 individuals were allowed to regurgitate on a cotton wool tip according to the protocol described in Waldner and Traugott (2012) at 0, 12, 24, 36, 48, 60, 72 and 96 hr postfeeding. After regurgitation, all beetles per given time point were freeze-killed and stored at -20°C . Exactly the same procedure at each time point was applied on a control tip without touching a beetle for checking potential DNA carry-over contaminations. All samples were stored at -20°C prior to DNA extraction and PCR. For the “faeces” treatment, 20 carabid beetles were placed after feeding in new Petri dishes containing a droplet of clean water. Carabids were checked for faeces production every 6 hr. Detected faeces were immediately frozen within the Petri dish at -20°C whereupon the corresponding carabid individual was

transferred into a new Petri dish. Faeces production was monitored until all beetles died.

2.3 | Molecular diet analysis

Regurgitate and faecal samples were directly lysed in 200 μ l TES Lysis Buffer (Macherey-Nagel, Düren, Germany) and 5 μ l Proteinase K (10 mg/ml) overnight at 56°C. The whole beetles were previously ground using three 4-mm stainless steel beads (Lemoine S.A.S, Rennes, France) within a volume of 620 μ l TES Lysis Buffer and 10 μ l Proteinase K (10 mg/ml) per beetle. Tissues were disrupted by a 1-min bead-beating step using a professional paint mixer (Fluid Management Inc., Wheeling, IL, USA). All samples were incubated overnight at 56°C. Respectively, 2, 6 and 2 lysate blanks (i.e., no DNA material) were carried out for the whole beetles, faecal and regurgitate treatments. DNA was extracted in batches of 92 samples using the BioSprint 96 DNA Blood Kit (Qiagen, Hilden, Germany) on a BioSprint 96 extraction robotic platform (Qiagen) following the manufacturer's instructions. DNA was finally diluted in 200 μ l TE buffer (0.1 M Tris, pH 8, 10 mM EDTA), and the extracts were stored at -28°C. To avoid contamination, DNA extractions were carried out in a separate pre-PCR laboratory using a UVC-equipped laminar flow hood. To check for sample-to-sample cross-contamination, four extraction negative controls (PCR-grade RNase-free water instead of lysate) were included within each batch of 92 samples. All of these controls tested negative using the diagnostic PCR assay described below.

The DNA extracts were screened with a multiplex PCR assay targeting three overlapping COI mtDNA fragments specific to *T. molitor* (GenBank Accession nos. HQ891143–HQ891145), that is 128, 332 and 612 bp (Oehm, Juen, Nagiller, Neuhauser, & Traugott, 2011; Sint et al., 2011). Additional information about the position of the different primers is provided in Appendix S1. The primer mix contained 6 μ M of primers Ten-mol-S210 (5'-TACCGTTATTCGTATGAGCAG TAG-3') and Ten-mol-A212 (5'-CGCTGGGTCAAAGAAGGAT-3') as well as 2 μ M of primers Ten-mol-S232 (5'-TAATAAGAAGAATTGTA GAAAACGGG-3') and Ten-mol-S231 (5'-TCATTTTTGGAGCGT GATCC-3') (Oehm et al., 2011; Sint et al., 2011). Each 10 μ l PCR consisted of 1.5 μ l template DNA, 5.0 μ l of 2x Multiplex PCR Kit reaction mix (Qiagen), 1.0 μ l of primer mix, 0.5 μ l of bovine serum albumin (BSA, 10 mg/ml) and 2.0 μ l of PCR-grade RNase-free water (Qiagen) to adjust the volume. Thermocycling was conducted in Eppendorf Mastercycler (Eppendorf, Hamburg, Germany), and cycling conditions were as follows: 15 min at 95°C, 35 cycles of 30 s at 94°C, 90 s at 63°C, 1 min at 72°C and final elongation 10 min at 72°C. To check for amplification success and DNA carry-over contamination, two positive (mealworm DNA) and two negative controls (PCR water instead of DNA) were included within each PCR, respectively.

The PCR products obtained were visualized using QIAxcel, an automated capillary electrophoresis system (Qiagen), with method AL320. The results were scored with BIOCALCULATOR FAST ANALYSIS Software version 3.0 (Qiagen), and the threshold was set to 0.07 relative

fluorescent units. Samples above this threshold and showing the expected fragment length were counted as positives. All DNA extracts that were tested negative in the first run were retested with general primers (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) in a second PCR to check for any amplifiable DNA (all of these samples tested positive). To ensure contamination-free conditions, PCR preparation and visualization of PCR products were carried out in two separate laboratories (workflow: from pre- to post-PCR areas).

2.4 | Statistical analyses

A generalized linear mixed model was built to fit a logistic regression on the DNA detection data. We integrated three fixed effects into the model: two qualitative factors, the marker size (128, 332, 612 bp) and the sample type (regurgitates, faeces or whole-body DNA extracts), and one continuous variable, the time postfeeding. To compensate for nonindependence in collection of faeces, individuals were included as a random effect. To match the sampling effort and the sampling time points between modalities, for faeces, we considered the endpoint of the sampling window in the statistical analyses. Moreover, not more than ten faecal samples per time point were considered to match the sampling effort for the other modalities (regurgitates and whole beetles) except in cases where available samples were less than ten. In this case, faecal samples from adjoining time points were grouped together (cf Table 1). The model was fitted using the "glmm" function from the R package GLMM (<https://cran.r-project.org/web/packages/glmm>). Models were fit using a Monte Carlo sample size of 1,024 with 10,000 iterations. The distribution of each of the model parameters was approximated to a normal distribution using the maximum goodness-of-fit estimation with the "fit-dist" function available in the R package FITDISTRPLUS (Delignette-Muller & Dutang, 2015). The variance in detectability rates explained by the model was estimated using the coefficient of determination method (Tjur, 2009). Tests of the differences between mean detectability rates for each of the qualitative factors (marker length and sample type) were conducted using a Z-test. The time point for a prey detection probability of 50% (i.e., the time point at which on average half of the individuals show positive for the target prey) was determined for each dietary sample and DNA fragment size. To compensate for false discovery rate, comparisons between fragments were based on 95% confidence limits (CI) as suggested by Greenstone, Payton, Weber, and Simmons (2013). All statistical analyses were conducted using the R software (R Core Team, 2013).

3 | RESULTS

Detectability of mealworm DNA in *Pterostichus melanarius* decreased with increasing postfeeding time and prey DNA fragment length for the three dietary samples (Figure 1, small vs. medium and small vs. large fragments: $p < .001$; medium vs. large fragment: $p = .08$), with postfeeding detection time intervals being longest for the shortest DNA fragment (Figure 1a–c). We also observed a significant effect

TABLE 1 Detection rates of small (128 bp), medium (332 bp) and large (612 bp) prey DNA fragments of the mealworm *Tenebrio molitor* fed to the carabid *Pterostichus melanarius* in whole beetles, regurgitates and faeces. *N* is the number of samples analysed per digestion time

Dietary sample	Digestion time (hr)	<i>N</i>	Detection rate per fragment size (%)		
			Small (128 bp)	Medium (332 bp)	Large (612 bp)
Whole beetles	0	20	95	90	95
	12	10	100	100	60
	24	10	100	80	40
	36	9	78	44	22
	48	10	100	40	40
	60	10	80	20	0
	72	10	70	10	0
Regurgitates	0	11	100	100	100
	12	10	100	100	80
	24	10	100	80	50
	36	10	100	30	30
	48	9	100	33	33
	60	10	70	10	0
	72	9	78	0	0
Faeces	6	30	97	83	73
	18	13	100	39	38
	30	19	100	68	47
	42	17	94	29	24
	54	9	78	22	11
	66	5	60	0	0

of the dietary sample type, with prey DNA detection success being significantly lower in faeces compared to regurgitates and whole beetles for all the three fragment sizes (Figure 1, in all cases $p < .001$). There was also a tendency for longer postfeeding detection periods in regurgitates compared to whole beetles (Figure 1a,b), but differences were not significant ($p = .6$). Our model fitted the data well for all of the three dietary samples: regurgitates (Figure 1a), whole beetles (Figure 1b) and faeces (Figure 1c), and explained 50% of the variance in DNA detectability. Raw data are presented in Table 1. For regurgitates, the 50% detection time was highest for the small fragment (94 hr), significantly lower for the medium fragment (42 hr) and again significantly lower for the longest fragment (30.6 hr; Figure 2). The 50% detection probabilities appeared to be consistently lower in faeces across all three fragment sizes (as low as 19 hr for the longest fragment) compared to regurgitates or whole bodies, but this was only significant when comparing the medium-length fragment between faeces and regurgitates (Figure 2).

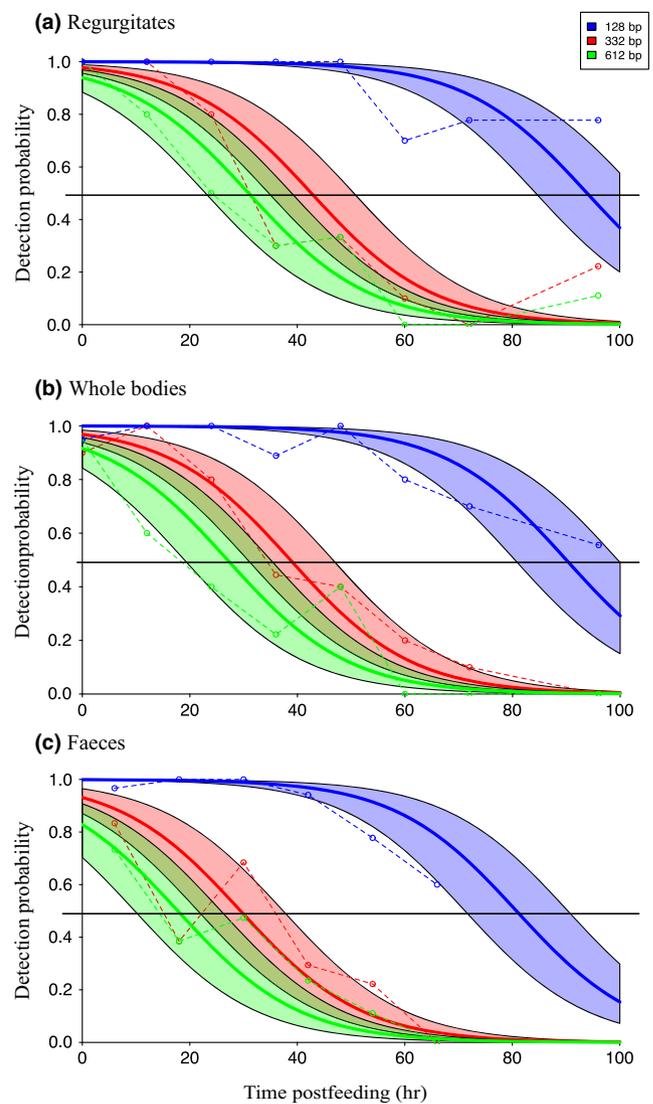


FIGURE 1 Prey DNA detection success in the predatory carabid beetle *Pterostichus melanarius* for regurgitates (a), whole bodies (b) and faeces (c). Detection rates are provided for the different time points examined within each dietary sample and for the three target DNA fragment sizes. Circles and dashed lines indicate actual target measures. Bold solid lines indicate the logistic regressions estimated from the glmm model and the shaded area the 95% confidence interval envelopes of the fit

4 | DISCUSSION

The length of time that prey DNA can be detected in a sample is determined by a range of interacting factors related to the environment, the predator–prey system and the molecular techniques used. These certainly affect results, but disentangling such effects without conducting comprehensive experiments that explicitly account for multiple factors is difficult (King et al., 2008; Welch, Schofield, Chapman, & Harwood, 2014). Here, we compared multiple dietary samples from one species of invertebrate consumer, in a controlled feeding experiment, and assessed how the combined effects of the type of dietary sample and DNA fragment size affect the prey DNA

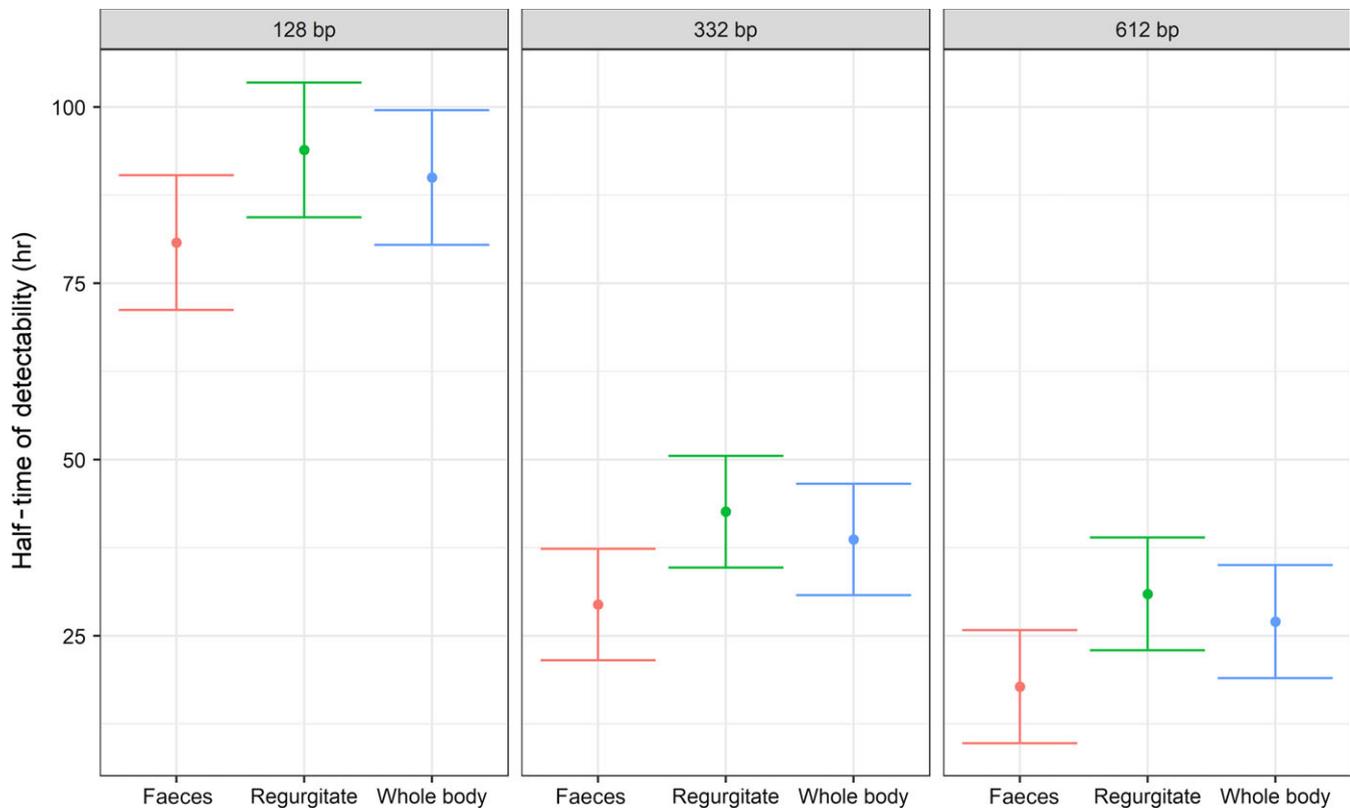


FIGURE 2 Estimated time points postfeeding for a 50% prey DNA detection probability (half-time of detectability) for the different types of dietary samples and DNA fragment sizes. Provided are the 50% prey detection probabilities in hours postfeeding. Vertical bars represent 95% confidence limits

detection probability since feeding. Our results show that each of these factors significantly affects the rate at which the probability of detecting prey DNA decreases over time. Consistent with our hypothesis, the time during which DNA could be detected was the longest for regurgitates, for each of the three tested prey DNA fragment sizes. While this was not significantly different from DNA detected from whole beetles, prey DNA contained in faeces was detectable for a significantly shorter time for all three fragment sizes.

Our results support the general assumption that regurgitates constitute a good alternative source of prey DNA (Waldner & Traugott, 2012; Wallinger et al., 2015) likely due to the higher relative concentration of prey DNA compared to whole-body extracts or faecal samples. Such an alternative could be particularly useful in manipulative food web experiments, where the removal and killing of the targeted predators during sampling could disturb the system under study. As 79% of predaceous land-dwelling arthropods use extra-oral digestion (Cohen, 1995), this approach is potentially applicable to a large array of taxa and ecological situations. Furthermore, by containing comparatively less predator DNA, regurgitates could also be a valuable source of dietary data in DNA metabarcoding studies involving the use of general primers (Waldner & Traugott, 2012).

Nevertheless, the use of regurgitates could entail some additional limitations such as the detection of only the most recent diet items, and probably represents merely a narrow fraction of individual's diet,

especially in generalist feeders with frequent switching behaviour such as carabids (Lövei & Sunderland, 1996). Thus, the choice of the most appropriate dietary sample will most likely consist in a trade-off between DNA detection rates and representativeness in terms of diet according to the focus of interest.

Faecal samples can have the advantage of providing a more integrated picture of individual's diet as single faeces usually contain the remains of several meals at a time (Deagle et al., 2005). Our results indicate that overall prey DNA detection in faeces was lower compared to regurgitates and whole bodies. Note, however, that this was true only for the medium-sized fragment when considering the 50% prey DNA detection probability, with significantly lower post-feeding interval found in faeces compared to regurgitates. Thus, despite lower detection rates, faeces remain overall a good source of dietary DNA at least in *Pterostichus melanarius* beetles. Similarly, earlier study in wolf spiders showed that prey DNA was detectable in spider faeces albeit at lower rates compared to whole-body DNA extracts (Sint et al., 2015). As spiders represent an important group of generalist feeders that typically do not regurgitate, the sole non-lethal dietary sample that could be collected is faeces. The important constraint in this case, however, is the necessity to collect faecal samples in the field. Even though this could be relatively easy for a range of large invertebrates (e.g., molluscs; large beetles), it is less practical for small invertebrates such as spiders or carabid beetles. In such a case, faecal samples could be rather obtained from field-

collected invertebrates in a Petri dish, prior to releasing them back in the field (cf. Sint et al., 2015).

Also, we cannot rule out the possibility that in our case, prey DNA detection success in faeces was lower simply due to the constraints of the experiment. As carabids were checked for faeces every 6 hr, faeces deposited earlier within that time frame could have experienced higher DNA degradation due to longer exposure to ambient temperature, thus resulting in increased variability in DNA prey detection success.

In a recent study, Unruh et al. (2016) even show that there is no difference in DNA detection between whole bodies and faeces in the insect predator *Forficula auricularia*. While the authors do not discuss the possible mechanisms behind this observation, results tend to suggest that faeces remain a viable nonlethal dietary source in certain situations, as detection rates are generally high.

Yet, when relying on faecal samples, it is important to have a good estimation about the time it takes for prey DNA to be completely eliminated from the digestive tract. As mentioned above, carabid beetles are generalist, mobile feeders with frequent switching behaviour (Lövei & Sunderland, 1996). This means that for some species, frequent diet shifts but long prey DNA retention periods may result in an overestimation of consumption rates or in a mismatch between diet composition and estimations of prey availability at the place where dietary samples were collected. This could be particularly problematic for estimating pest consumption rates in the context of conservation biological control for instance and may potentially explain why in some cases, high consumption rates do not necessarily lead to a decrease in pest populations (Straub & Snyder, 2006). Within carabid beetles, species could differ fundamentally in the anatomy of their digestive tracts (Thiele, 1977), suggesting potential variations in prey DNA transit/persistence across the digestive system (cf. Von Berg, Traugott, & Symondson, 2008). Yet, the question about the temporal aspects of DNA digestion in insects in general is currently poorly addressed. For instance, results have shown that ^{14}C -inulin-labelled animal prey in another predatory carabid, *Calosoma calidum*, could still be detected in faeces up to 5 days postfeeding (Cheeseman & Gillott, 1987). It would be interesting to confront these findings with observations about prey DNA transit in order to be able to estimate the magnitude of the potential bias due to extended DNA transit times. In particular, we do not know whether this problem could be exacerbated in herbivorous species as the digestion process of plant DNA in carabids can last much longer compared to animal DNA (Staudacher, Wallinger, Schallhart, & Traugott, 2011; Wallinger et al., 2013, 2015).

Here, we also show that prey DNA detection continuously decreases over time for all the three types of dietary samples, with longer fragments (332 and 612 bp) decaying more rapidly compared to the shorter one (128 bp). These results meet our expectations and corroborate the general idea that digested DNA molecules break down relatively quickly and that the size of the targeted prey DNA fragment affects postfeeding prey DNA detection (Agustí et al., 2003; Von Berg et al., 2008). In line with previous studies, our results support the idea that targeting short- to medium-sized DNA

fragments in DNA diet analysis is essential in order to maximize the prey detection (Deagle, Eveson, & Jarman, 2006; Valentini et al., 2009). Nonetheless, if a recent feeding event is the focus, then targeting longer fragments might actually be a better strategy to ensure that only the most recent prey items are detected. Additionally, as in DNA metabarcoding diet analysis, there is generally a trade-off between DNA fragment length and taxonomic resolution, targeting longer DNA fragments—within a certain range—could indeed improve the taxonomic discrimination of prey species (Pompanon et al., 2012). In this study, the most important observed source of variation in terms of prey DNA detection, besides time postfeeding, is DNA fragment size. This could have profound implications in metabarcoding studies where the DNA fragment size usually needs to be optimized in order to meet criteria for both optimal detectability and taxonomic resolution (Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012). It would be interesting to simultaneously explore the decay rate of a larger array of DNA fragments of different lengths in order to assess whether a general relationship between DNA length and detectability can be drawn despite the many other sources of variability detected in previous studies. One might speculate that a consistent relationship between DNA detection success and DNA fragment size could be further used as a raw predictor of postfeeding prey DNA detection periods based solely on prey DNA fragment length.

In general, our findings show that quantitative analyses of diet based on different DNA fragment sizes or on different dietary samples are not directly comparable. Our study suggests that for estimating and comparing consumption rates for the same species between studies using different DNA fragment sizes or different dietary samples (whole beetles/regurgitates vs. faeces), values should be corrected after taking into account differences in detection probabilities (e.g., Greenstone et al., 2010). Nevertheless, prey DNA detection depends on numerous additional factors including species identity of the prey or the predator (Hosseini, Schmidt, & Keller, 2008; Wallinger et al., 2013), the feeding mode (Greenstone, Rowley, Weber, Payton, & Hawthorne, 2007; Greenstone et al., 2013), the time since the last meal, the number/size or the quality of prey consumed (Eitzinger, Unger, Traugott, & Scheu, 2014; Harper et al., 2005; Hoogendoorn & Heimpel, 2001), which we did not investigate here. The next step therefore would be the integration of multiple sources of variation in a complex multispecies, multifactorial experimental design where the different sources of variation could be quantified at once, and hierarchized (Welch et al., 2014).

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CONFLICTS OF INTEREST

The authors have declared that no competing interests exist.

AUTHOR CONTRIBUTIONS

M.T., S.K. and M.P. designed the experiment. S.K. realized the field work and the feeding experiment. R.M. carried out molecular analyses. O.R.R. and E.C. realized data analyses. S.K. wrote the manuscript with input from all the authors.

DATA ACCESSIBILITY

All the data used in this manuscript are included in the figures and the table presented within the manuscript.

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SUPPORTING INFORMATION

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