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SPECIAL ISSUE: MOLECULAR DETECTION OF TROPHIC INTERACTIONS

Effects of prey quality and predator body size on prey DNA detection success in a centipede predator

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Abstract

Predator body size and prey quality are important factors driving prey choice and consumption rates. Both factors might affect prey detection success in PCR-based gut content analysis, potentially resulting in over- or underestimation of feeding rates. Experimental evidence, however, is scarce. We examined how body size and prey quality affect prey DNA detection success in centipede predators. Due to metabolic rates increasing with body size, we hypothesized that prey DNA detection intervals will be shorter in large predators than in smaller ones. Moreover, we hypothesized that prey detection intervals of high-quality prey, defined by low carbon-to-nitrogen ratio will be shorter than in low-quality prey due to faster assimilation. Small, medium and large individuals of centipedes *Lithobius* spp. (Lithobiidae, Chilopoda) were fed Collembola and allowed to digest prey for up to 168 h post-feeding. To test our second hypothesis, medium-sized lithobiids were fed with either Diptera or Lumbricidae. No significant differences in 50% prey DNA detection success time intervals for a 272-bp prey DNA fragment were found between the predator size groups, indicating that body size does not affect prey DNA detection success. Post-feeding detection intervals were significantly shorter in Lumbricidae and Diptera compared to Collembola prey, apparently supporting the second hypothesis. However, sensitivity of diagnostic PCR differed between prey types, and quantitative PCR revealed that concentration of targeted DNA varied significantly between prey types. This suggests that both DNA concentration and assay sensitivity need to be considered when assessing prey quality effects on prey DNA detection success.

Keywords: Collembola, Diptera, feeding experiment, gut content, predator–prey, qPCR

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Introduction

DNA-based gut content analysis has become a widely used tool to unravel trophic interactions in the field (King *et al.* 2008; Pompanon *et al.* 2012; Traugott *et al.* 2013). This applies in particular to soil food webs where direct observation is hindered by the minute size of the animals, a wide spectrum of possible prey which often provide no microscopically discernible remains for gut content analysis and the opaque characteristics of the habitat (Juen & Traugott 2007; Weber & Lundgren 2011; Heidemann *et al.* 2011). Analysing prey DNA in the gut of soil animals *post-mortem* allows studying the feeding history under field conditions, omitting effects of disturbances or limitations of laboratory experiments.

When analysing data and interpreting results from gut content analyses, methodological (e.g. sensitivity and specificity of PCR assays), environmental (e.g. ambient temperature) and biological/physiological factors (e.g. feeding mode, body size) need to be considered. While there are several studies addressing the influence of these factors (Greenstone *et al.* 2007; Hosseini *et al.* 2008; Sint *et al.* 2011), we lack knowledge how body size within a predator species affects prey DNA detection success.

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Body size of animals has major implications for biological processes including those associated with feeding and metabolism (Peters 1983; Cohen *et al.* 1993; Otto *et al.* 2007). Predators are usually larger than their prey, and large predators are able to feed on a wider range of prey sizes than small ones, exploiting possible prey communities more efficiently (Cohen *et al.* 1993; Brose *et al.* 2006). Throughout the animal kingdom, metabolic rate scales to the ¾ power of animal body mass (Kleiber & Rogers 1961). The increase in metabolism with body size also holds true for digestive processes (Secor 2009), suggesting that large consumers digest a standardized food item faster than small consumers. Thus, larger predators are able to consume more prey per time unit than small individuals. This, however, has implications for molecular gut content analysis, where binary data indicate the presence or absence of prey DNA but do not reflect the amount of ingested prey. Faster digestion of prey, resulting in lower detection rates of prey DNA, may therefore lead to incorrect assumptions on feeding frequency and consequently predation impact of large predators.

Feeding experiments with predator taxa of varying body masses shed some light on body size-induced variation in prey DNA detection success (Greenstone *et al.* 2007; Lundgren & Weber 2010; Waldner *et al.* 2013); however, the effect of body size cannot be separated from the impact of taxon-specific characteristics, such as feeding mode and the efficiency of the alimentary canal.

Prey identity is another factor potentially influencing prey DNA detection in predators. Generalist predators select prey depending on factors such as body size, abundance, palatability or the nutritional requirements of predators (Eitzinger & Traugott 2011; Kalinkat *et al.* 2011; Schmidt *et al.* 2012). The quality of prey tissue is likely to also affect prey DNA amplification success and consequently the molecular assessment of consumption rates. Prey of high quality, indicated by a low carbon-to-nitrogen (C-to-N) ratio (as used in e.g. Haubert *et al.* 2005), is assimilated faster than low-quality food sources (Jaeger & Barnard 1981; Mitra & Flynn 2007). This results in shorter gut passage times of high-quality food, eventually shortening post-feeding prey DNA detection intervals.

In the present study, we assessed the influence of predator body size and prey quality on prey DNA detection success in the generalist centipede predator *Lithobius* spp. (Lithobiidae, Chilopoda). We hypothesize that (i) predator body size negatively affects post-feeding prey DNA detection intervals and that (ii) high-quality prey (low C-to-N ratio) will be digested faster than those of low quality (high C-to-N ratio), that is, prey DNA detection periods will be significantly shorter in the former than in the latter prey type.

Centipedes within the genus *Lithobius* were used as model predators as they are widespread and occur in high numbers in the litter layer of temperate forests (Lewis 1981). Up to eight species may coexist in one habitat, differing in body size and preference for microhabitats. Lithobiids perform a sit-and-wait hunting strategy, using their poison fangs to kill a wide spectrum of prey particularly Collembola, Diptera larvae and Lumbricidae (Lewis 1981; Poser 1988; Eitzinger *et al.* 2013). Due to similar hunting modes in small and large species, lithobiids represent ideal model organisms to study effects of body size on prey DNA detection success.

Materials and methods

Feeding experiments

Specimens of adult *Lithobius aulacopus* Latzel, 1880; *L. crassipes* L. Koch, 1862; *L. dentatus* C.L. Koch, 1844; *L. mutabilis* L. Koch, 1862; *L. muticus* L. Koch, 1847; *L. nodulipes* Latzel, 1880; and *L. piceus* L. Koch, 1862 were collected by sieving of litter in beech forests in the vicinity of Göttingen, Germany, in summer and autumn 2011. These species are generalist predators with similar feeding behaviour but differing in body size and the habitat they colonize in soil (Eason 1964; Lewis 1981). Among collected specimens, which could not be assigned to one of these species, there were few individuals of the post-larval stage PL4, showing all characteristics (including body size) of adult animals with the exception of being fertile (Eason 1964). We therefore did not include 'development stage' as additional factor in our experiment. The animals were starved for 1 week and separated into three size classes (small, 0.1–5 mg; medium, 5.1–15 mg; large, 20–30 mg). Individuals with body masses between these size classes were not used for this experiment. They were kept in transparent glass vessels (7 cm diameter) with a moist bottom of plaster-of-Paris, rumpled tissue serving as refuge at constant 15 °C and a day/night cycle of 12:12 h. This temperature and light regime represents mean day–night temperature in field conditions in central Germany in September/October and March/April. A mix of freeze-killed mealworms *Tenebrio molitor* Linnaeus, 1758 and larvae of honeycomb moths *Galleria mellonella* (Linnaeus, 1758) served as food. One week prior to the feeding experiments, the predators were starved to ensure that no prey DNA was present in their guts and that the centipedes will readily accept prey. Before start of the experiment, the body mass of each predator was determined to the nearest 0.01 mg.

Two different feeding experiments were conducted to investigate (i) the effect of predator body mass; and

(ii) prey quality on prey DNA detection success. We used three prey types of different prey quality: adult *Sinella curviseta* (Brook, 1882) (Collembola, Entomobryidae) and adult *Drosophila melanogaster* Meigen, 1830 (Diptera, Drosophilidae) as sclerotized prey of low quality, that is, high C-to-N ratio of 6.36 and 6.64, respectively, and *Lumbricus terrestris* Linnaeus, 1758 (Oligochaeta, Lumbricidae) as soft-tissued prey with low C-to-N ratio (4.92), that is, high protein content. In the first feeding trial, lithobiids of the three size classes (minimum number of 130 individuals each) were offered three dead individuals of *S. curviseta*. In the second experiment, only medium-sized predators (minimum number of 130 centipedes for each predator–prey combination) were fed with two individuals of *D. melanogaster* and small pieces of *L. terrestris*. Prey in each of the two experiments was killed by freezing and served in portions of approximately 0.8 ± 0.1 mg. The lithobiids were allowed to feed for 2 h in the climate chamber, and thereafter, their body mass was determined again and they were placed in a new glass vessel. Predators which had not or only partially consumed the prey were excluded from the experiment.

For each of the three prey types and for each predator size class, batches of a minimum of ten centipedes were individually frozen at −20 °C in 1.5-mL reaction tubes after digesting their meal for 0, 16, 24, 32, 40, 48, 56, 72, 88, 104, 120, 144 and 168 h.

To avoid different DNA digestion rates due to different levels of gut filling (i.e. differences in satiation) and to reach maximum digestive activity, we offered dead specimens of the isopod *Trichorhina tomentosa* (Budde-Lund, 1893) *ad libitum* to the centipedes as additional prey after the second measurement of body mass directly after the 2-h feeding period.

DNA extraction and diagnostic PCR

Prior to DNA extraction, the frozen predators were checked for attached prey remains and phoretic mites using a dissecting microscope; additionally, their body length was measured. Whole lithobiids were subjected to a CTAB-based DNA extraction protocol (Juen & Traugott 2005) and purified using Geneclean Turbo Kit (MP Biomedicals, Solon, OH, USA) yielding 150 μL of final DNA extract. One blank sample was included per 47 extracts to check for DNA carry-over contamination. Successful DNA extraction was confirmed by PCR using universal invertebrate primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Each 10 μL PCR contained 5 μL PCR SuperHot Mastermix (2×), 1.25 mM $MgCl_2$ (both Geneaxxon, Ulm, Germany), 0.5 μL bovine serum albumin (BSA, 3%; Roth, Karlsruhe, Germany), 0.5 μM of each primer and 3 μL of DNA extract. Thermocycling conditions were 95 °C for 10 min followed by 35 cycles of 95 °C for 30 s, 48 °C for 30 s, 72 °C for 90 s and a final elongation of 10 min at 72 °C. PCR products were separated in 1% ethidium bromide-stained agarose gels and visualized under UV light. Samples testing positive with the universal invertebrate primers were then screened for DNA of the respective prey: for Collembola, Diptera and Lumbricidae group-specific primers Col3F/Col5R (272 bp; Kuusk & Agustí 2008), DIP S16/DIP A17 (198 bp; Eitzinger *et al.* 2013) and 185F/14233R (225–236 bp; Harper *et al.* 2005) were used, respectively. PCR mixes and thermocycling conditions were the same as above, only differing in the primers used, the elongation step at 72 °C for 45 s and the following annealing temperatures: Col3F/Col5R 60 °C, DIP S16/DIP A17 60 °C and 185F/14233R 65 °C. PCR products were separated using the capillary electrophoresis system QIAxcel (Qiagen, Hilden, Germany); fragments of the expected size and a relative fluorescent value ≥0.1 RFU were scored positive. Samples yielding no band in these PCR were retested once.

To investigate the sensitivity of singleplex PCR assays, we employed a dilution series for each of the three prey species. We determined template DNA copy number of purified PCR products of prey DNA following guidelines by Sint *et al.* (2012). The number of copies was adjusted to 100 000 amplicon copies per microlitre and then twofold serially diluted. The serially diluted target DNA was then used as template in the singleplex PCR assays at concentrations of 20 000/10 000/5000/2500/1250/625/313/156/79/40/20/10/5/3 and 2 copies of target DNA per reaction.

Quantitative real-time PCR (qPCR)

To quantify the amount of prey DNA present before and after different time points post-feeding in the gut content in medium-sized predators, we established a qPCR protocol using the same primers as for the diagnostic PCR described above. The PCR mix consisted of 7 μL PCR water, 0.25 μM of each primer, 10 μL Kapa SYBR FAST Mix (Kapa Biosystems Inc., Woburn, MA, USA) and 2 μL of DNA extract. Thermocycling in Stratagene Mx3005P (Agilent Technologies Inc., Santa Clara, CA, USA) started with 95 °C for 5 min followed by 40 main cycles of 95 °C for 30 s, the primer-specific annealing temperature (see above) for 30 s and 72 °C for 45 s. Subsequent dissociation curve analysis consisted of 95 °C for 60 s, 55 °C for 30 s and 95 °C for 30 s. To standardize the DNA quantification, eight steps of a 10-fold dilution series of target DNA of *S. curviseta* (1.54–1.54 × 10^{-7} ng/μL), *L. terrestris* (6.54 × 10^{-1}–6.54 × 10^{-8} ng/μL) and *D. melanogaster* (2.43–2.43 × 10^{-7} ng/μL), along with two negative controls (PCR

water instead of DNA), were run with every batch of 38 samples. Only samples showing a single peak of the expected PCR product in the dissociation curve were counted as positive, and extracts which tested negative were retested once.

Statistical analysis

LOGIT analyses were carried out to describe the changes in prey DNA detection success over time (Field 2005). The time point for 50% prey detection probability and the corresponding 95% confidence limits were determined; nonoverlapping confidence intervals were interpreted as being significantly different. All analyses were performed using SPSS (version 18). Additionally, to account for possible effects of predator species and single predator body mass on prey DNA detection probability, we calculated a linear mixed-effect model in R 2.12.2 (R Development Core Team 2011) using the package 'lme4' with the family set to 'binomial'.

The relationship between digestion time and prey DNA quantity as measured by qPCR was calculated using nonlinear regression in SIGMAPLOT 11.0 (Systat Software, Chicago, IL, USA). To analyse relationships between prey DNA detection success and prey type, overall DNA quantity (predator + prey) and quantity of prey DNA, we calculated a generalized linear model (GLM) in R 2.12.2 using the function glm {stats} with subsequent step function. Prey DNA detection success was coded as binary 1/0 (prey DNA present or absent). DNA quantity was $\log_{10}$-transformed prior to the calculation.

Results

Effect of feeding on predator body mass

After feeding, predator body mass increased by 0.18 ± 0.83 (SD) mg (large lithobiids), 0.40 ± 0.44 (medium lithobiids), 0.20 ± 0.24 (small lithobiids), 0.70 ± 0.53 mg (medium lithobiids fed with Diptera) and 0.40 ± 0.49 mg (medium lithobiids fed with Lumbricidae). Predator biomass increase was significant in each of the experiments except for the combination of Collembola with large centipedes (Table 1). However, 104 individuals (14.9% of the fed lithobiids) lost weight, while in 50 specimens (7.2%), body mass did not change.

Prey DNA detection success

The singleplex PCR assays proved to be specific and highly sensitive, amplifying DNA of the target prey species only and at low template concentrations of 625 (Diptera), 20 (Collembola) and 10 (Lumbricidae) copies of template DNA per PCR.

After feeding for 2 h, 100% of the lithobiids fed with Collembola or Diptera and 80% of the predators fed with Lumbricidae tested positive for prey DNA. DNA detection success decreased with digestion time in each of the prey taxa and predator size classes (Fig. 1a–e). The decline in prey detection in medium-sized lithobiids was reflected by Pearson's chi squares for Collembola ($\chi^2 = 18.98$, $P = 0.062$), Diptera ($\chi^2 = 33.70$, $P < 0.0001$) and Lumbricidae ($\chi^2 = 11.96$, $P = 0.367$) as well as in large ($\chi^2 = 18.50$, $P = 0.071$) and small lithobiids ($\chi^2 = 15.22$, $P = 0.173$). Collembola DNA was detected at all time points until 168 h post-feeding in each of the three predator size classes, while Diptera DNA was detected up to 144 h post-feeding. Prey DNA detection rates of Lumbricidae decreased fastest over time; their DNA could only be detected up to 40 h post-feeding.

The time span for 50% prey detection probability differed between each of the three prey types (Fig. 1a,d,e): it was shortest in Lumbricidae with 21.6 h [lower (lCL) and upper 95% confidence limits (uCL) of 11.1 and

Table 1 Mean lithobiid body mass and standard deviation (SE) before and after feeding on standardized (0.8 mg) prey items for 2 h

Feeding trial	Mean body mass before feeding ± SE (mg)	Mean body mass after feeding ± SE (mg)	*P*-value of paired Student's *t*-test
Collembola – large predator ($n = 141$)	25.1 ± 0.37	25.3 ± 0.37	0.159 n.s.
Collembola – small predator ($n = 142$)	3.5 ± 0.09	3.7 ± 0.09	**<0.001**
Collembola – medium predator ($n = 152$)	8.9 ± 0.23	9.2 ± 0.23	**<0.001**
Diptera – medium predator ($n = 130$)	10.2 ± 0.29	10.8 ± 0.29	**<0.001**
Lumbricidae – medium predator ($n = 132$)	9.8 ± 0.26	10.2 ± 0.26	**<0.001**

Significant effects are highlighted in bold.

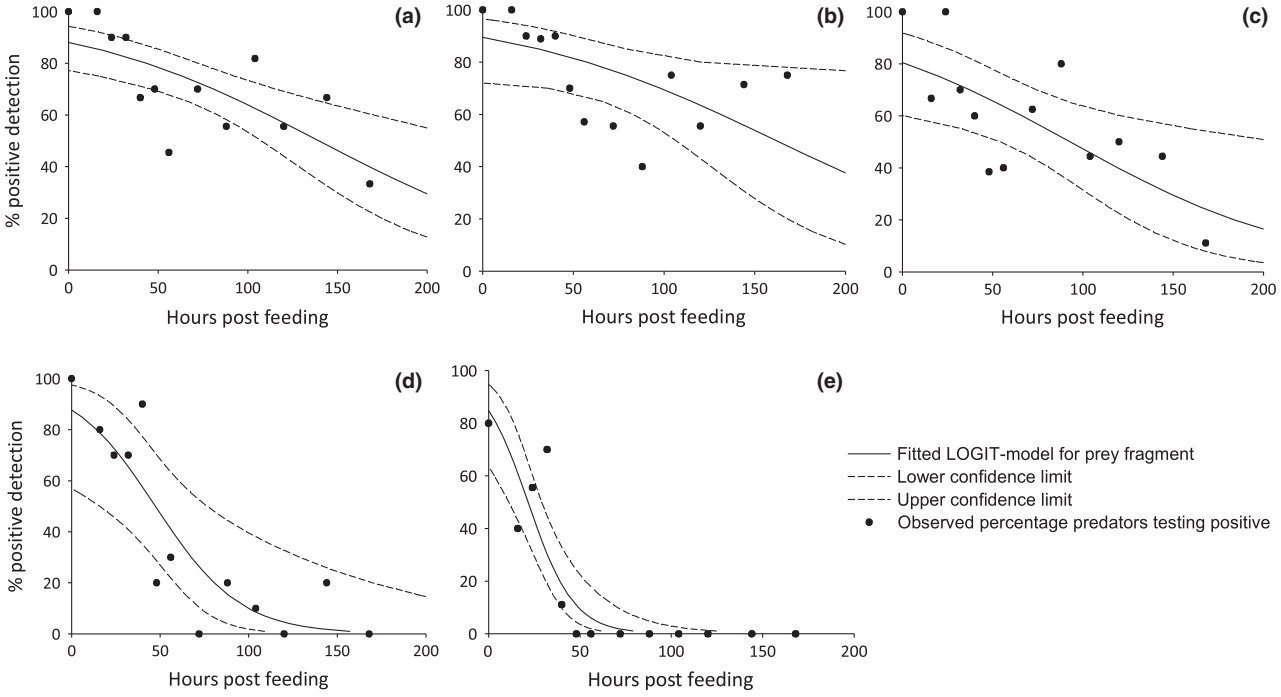

Fig. 1 Fitted model of prey DNA detection rates in lithobiid predators at 13 time points post-feeding: (a) small predator (0.1–5 mg), (b, d, e) medium predator (5.1–10 mg) and (c) large predator (20–30 mg). (a, b, c) Collembola prey (*Sinella curviseta*), (d) Diptera prey (*Drosophila melanogaster*) and (e) Lumbricidae prey (*Lumbricus terrestris*). Dashed lines represent 95% confidence limits.

29.1 h, respectively], medium in Diptera with 47.4 h (lCL 15.9 h, uCL 76.4 h) and significantly longer in Collembola with 161.8 h (lCL 106.5 h, uCL 1632.9 h). In contrast, the 50% prey detection probability did not differ significantly between the three size classes of lithobiids fed with Collembola (large centipedes 92.6 h, lCL 54.2 h, uCL 208.6 h; small centipedes 139.6 h, lCL 107.5 h, uCL230.1 h). Results of linear mixed-effect model also showed that neither fixed factor 'predator body size' nor random factor 'predator species' affected prey DNA detection probability (Table S1, Supporting information).

Quantification of prey DNA

Compared to the DNA concentration in the prey before it was offered to the predators, the quantity of prey DNA in lithobiids examined after the 2-h feeding phase was markedly lower (Table 2). For each of the three prey taxa, the quantity of prey DNA decreased rapidly with the duration of digestion yielding low prey DNA concentrations at time points beyond 32 h (Fig. 2a–c). Remarkably, DNA quantities scattered widely at each of the time points post-feeding, (Fig. 2a–c; nonlinear regressions for Collembola $R^2 = 0.17$, $P = 0.95$, Diptera $R^2 = 0.26$, $P = 0.79$ and Lumbricidae $R^2 = 0.19$, $P = 0.07$). Many samples had to be excluded from the analysis due to multiple and nonspecific amplification and/or production of primer dimers. Nevertheless, GLM analysis of the pooled qPCR data showed prey DNA amplification success to be significantly correlated with prey DNA quantity and prey type with particularly high detection success in Diptera (Table 3 and Table S2, Supporting information).

Discussion

We investigated two hypotheses, that is, that post-feeding prey DNA detection intervals are prolonged in low-quality prey and in small predator individuals. In the *Lithobius*–Collembola predator–prey system investigated here, predator body size did not significantly affect prey DNA detection success, conflicting with our first hypothesis. To our knowledge, only Lundgren & Weber (2010) also examined the effect of predator body size on prey DNA detection success using larvae of the coccinellid beetle *Coleomegilla maculata*. In these experiments, large late-instar larvae digested their prey, eggs of Colorado potato beetle *Leptinotarsa decemlineata*, more efficiently than smaller instar larvae, as indicated by a more rapid decline of prey DNA quantity in the former compared to the latter. However, when these ladybird beetle larvae were fed with *Aphis glycines*, predator body size did not affect prey DNA recovery success. The current finding and the findings by Lundgren & Weber (2010) indicate that effects of predator size on

Table 2 Mean prey DNA quantity and prey DNA copy number (±SE) of Collembola, Diptera and Lumbricidae prey items before feeding to the centipedes and after 2 h of digestion

	Before feeding	After feeding (2 h)			Mean difference DNA quantity/copies (before–after feeding)
Prey type	Mean DNA quantity ± SE (ng/μL)	Mean DNA copy number ± SE	Mean DNA quantity ± SE (ng/μL)	Mean DNA copy number ± SE	DNA quantity/DNA copies
Collembola	$5.60 \times 10^{-3} \pm 2.87 \times 10^{-3}$	$2.00 \times 10^{7} \pm 1.03 \times 10^{7}$	$6.86 \times 10^{-4} \pm 4.29 \times 10^{-4}$	$2.46 \times 10^{6} \pm 1.54 \times 10^{6}$	$4.91 \times 10^{-3}/1.76 \times 10^{7}$
Diptera	$2.45 \times 10^{-1} \pm 3.11 \times 10^{-2}$	$1.19 \times 10^{9} \pm 1.51 \times 10^{8}$	$6.90 \times 10^{-4} \pm 2.68 \times 10^{-4}$	$3.36 \times 10^{6} \pm 1.31 \times 10^{6}$	$2.44 \times 10^{-1}/1.19 \times 10^{9}$
Lumbricidae	$4.21 \times 10^{-5} \pm 1.86 \times 10^{-5}$	$1.76 \times 10^{5} \pm 7.78 \times 10^{4}$	$9.60 \times 10^{-7} \pm 4.76 \times 10^{-7}$	$4.01 \times 10^{3} \pm 1.99 \times 10^{3}$	$4.11 \times 10^{-5}/1.72 \times 10^{5}$

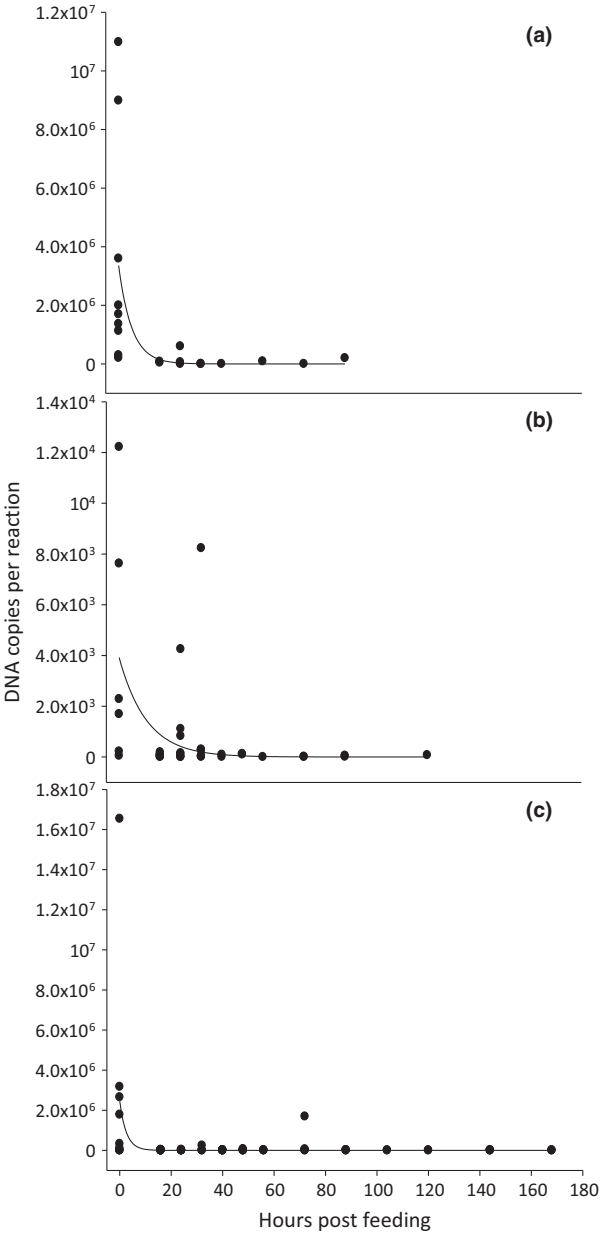

Fig. 2 Quantification of prey DNA of (a) Diptera (*Drosophila melanogaster*), (b) Lumbricidae (*Lumbricus terrestris*) and (c) Collembola (*Sinella curviseta*) in medium-sized lithobiid predators at 13 time points post-feeding. Trend line indicates the decay of prey DNA calculated by linear regression.

prey DNA detection success are affected by characteristics of both the predator and the prey.

The long post-feeding prey DNA detection intervals for Collembola in the current experiment which were derived by amplification of the relatively short 272-bp DNA fragment complicated the analysis of the body size experiment as the 50% prey detection probabilities were characterized by wide 95% confidence limits. Even though the 272-bp fragment is the longest in this study,

Table 3 Generalized linear model (GLM) on the effect of overall DNA quantity (predator + prey) and quantity of prey DNA, prey type (Collembola, Diptera, Lumbricidae) and the two-way interactions on the detection success of prey DNA in lithobiid predators via diagnostic PCR

Variable	d.f.	Deviance	Resid. d.f.	Resid. dev	*P* (>|chi|)
NULL		121	136.10		
Log_{10} DNA quantity overall	1	1.9287	120	134.17	0.164
Log_{10} prey DNA quantity	1	4.1990	119	129.97	**0.04**
Prey type	2	10.7440	117	119.23	**0.005**
Log_{10} DNA quantity overall × prey type	2	6.4338	115	112.8	**0.04**
Log_{10} prey DNA quantity × prey type	2	5.5666	113	107.23	0.062

Significant effects are highlighted in bold.

it is relatively short compared to fragments used in other studies (e.g. Waldner *et al.* 2013). Longer fragments have been shown to have shorter detection times due to faster enzymatic breakdown as compared to shorter fragments (Agusti *et al.* 1999; Von Berg *et al.* 2008). The application of longer prey DNA fragments would have possibly allowed for a more detailed characterization of post-feeding detection intervals.

Overall, post-feeding prey DNA detection times in lithobiid predators are long compared to predatory insects and spiders (Greenstone & Shufran 2003; Gagnon *et al.* 2011; Waldner *et al.* 2013), allowing successful amplification of prey DNA up to 168 h (7 days) post-feeding. This long post-feeding detection intervals are important to be considered when interpreting prey DNA detection in field-collected lithobiids, as feeding frequency and consequently predation impact could be overestimated (McMillan *et al.* 2007; Gagnon *et al.* 2011). Waldner *et al.* (2013) detected prey DNA of the scarabaeid beetle *Amphimallon solstitiale* in lithobiid predators up to 60 h post-feeding, which is on average three times longer than for the beetle predators tested in this study. Long prey retention times also have been reported in the feeding studies conducted by Poser (1990), in which an ELISA-based approach allowed tracking prey protein for even 20 days after the feeding event. Prolonged detection times have been shown in both, chewing and fluid feeding arthropod predators, (Juen & Traugott 2005; Sheppard *et al.* 2005; Greenstone *et al.* 2007; Kuusk & Ekbom 2010; Pumariño *et al.* 2011) and may depend on gut structure and physiology of animals. Also, predator species did not account for significant differences in positive prey DNA detection rates, indicating uniform prey digestion irrespective of lithobiid species and life stages (Table S1, Supporting information).

Lithobius chews its prey and ingests solid particles, but supposedly uses also its poison to predigest prey (Lewis 1981). Unlike spiders, the alimentary canal of centipedes is rather straight, having no diverticula serving as food reservoir (Rosenberg 2009).

The unusually long detection times of lithobiids may be ascribed to three factors: first, the low ambient temperatures of 15 °C slowed down metabolism and in particular digestive activity (Von Berg *et al.* 2008). This change of metabolic rate may be enhanced by the second factor, starvation: to void their guts, centipedes were starved for 7 days prior to experiment. Lithobiids regularly are starving during summer and winter (Pfleiderer-Gruber 1986), when drought and low temperatures are responsible for low prey activity, which is supposed to slow down their digestive activity (Hassett & Landry 1990). The effect of starvation, however, may also depend on the development stage of consumer, leading, for example, to different effects (i.e. increase and decrease) in different larval stages of carabid beetles (Lövei *et al.* 1985). Finally, feeding the lithobiids *ad libitum* with nontarget isopod prey ('chaser prey') could have provoked longer DNA retention times in medium and large predators (Symondson & Liddell 1995; Weber & Lundgren 2009). Chaser prey was offered to reach maximal digestive activity in all predator body size classes, of which only medium and particularly large individuals occasionally fed on this additional prey. As detection periods between all size classes, which presumably all had full guts, do not differ significantly, chaser prey, however, cannot be the sole reason for longer DNA detectability.

In the second experiment, we investigated the effect of prey quality on DNA detection success. Several studies have shown that post-feeding prey DNA detection intervals are related to the prey species (Harwood *et al.* 2007; Gagnon *et al.* 2011; Kuusk & Ekbom 2010). Moreover, it has been demonstrated that food quality has a great impact on digestion and gut transit times (Karasov *et al.* 2011). Animals feeding on low-quality food, such as phytoplankton or sclerotized arthropods, have extended digestion times to maximize nutrient uptake (Jaeger & Barnard 1981; Tirelli & Mayzaud 2005; Karasov *et al.* 2011; but see Mitra & Flynn 2007), suggesting longer post-feeding detection intervals of prey DNA than in high-quality prey. To study the effect of prey DNA quantity on time-dependent prey DNA detection success in predators, we therefore, for the first time, employed both diagnostic PCR and quantitative

real-time PCR. We found that in protein-rich prey of Lumbricidae, DNA detection intervals were significantly shorter than in Diptera and Collembola prey, apparently supporting our second hypothesis, and this was confirmed by generalized linear model (GLM) analysis of the pooled data. However, the qPCR results showed a different picture, with prey DNA quantity of each of the three prey taxa decreasing to a similar extent over time, indicating no effect of prey quality. Two reasons might have been responsible for the discrepancy of amplification success in diagnostic and quantitative PCR. (i) Despite using the same mass of prey offered, the target DNA concentration differed strongly between the three prey types. Lumbricid prey presumably consisted of more fluid tissue desiccating while being exposed to the predator. This would also explain the only small increase in body mass of lithobiids fed with earthworms. The number of *Drosophila* template DNA was over 5800 times higher than that for *Lumbricus* and 44 times higher than that for *Sinella* (Table 2). This indicates a greater likelihood of successful detection of dipteran prey. (ii) The sensitivity of the diagnostic PCR assays targeting Collembola and Lumbricidae prey was higher than the assay used to amplify Diptera prey DNA. Combining these two effects, an increasing amplification success in singleplex PCR in the order Collembola > Diptera > Lumbricidae is expected, and this is consistent with the present findings.

Target gene and gene copy number are important factors in molecular gut content analysis, and use of multicopy genes (e.g. cytochrome *c* oxidase subunit I, 18S rDNA) has been recommended to increase detection probability (Symondson 2002; Gariepy *et al.* 2007). However, the number of these genes can differ largely between different prey taxa and even between different body parts of individual species (Alberts *et al.* 2002). Using two different genes for prey detection therefore complicates comparisons. Additional analysis of prey DNA detection using quantitative PCR allowed examining the efficiency of the diagnostic PCR. GLM results revealed that detection of prey DNA depends on prey DNA quantity, indicating higher probability of positive detection at higher amounts of prey DNA. This might explain the lower proportion of centipedes tested positive for lumbricid prey immediately after feeding. However, prey DNA quantity did not significantly decrease with time in the three studied prey taxa. In fact, prey DNA quantity dropped within the first 32 h post-feeding, then levelling off close to detection threshold. However, the quantity of prey DNA was highly variable at specific time points post-feeding and characterized by high dropout rates of samples due to primer dimers and unspecific amplification, complicating the analysis. Rapid decrease in prey DNA template numbers and high variability in prey DNA concentrations have also been observed in other feeding studies employing qPCR, indicating that these findings are of general importance (Weber & Lundgren 2009; Durbin *et al.* 2011).

DNA has been shown to be a valuable marker in studying trophic interactions qualitatively and quantitatively (King *et al.* 2008; Pompanon *et al.* 2012). High sensitivity and specificity of PCR assays allows examination of the prey spectrum and prey preference of predators in unprecedented detail; however, the parameters affecting the detection success of prey DNA in a predator's gut are still poorly understood, necessitating further experiments. Such factors include predator body size and prey quality, which have been shown to drive predation (Eubanks & Denno 2000; Vucic-Pestic *et al.* 2010). In the present study, both factors did not affect prey DNA detection success suggesting that other factors, such as DNA copy number and assay sensitivity, may be more important. Laboratory feeding studies, such as the present one, should guide us how to interpret field-derived data on prey DNA detection. Results of the present study stress the importance of standardizing PCR assays. For example, the development and application of a new 18S rDNA-based PCR assay to detect Lumbricidae prey, featuring similar detection sensitivity than the other assays for Diptera and Collembola, would facilitate comparisons between the three prey taxa. Further, the results showed that the combined application of diagnostic and quantitative PCR helps interpreting prey DNA detection rates, thereby allowing to identify factors which are actually driving predator–prey interactions.

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Data accessibility

Predator biomass and prey DNA detection data: DRYAD entry doi:10.5061/dryad.ss17b.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Linear mixed-effect model on the effect of fixed factors predator body mass and digestion time (0–168 h post-feeding) and random factor predator species on the detection of prey DNA in lithobiid predators via diagnostic PCR.

Table S2 Summary of generalized linear model (GLM) on the effect of overall DNA quantity (predator + prey) and quantity of prey DNA, prey type (Collembola, Diptera, Lumbricidae) and the two-way interactions on the detection success of prey DNA in lithobiid predators via diagnostic PCR.