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

STEFANIYA KAMENOVA[1,2,3], REBECCA MAYER[4], ERIC COISSAC[5,6], MANUEL PLANTEGENEST[2] and MICHAEL TRAUGOTT[4]

[1]Centre d'Etudes Biologiques de Chizé, Villiers-en-Bois, France, [2]Agrocampus Ouest UMR1349 IGEPP, 65 rue de Saint-Brieuc, CS 84215, F-35042 Rennes, France, [3]Integrative Biology, University of Guelph, Guelph, ON, Canada, [4]Mountain Agriculture Research Unit, Institute of Ecology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria, [5]Université Grenoble-Alpes, Laboratoire d'Ecologie Alpine (LECA), F-38000 Grenoble, France, [6]Centre National de la Recherche Scientifique, Laboratoire d'Ecologie Alpine (LECA), F-38000 Grenoble, France

Corresponding author: Stefaniya Kamenova
Integrative Biology, University of Guelph, 50 Stone Road East, N1G 2W1, Guelph, ON, Canada
stefaniya.kamenova@gmail.com

Keywords: diagnostic PCR, molecular diet analysis, prey detection, Carabidae, trophic interactions

Running title: DNA decay in three types of dietary samples

Abstract

Molecular diet analysis is rapidly popularizing among ecologists, especially with regard to methodologically challenging groups such as invertebrate generalist predators. Prey DNA detection success is known to be dependent on multiple factors among which the type of dietary sample has rarely been addressed. Here, we address this knowledge gap by comparing prey DNA detection success from three types of dietary samples. In a controlled feeding experiment, and using the carabid beetle *Pterostichus melanarius* as a model predator, we collected regurgitates, feces and whole gut contents at different time points post-feeding. All dietary samples were analyzed by multiplex PCR targeting three DNA fragments of different length (128 bp, 332 bp and 612 bp). Our results show that both the type of dietary sample and the DNA fragment size explain a significant part of the variation found in prey DNA detectability. Specifically, despite a significant decrease in prey DNA detectability with increasing time post-feeding, we observed for both regurgitates and whole bodies significantly higher detection rates for all prey DNA fragment sizes compared to feces. Based on these observations, we conclude that regurgitates and whole body DNA extracts provide similar information when prey DNA is targeted by diagnostic PCR, whereas prey DNA detections success in feces is still good enough to use this approach in ecological studies. Therefore, regurgitates and feces constitute an excellent, non-lethal source for dietary information that could be applied to field studies in situations when invertebrate predators should not be harmed.

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 (Traugott *et al*. 2013; Clare 2015). 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 *et al*. 2015; Mollot *et al*. 2014) or other types of dietary samples such as feces, regurgitates, or the entire consumer's body (*e.g.* Ibanez *et al*. 2013; Kartzinel *et al*. 2015; Thalinger *et al*. 2016; Wallinger *et al*. 2015). The success of DNA-based approaches to analyze trophic interactions is mainly due to the fact that they require little technical training while allowing the direct and accurate identification of trophic links from minute amount of any starting material, even of very small-sized organisms such as mites (Pérez-Sayas *et al*. 2015) or zooplankton (Durbin *et al*. 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.* Valentini *et al*. 2009; Pompanon *et al*. 2012; Sint *et al*. 2011). DNA-based diet analysis has a particularly long history in studies involving 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). But DNA techniques are 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 *et al*. 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 avoids laborious dissections. But besides the drawbacks of a lethal approach, 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.* Shehzad *et al*. 2012; Piñol *et al*. 2014). Waldner & Traugott (2012) demonstrated that regurgitates, a fluid mixture containing semi-digested 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 feces, although their use as a dietary source in invertebrates is still uncommon (*e.g*. Ibanez *et al*. 2013; Redd *et al*. 2014; Sint *et al*. 2015). Usually, both regurgitates and feces seem to provide similar or better detection rates compared to whole body extracts (Durbin *et al*. 2012; Egeter *et al*. 2015; Unruch *et al*. 2016), and contain comparatively much less consumer DNA, making them an ideal source for metabarcoding diet analysis. Nonetheless, to date we lack a comparative and quantitative assessment of the respective efficiency in detection success between whole bodies, regurgitates and feces as well as prospective interactions with other sources of non-dietary 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 predator tissues, regurgitates and feces. Samples were generated in a controlled feeding experiment involving a

widespread carabid predator, *Pterostichus melanarius* (Coleoptera: Carabidae). Beetles were fed with a single known prey species and dietary samples were collected at several time points post-feeding. Collected samples were screened by diagnostic PCR for three prey DNA fragments of contrasting size: 128 bp, 332 bp, and 612 bp, respectively. We hypothesized (i) similar or better DNA detection probability in regurgitates compared to whole beetles due to lesser degradation of prey DNA; (ii) inferior detection probability in feces compared to regurgitates and whole bodies as they represent the final stage of the digestion process; and (iii) a decrease in DNA prey detection success with increasing DNA fragment size and the time post-feeding for all types of samples.

Material & Methods

Sampling and maintenance of predators

P. 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-hour trapping sessions occurred in July – August 2013 until a sufficient number of individuals had been collected. All living beetles were brought to the laboratory where they were identified to the 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).

Feeding experiment

Prior to the feeding experiment, beetles were starved for 96 h 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. Carabids were allowed to feed for one hour in a dark climatic chamber at 20°. After feeding, all beetles were transferred into fresh Petri dishes with no food and stored at room temperature. Beetles were continuously provided with water during the experiment.

For the “whole beetle” treatment, batches of 10 randomly chosen carabids were frozen in 2-mL reaction tubes by immersion in liquid nitrogen at 0, 12, 24, 36, 48, 60, 72 and 96 h post-feeding. Thirteen starved beetles were never allowed to feed and they were freeze-killed at 0 h to be used as negative controls. For the “regurgitate” treatment, batches of 10 randomly chosen individuals were allowed to regurgitate on a cotton wool tip according to the protocol described in Waldner & Traugott (2012) at 0, 12, 24, 36, 48, 60, 72 and 96 h post-feeding. 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 “feces” treatment, 20 carabid beetles were placed after feeding in new Petri dishes with a droplet of clean water. They were firstly checked for feces production at every 3 hours, and then at every 6 hours as droppings have become scarcer. Detected feces were immediately frozen within the Petri dish at -20°C whereas the corresponding

carabid individual was transferred into a new Petri dish. Feces production was monitored until all beetles died.

Molecular gut content analysis

Regurgitate and fecal samples were directly lysed in 200 µl TES Lysis Buffer (Macherey-Nagel, Germany) and 5 µl Proteinase K (10 mg/mL) overnight at 56°C. The whole beetles were previously grinded using three 4 mm stainless steel beads (Lemoine S.A.S, Rennes, France) within a volume of 620 µl TES Lysis Buffer and 10 µl Proteinase K (10 mg/mL) per beetle. Tissues were disrupted by a 1 minute bead-beating step using a professional paint mixer. 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, fecal 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 instruction. DNA was finally diluted in 200 µl 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 done 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 DNA fragments of different lengths of *T. molitor,* i.e. 128 bp, 332 bp, and 612 bp. The primer mix contained 6 µM of primers Ten-mol-S210 (5'-TACCGTTATTCGTATGAGCAGTAT-

3') and Ten-mol-A212 (5'- CGCTGGGTCAAAGAAGGAT-3') as well as 2 µM of primers Ten-mol-S232 (5'-TAATAAGAAGAATTGTAGAAAACGGG-3') and Ten-mol-S231 (5'-TCATTTTTGGAGCGTGATCC-3') (Oehm *et al*. 2011; Sint *et al*. 2011). Each 10 µl PCR consisted of 1.5 µl template DNA, 5.0 µl of 2x Multiplex PCR Kit reaction mix (Qiagen), 1.0 µl of primer mix, 0.5 µl of bovine serum albumin (BSA, 10 mg ml^{-1}), and 2.0 µl of PCR-grade RNase-free water (Qiagen) to adjust the volume. Cycling conditions were 15 min at 95 °C, 35 cycles of 30 sec at 94 °C, 90 sec 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 re-tested with general primers (Folmer *et al*. 1994) in a second PCR to check if any DNA is amplifiable. To ensure contamination-free conditions, PCR preparation and visualization of PCR products were done in two separate laboratories (workflow: from pre- to post-PCR areas).

Statistical analyses

All statistical analyses were run using the R software (R Core Team 2013). A generalized linear mixed model was built to fit a logistic regression on the DNA

185 detection data. We integrated three fixed effects into the model: two qualitative factors,
186 the marker size (128 bp, 332 bp, 612 bp) and the sample type (regurgitates, faeces or
187 whole body DNA extracts), and one continuous variable, the time post-feeding.
188 Individuals were included as a random effect. The model was fitted using the *glmm*
189 function from the *glmm* R package (https://cran.r-project.org/web/packages/glmm). We
190 run 1024 independent fittings of the model, with 10,000 iterations for each run. The
191 distribution of each of the model parameters was approximated to a normal distribution
192 using the maximum goodness-of-fit estimation provided by the *fitdist* function of the
193 *fitdistrplus* R package (Delignette-Muller & Dutang 2015). The variance in detectability
194 rates explained by the model was estimated using the coefficient of determination
195 method (Tjur 2009). Significance of the differences in detectability rates associated to
196 the modalities of the qualitative factors (marker length and sample type) were assessed
197 through the testing of differences in the mean of their associated parameter in the
198 model using a Z-test. The time point for a prey detection probability of 50% (i.e. the time
199 point at which on average half of the individuals show positive for the target prey) was
200 determined for each dietary sample and DNA fragment size. Comparisons between
201 fragments were based on 95% confidence limits (CI).
202
203 **Results**
204 Detectability of mealworm DNA in *P. melanarius* significantly decreased with
205 increasing post-feeding time and prey DNA fragment length for the three dietary
206 samples (Fig. 1, small *vs* medium and small vs large fragments: $p<0.001$; medium *vs*
207 large fragment: $p=0.008$), with post-feeding detection intervals being longest for the

208 shortest DNA fragment (Fig. 1 A, B, C). We also observed a significant effect of the
209 dietary sample type, with prey DNA detection success being significantly lower in feces
210 compared to regurgitates and whole beetles for all the three fragment sizes (Fig. 1, in all
211 cases $p<0.001$). There was also a tendency for longer post-feeding detection periods in
212 regurgitates compared to whole beetles (Fig. 1A, B) but differences were not significant
213 ($p=0.4$). Our model fitted well the data for all of the three dietary samples: regurgitates
214 (Fig. 1A), whole beetles (Fig. 1B) and feces (Fig. 1C), and explained 52% of the
215 variance in DNA detectability. Raw data are presented in Table 1. For the small prey
216 DNA fragment, 50% retention times was the highest for regurgitates (94 hours) but the
217 value significantly dropped by more than half for the medium fragment (42 hours) and
218 was significantly shortest for the largest prey DNA fragment (30.6 hours; Table 2). In
219 feces 50% detection probabilities were the lowest for all the three DNA fragment sizes,
220 with only 19 hours for the largest DNA fragment (612 pb) and a significantly shorter
221 detection probability for the medium prey DNA fragment when compared to the
222 regurgitate samples (Table 2).
223
224 **Discussion**
225 Prey DNA detection success is determined by a range of interacting factors,
226 which might be difficult to disentangle without conducting comprehensive experiments
227 that explicitly account for factor multiplicity. Here, by comparing multiple dietary samples
228 from one species of invertebrate consumer in a controlled feeding experiment we
229 assess the joint effects of the type of dietary sample and DNA fragment size on the
230 post-feeding prey DNA detection probability. Our results show that both of these factors

231 significantly affect the quickness in the temporal decrease in prey DNA detection
232 probability. Consistent with our hypothesis, the DNA detection rate was the highest for
233 regurgitates, for all the three tested DNA fragment sizes. Additionally, prey DNA in feces
234 was the least detectable and detection rate decayed most rapidly with time for the three
235 fragment sizes. Concurrently, no significant differences in DNA detection success were
236 observed between regurgitates and whole beetles. Our results reinforce the general
237 idea that regurgitates constitute a good alternative to DNA extracts from whole
238 individuals as source of prey DNA (Waldner & Traugott 2012; Wallinger *et al*. 2015).
239 Such alternative could be particularly useful in manipulative food web experiments,
240 where the mortality of the target species could disturb the system under study. As 79%
241 of predaceous land-dwelling arthropods use extra-oral digestion (Cohen 1995), this
242 approach is potentially applicable to a large array of taxa and ecological situations.
243 Finally, by containing comparatively less predator DNA, regurgitates could also be a
244 valuable source of dietary data in metabarcoding studies involving the use of general
245 primers (Waldner & Traugott, 2012). Considering feces, our results show that prey DNA
246 detection success was lower compared to regurgitates and whole bodies. Note,
247 however, that when considering the 50% prey DNA detection probability, only for the
248 medium sized fragment a significantly lower post-feeding interval could be found in
249 faeces compared to regurgitates. This indicates that faeces overall are a good source of
250 dietary DNA in this carabid beetle. These observations are in line with previous results
251 in wolf spiders showing that prey DNA was detectable in spider faeces albeit in lower
252 rates compared to whole body DNA extracts (Sint *et al*. 2015). Hence, feces remain an
253 interesting non-lethal dietary source in certain situations, as detection rates are

254 generally high. For instance, spiders are typically an important group of generalist
255 feeders that usually do not regurgitate and the sole non-lethal dietary sample that could
256 be collected are feces. Although, as prey DNA concentrations tend to be lower, it would
257 be advisable to concentrate extracted DNA in order to improve DNA detectability. But
258 low prey DNA concentrations in feces may not always be the rule depending on the
259 taxon under study. In a recent paper, Unruch et al. (2016) showed no differences in
260 DNA detection success between whole bodies and feces in the insect predator *Forficula*
261 *auricularia*. While the authors do not discuss the possible mechanisms behind this
262 observation, results tend to suggest that feces could be at least as good dietary source
263 as whole body extracts for organisms such as *F. auricularia*. Finally, we cannot exclude
264 that in our case DNA in feces could also have been less well preserved here due to the
265 constraints of the experiment. As carabids were checked for feces every 6 hours by the
266 end of the experiment, one may expect that feces deposited earlier within that
267 timeframe would experience higher DNA degradation due to longer exposure to ambient
268 temperatures and atmosphere. This might have negatively affected the prey DNA
269 amplification success and increased the variability in the fecal diet data. In this line, it is
270 essential to bring attention to the fact that we still lack a good understanding about the
271 temporal window of a full prey DNA transfer across the digestive tract in insects. Results
272 have shown that ^{14}C-inulin labelled prey in carabid beetles could still be detected in
273 feces up to five days post-feeding (Cheeseman and Gillott 1987). For generalist feeders
274 with frequent switching behavior such as carabids (Lövei & Sunderland, 1996), temporal
275 aspects of the digestion process should be taken into account in manipulative food web
276 experiments as we ignore at what extend a dietary sample reflects the most recent

277 feeding event. We also ignore whether this problem could be exacerbated in
278 herbivorous species as plant DNA can survive much longer the digestion process in
279 insects as compared to animal DNA (Staudacher *et al*. 2011; Wallinger *et al*. 2013,
280 2015). A more general insight into the digestion physiology of carabid beetles with
281 regard to prey DNA decay will certainly improve the interpretation of DNA-based dietary
282 information.

283 Here, we also show that prey DNA detection success continuously decreases
284 over time for all the three types of dietary samples, with longer fragments (332-612 bp)
285 decaying more rapidly compared to the shorter one (128 bp). These results meet our
286 expectations and corroborate the general idea that digested DNA molecules break
287 down relatively quickly and that the size of the targeted prey DNA fragment affects post-
288 feeding prey DNA detection success (Agustí *et al*. 2003; von Berg *et al*. 2008). In line
289 with previous studies, our results reinforce the idea that targeting short to medium size
290 DNA fragments in DNA diet analysis is essential in order to maximize the prey detection
291 success (Deagle *et al*. 2006; Valentini *et al*. 2009). Nonetheless, if a recent feeding
292 event is the focus, then targeting longer fragments might actually be better. Additionally,
293 as in metabarcoding diet analysis there is generally a trade-off between DNA fragment
294 length and taxonomic resolution, targeting longer DNA fragments – within a certain
295 range - could indeed improve the taxonomic discrimination of prey species (Waldner et
296 al. 2013). In this study, the most important observed source of variation in terms of prey
297 DNA detection success is DNA fragment size. This could have profound implications in
298 metabarcoding studies where the DNA fragment size needs usually to be optimized in
299 order to meet criteria for both optimal detectability and taxonomic resolution (Taberlet *et*

al. 2012). It would be interesting to simultaneously explore the decaying rate of detection probability of a larger array of DNA fragment 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 the DNA detection rate based solely on DNA length.

In general, our findings evidence that quantitative analyses of diet based on different DNA fragments or on different dietary samples are not directly comparable. Our study suggests that consumption frequency should be corrected to account for differences in detection sensibility – related in our case to the type of dietary sample and the DNA fragment size. Such corrective approach would allow direct comparisons across molecular diet studies based on different kind of samples. Nevertheless, prey DNA detection success depends on numerous additional factors including species identity of the prey or the predator (Hosseini *et al*. 2008; Wallinger *et al*. 2013), the time since the last meal, the number/size or the quality of prey consumed (Hoogendoorn & Heimpel 2001; Harper *et al*. 2005; Eitzinger *et al*. 2014), which we did not investigated here. The very 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).

Acknowledgements

We thank Paul Abrams for his invaluable help with early analyses of data presented here. This study was founded by the French National Research Agency (Peerless project ANR-12-AGRO-0006). S.K. was funded by the Atlantic Canada Opportunities Agency. R.M. was supported by a grant of the regional government of Tyrol ("Land Tirol") provided by the Mountain Agriculture Research Unit of the University of Innsbruck.

Data Accessibility

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

Author Contributions

MT, SK and MP designed the experiment. SK realized the field work and the feeding experiment. RM carried out molecular analyses. EC realized data analyses. SK wrote the manuscript with input from all the authors.

Conflicts of Interest: The authors have declared that no competing interests exist.

Bibliography

Agustí N, Shayler SP, Harwood JD, Vaughan IP, Sunderland KD, Symondson WOC (2003) Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. *Molecular Ecology*, **12**, 3467e3475.

Cheeseman MT, Gillott C (1987) Organization of protein digestion in adult *Calosoma calidum* (Coleoptera: Carabidae). *Journal of Insect Physiology*, **33**, 1-8.

Clare EL (2015) Molecular detection of trophic interactions: Emerging trends, distinct advantages, significant considerations and conservation applications. *Evolutionary Applications*, **7**, 1144-1157.

Cohen AC (1995) Extra-oral digestion in predaceous terrestrial Arthropoda. *Annual review of entomology*, **40**, 85–103.

Deagle BE, Eveson JP, Jarman SN (2006) Quantification of damage in DNA recovered from highly degraded samples–a case study on DNA in faeces. *Frontiers in Zoology*, **3**, 1.

Delignette-Muller ML, Dutang C (2015) fitdistrplus: An R package for fitting distributions. *Journal of statistical software*, **4**, 1-34.

Durbin EG, Casas MC, Rynearson TA (2012) Copepod feeding and digestion rates using prey DNA and qPCR. *Journal of Plankton Research*, **34**, 72-82.

Egeter B, Bishop PJ, Robertson BC (2015) Detecting frogs as prey in the diets of introduced mammals: a comparison between morphological and DNA-based diet analyses. *Molecular Ecology Resources*, **15**, 306–316.

Eitzinger B, Unger EM, Traugott M, Scheu S (2014) Effects of prey quality and predator body size on prey DNA detection success in a centipede predator. *Molecular Ecology*, **23**, 3767-3776.

Folmer O, Black M, Hoeh W et al. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294-299.

Harper GL, King RA, Dodd CS, Harwood JD, Glen DM et al. (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology*, **14**, 819-827.

Hoogendoorn M, Heimpel GE (2001) PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. *Molecular Ecology*, **10**, 2059-2067.

Hosseini R, Schmidt O, Keller MA (2008) Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. *Entomologia Experimentalis et Applicata*, **126**, 194–202.

Ibanez S, Manneville O, Miquel C *et al.* (2013) Plant functional traits reveal the relative contribution of habitat and food preferences to the diet of grasshoppers. *Oecologia*, **173**, 1459–1470.

Kartzinel TR, Chen PA, Coverdale TC *et al.* (2015) DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences*, **112**, 8019–8024 (2015).

King RA, Read DS, Traugott M, Symondson WOC (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, 17, 947-963.

Leray M, Meyer CP, Mills SC (2015) Metabarcoding dietary analysis of coral dwelling predatory fish demonstrates the minor contribution of coral mutualists to their highly partitioned, generalist diet. *PeerJ*, **3,** e1047.

Lövei GL, Sunderland KD (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual review of entomology*, **41**, 231–256.

Mollot G, Duyck P-F, Lefeuvre P *et al.* (2014) Cover Cropping Alters the Diet of Arthropods in a Banana Plantation: A Metabarcoding Approach. *PLoS ONE*, **9**, e93740.

Oehm J, Juen A, Nagiller K et al. (2011) Molecular scatology: how to improve prey DNA detection success in avian faeces? Improving prey DNA detection in avian faeces. *Molecular Ecology Resources*, **11**, 620–628 (2011).

Pérez-Sayas, C, Pina T, Gómez-Martínez MA *et al.* (2015) Disentangling mite predator-prey relationships by multiplex PCR. *Molecular Ecology Resources*, **15**, 1330–1345.

Piñol J, San Andrés V, Clare EL et al. (2014) A pragmatic approach to the analysis of diets of generalist predators: the use of next-generation sequencing with no blocking probes. *Molecular Ecology Resources*, **14**, 18–26.

Pompanon F, Deagle BE, Symondson WOC *et al.* (2012) Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, **21**, 1931–1950.

Redd KS, Ling SD, Frusher SD, Jarman S, Johnson CR (2014) Using molecular prey detection to quantify rock lobster predation on barrens-forming sea urchins. *Molecular Ecology*, **23**, 3849–3869.

Shehzad W, Riaz T, Nawaz MA *et al.* (2012) Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. *Molecular Ecology*, **21**, 1951–1965.

Sint D, Raso L, Kaufmann R et al. (2011) Optimizing methods for PCR-based analysis of predation. *Molecular Ecology Resources*, **11**, 795–801.

Sint D, Thurner I, Kaufmann R, Traugott M (2015) Sparing spiders: Faeces as a non-invasive source of DNA. *Frontiers in Zoology*, *12*, doi:10.1186/s12983-015-0096-y

Staudacher K, Wallinger C, Schallhart N, Traugott M (2011) Detecting ingested plant DNA in soil-living insect larvae. *Soil Biology & Biochemistry*, **43**, 346-350.

Symondson, W.O.C., 2012. The molecular revolution: using polymerase chain reaction based methods to explore the role of predators in terrestrial food webs. In: Gurr, G.M., Wratten, S.D., Snyder, W.E., Read, D.M.Y. (Eds.), Biodiversity and Insect Pests: Key Issues for Sustainable Management. Wiley and Sons, pp. 166–184.

Thalinger B, Oehm J, Mayr H *et al.* (2016) Molecular prey identification in Central European piscivores. *Molecular Ecology Resources*, **16**, 123–137.

Tjur T (2009) Coefficients of Determination in Logistic Regression Models—A New Proposal: The Coefficient of Discrimination. *The American statistician*, **63**, 366–372.

Traugott M, Kamenova S, Ruess L et al. (2013) Empirically characterising trophic networks: what emerging DNA-based methods, stable isotope and fatty acid analyses can offer. *Advances in Ecological Research*, **49**, 177.

Unruh TR, Miliczky ER, Horton DR *et al.* (2016) Gut content analysis of arthropod predators of codling moth in Washington apple orchards. *Biological Control,* doi:10.1016/j.biocontrol.2016.05.014

Valentini A, Miquel C, Muhammad AN et al. (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. *Molecular Ecology Resources*, **9**, 51-60.

Von Berg K, Traugott M, Symondson WOC (2008) The effects of temperature on detection of prey DNA in two species of carabid beetle. *Bulletin of Entomological Research* **98,** 263-269.

Waldner T, Sint D, Juen A et al. (2013) The effect of predator identity on post-feeding prey DNA detection success in soil-dwelling macro-invertebrates. *Soil Biology and Biochemistry*, **63**, 116–123.

Waldner T, Traugott M (2012) DNA-based analysis of regurgitates: a noninvasive approach to examine the diet of invertebrate consumers. *Molecular Ecology Resources*, **12**, 669–675.

Wallinger C, Sint D, Baier F et al. (2015) Detection of seed DNA in regurgitates of granivorous carabid beetles. *Bulletin of Entomological Research*, **1**, 1-8.

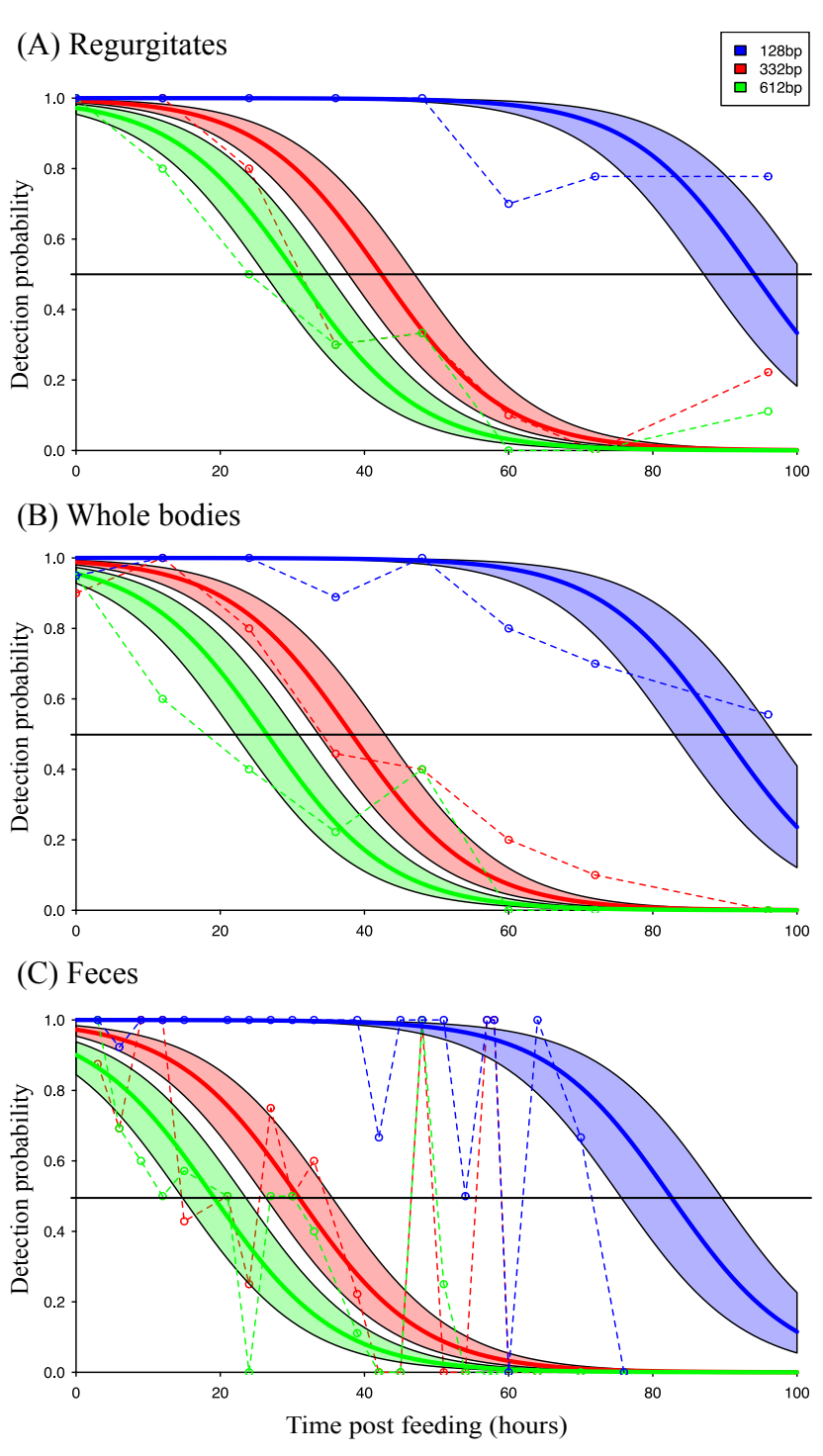
Wallinger C, Staudacher K, Schallhart N, Peter E, Dresch P, Juen A, Traugott M (2013). The effect of plant identity and the level of plant decay on molecular gut content analysis in a herbivorous soil insect. *Molecular Ecology Resources*, **13**, 75–83.

Welch KD, Schofield MR, Chapman EG, Harwood JD (2014) Comparing rates of springtail predation by web-building spiders using Bayesian inference. *Molecular Ecology*, **23**, 3814-3825.

Figure 1 Prey DNA detection success in the predatory carabid beetle *Pterostichus melanarius* for regurgitates (A), whole bodies (B) and feces (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 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. The horizontal line represents the 50% prey DNA detection probability. Corresponding lower and upper 95% confidence limits are presented in Table 2.

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 feces. *N* is the number of samples analyzed per digestion time.

Table 2 Estimated time points post-feeding for a 50% prey DNA detection probability for the different types of dietary samples and DNA fragment sizes. Provided are the 50% prey detection probabilities in hours post-feeding. The numbers in parentheses refer to the corresponding lower and upper 95% confidence limits.


(A) Regurgitates
128bp
332bp
612bp
Detection probability
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
(B) Whole bodies
Detection probability
(C) Feces
Detection probability
Time post feeding (hours)

			Detection rate per fragment size (%)		
Dietary sample	Digestion time (h)	*n*	Small (128 bp)	Medium (332 bp)	Large (612 bp)
Whole bodies	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
	96	9	56	0	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
	96	9	78	22	11
Feces	3	8	100	87,5	100
	6	13	92	69	69
	9	5	100	100	60
	12	4	100	100	50
	15	7	100	43	57
	21	2	100	50	50
	24	4	100	25	0
	27	12	100	75	50
	30	2	100	50	50
	33	5	100	60	40
	39	9	100	22	11
	42	3	67	0	0
	45	2	100	0	0
	48	3	100	100	100
	51	4	100	0	25
	54	2	50	0	0
	57	1	100	100	0
	58	1	100	100	0
	60	1	0	0	0
	64	1	100	0	0
	70	3	67	0	0
	76	1	0	0	0

Dietary sample	Small fragment	Medium fragment	Large fragment
	h	h	h
Regurgitates	94 (87/101)	42.4 (37.7/47)	30.6 (26.1/35)
Whole body	89.9 (82.9/96.8)	38.2 (33.6/42.8)	26.4 (22/30.8)
Feces	82.4 (75.5/89.4)	30.7 (26.1/35.4)	19 (14.6/23.4)