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# Variation in the detectability of predator-prey interactions may lead to misleading conclusions in trophic ecology studies

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## Research Article

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# Abstract

Molecular gut content analysis has revolutionised trophic ecology and provided precious insights for biological control. It allowed for the determination of the diet of many organisms and the identification of predators of specific pests in natural conditions. However, DNA detectability depends on many parameters, notably on digestion rates that vary according to the predator-prey pair considered and the amount of prey consumed. When not considered, these factors may lead to an underestimation of the frequency of certain interactions compared with those for which the detection time window is wide, and to distortions in the conclusions of ecological studies.

Here we study to which extent the identity of the prey and the predator affects the detection half-life of their interaction when using a diagnostic multiplex PCR. We focused on an omnivorous family of arthropods, carabid beetles, regarded as major natural enemies in many agroecosystems. To this purpose, the effects of digestion time, predator size, and voracity on DNA detectability were assessed for five common prey types. Based on this study, a correction factor was generated for each species of predator and type of prey. Finally, predation intensity indexes of carabid communities on the five prey types were calculated with and without the correction factor on a dataset containing observations from conventional and conservation winter wheat fields to illustrate the extent to which the inclusion of a digestion-based correction factor may alter the study's conclusions.

## Key Messages

- For many trophic interactions, the digestion process and its impact on DNA detection are unknown
- Size and voracity of the predator and prey identity impact digestion rates
- Assessment of predation intensity on agricultural pests is modified when considering digestion rates

## Introduction

With the banning of several phytochemical products, such as neonicotinoids, and the ongoing agroecological transition, modern agriculture has undergone a paradigm shift. Agroecological management of pests is notably based on effective conservation biological control (Letourneau et al. 2009). Extensive agricultural practices and complex habitats, at landscape or field scale, have been shown to favour natural enemies' abundance and diversity, and consequently their pest control service (Birkhofer et al. 2011; Rusch et al. 2016; Staudacher et al. 2018). However, the identification of effective natural enemies of pests among this diversity and assessment of their regulation potential are still required to predict their effectiveness in pest control (Greenstone et al. 2010), especially for generalist predators feeding on multiple resources.

Molecular gut content analyses have been effectively used to unravel trophic interactions (Birkhofer et al. 2017; Schmidt et al. 2021) and taxon-specific methods, such as diagnostic PCR, have been particularly helpful to identify predators of specific pests (Chen et al. 2000; Greenstone et al. 2010; Roince et al. 2012; Renkema et al. 2014; Rowley et al. 2017). Technological advances, such as multiplex PCR or high-throughput sequencing, make it possible to test predation on multiple prey at once, providing insights into the complexity of trophic networks. It also enables us to study the trade-off between pest control and other ecosystem services, hindered by the consumption of their suppliers (Roubinet et al. 2017; Staudacher et al. 2018) by opportunistic predators.

However, molecular approaches have several drawbacks. Indeed, detectability of prey DNA in the gut content of predators depends on multiple factors other than the act of predation itself (Sint et al. 2011; Greenstone et al. 2014), including the amount of DNA present in the sample (Juen and Traugott 2005), primer sensitivity (De León et al. 2006; Kheirodin et al. 2022) or amplicon size (Juen and Traugott 2005; Berg et al. 2008; Sint et al. 2011). DNA detectability is also affected by digestion rates, depending on both abiotic and biotic factors (Greenstone et al. 2014). The longer the digestion time is, the higher the chance of detecting prey DNA (Chen et al. 2000; Hosseini et al. 2008; Greenstone et al. 2010). Many factors have been suggested to modify detection rates, but studies are frequently inconsistent. Increasing temperature accelerates the rate of digestion and consequently lowers prey DNA detectability (Berg et al., 2008; Sint et al., 2011; but also Hosseini, Schmidt and Keller, 2008). Variability is also observed among predator taxa (Chen et al. 2000; Greenstone et al. 2010; Kobayashi et al. 2011; Kheirodin et al. 2020), among predators with different digestive systems (Hosseini et al. 2008), depending on sex and developmental stages

(Waldner et al., 2013; but Hoogendoorn and Heimpel, 2001) or amount of food ingested (King et al., 2010; Waldner et al., 2013; but Hoogendoorn and Heimpel, 2001; Juen and Traugott, 2005). Prey taxon is also a major source of variation for DNA detectability (reviewed in Greenstone et al., 2014) and the consumption of multiple prey can also change DNA detectability (Sint et al. 2018).

All of these limitations make it difficult to use molecular analysis to quantify predation. The obtained data is mostly qualitative (presence or absence of prey DNA in predator's gut) or not easily translated into the quantity of prey consumed (amount of DNA in the sample) and the detection rate of prey DNA in the predator pool is commonly used as a proxy for regulation intensity (Roubinet et al. 2015; Staudacher et al. 2018; Reich et al. 2020). Notable refinements on regulation proxies based on molecular analyses have been made by considering the respective abundance of predators in the ecosystem (Feit et al. 2019; Jones et al. 2021), adjusting prey detection rate with digestion rates, such as detection half-lives (Greenstone et al. 2010; Kheirodin et al. 2020; Uiterwaal and DeLong 2020; Andow and Paula 2023), or weighting by predator metabolic rates (Feit et al. 2019). Yet, digestion or metabolic correction factors are limited to a few specific predator-prey pairs, since their acquisition is time and cost-intensive (Greenstone et al. 2014; Uiterwaal and DeLong 2020).

Carabids are one of the most abundant and diverse families of natural enemies in temperate agroecosystems. These arthropods are characterized by an omnivorous and opportunistic diet that includes a wide range of pests and weed seeds (Lövei and Sunderland 1996; Kromp 1999). Molecular analysis of carabids' gut content confirmed those trophic links (Roubinet et al. 2017; Frei et al. 2019), but also revealed the consumption of other ecosystem services providers such as decomposers or other natural enemies (Davey et al. 2013; Frei et al. 2019). In a multiservice approach, this generalist diet questions the balance between the provision of pest control service and the potential disservice resulting from predation on other auxiliaries. Assessing the predation intensity of carabids on pest and non-pest prey might be the key to the answer.

This study aims to quantify the effects of several important factors on prey DNA detectability in the gut of carabids. Four hypotheses will be tested: (i) DNA detectability decreases with the digestion time interval since prey consumption. (ii) DNA detectability varies with prey taxon. Indeed, a wide gradient of detectability half-lives has been observed, with values ranging from less than 20 hours for aphids (Sheppard et al. 2005; Berg et al. 2008) to as much as 90 hours for some earthworm species (Harper et al. 2005; King et al. 2010), on a unique predator species, *Pterostichus melanarius*. (iii) DNA detectability increases with predator voracity. (iv) DNA detectability increases with predator size. This is because small predators are expected to have higher metabolic rates and digest faster than large predators, resulting in lower DNA detectability. Our expectancy is that carabid size strongly determines metabolic rate on the one hand and voracity on the other. Therefore, carabid size could account for most of the effects of carabid taxonomic identity on DNA detectability. If confirmed, it could considerably reduce future experimental efforts to estimate digestion correction factors, by allowing predator species to be replaced by their size. This would avoid the need to assess individual digestion rates for each species.

The factors influencing prey DNA detectability will finally be used to correct the predation intensity of carabid communities on the five prey types studied in the field. This will allow us to assess the extent to which digestion-based correction factors affect our conclusions about the biological control potential and disservices provided by carabids.

## Material & Method

### Factors of variation in DNA detectability

#### Carabid sampling

Carabid beetles were collected in 2 experimental fields at Le Rheu, west of Rennes, Brittany, Western France. The first field was planted with conventional wheat and the second consisted of 6 plots, cultivated with wheat, potato and wheat-pea intercrop under conventional and conservation systems. 80 dry pitfall traps were opened and emptied once a day over three days in April, May and June 2021.

Collected carabid beetles were identified with Roger *et al.*'s identification key (Roger et al., 2013) and isolated in individual Petri dishes containing moistened filter paper at the bottom, moistened cotton and a hiding place to limit stress. Petri dishes were stored in a climate chamber under controlled conditions ( $18 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  RH and LD 16:8h photoperiod). Before the experiment, carabid beetles were fed with larvae of the miller beetle, *Tenebrio molitor*.

## Prey sampling

The prey species used for the feeding trials were chosen to be representative of the five prey types detected by the multiplex used in Sacco–Martret de Prévaille et al. (2024), *i.e.*, aphids and slugs for pests, springtails and earthworms for decomposers, and spiders for predators. *Acyrtosiphon pisum* aphids and *Folsomia candida* springtails were obtained from laboratory strains. Slugs were handpicked and identified with the Rowson (2014) identification key. Spiders were collected in the same dry pitfall traps as carabid beetles and identified with the Laurent, Ridel and Pétilon (2022) identification key. Earthworms were collected by soil sorting and identified by an expert. *Deroceras reticulatum*, *Pardosa tenuipes* and *Lumbricus terrestris* were chosen for the feeding trial because they were the most abundant species of their taxonomic group in the captures. After identification, all prey were stored at  $-20^\circ\text{C}$ .

## Feeding trial

Before the feeding trial, carabid beetles were starved for 7 days to maximize their food intake. Carabids were deprived of food but were provided water with moistened cotton.

Before the feeding trial, the prey were defrosted. Each carabid was given a single prey type *ad libitum* for 2 hours and observed until it had begun to consume it (scheme of the feeding trial in Online Resource 1). Feeding, regurgitation and defecation behaviours were noted. Individuals were weighed before and after the feeding trial with a precision scale (Medler Toledo XS105) and the difference between the two measurements is then referred to as voracity. Carabids that did not feed during the 2 hours were removed from the rest of the experiment. 38 carabids received no prey and served as controls.

After the feeding phase, carabids were allowed to digest for a fixed time interval (0h, 3h, 6h, 18h, 24h, 48h or 72h). Carabids in the 0h-time interval were frozen at  $-20^\circ\text{C}$  immediately after the feeding phase. The remaining carabids were stored in a climate chamber under controlled conditions ( $18 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  RH and LD 16:8h photoperiod) for the designated time and then frozen at  $-20^\circ\text{C}$ . The distribution of samples depending on digestion time interval, prey and carabid species or their size is presented in Online Resources 2 and 3, respectively.

## Molecular analysis

To remove potential prey DNA on the predator body surface, carabids were washed successively in a solution of 1–1.5% sodium hypochlorite (Sigma-Aldrich) and 0.02% Tween for 30s, a solution of molecular grade water with 0.02% Tween for 30s and a solution of molecular grade water for 30s and isolated into a 2mL-individual Eppendorf tube.

Cuticles and soft tissues were frozen with liquid nitrogen, crushed and homogenised with 3-5mm-stainless steel beads (two cycles of 3 minutes in the paint stirrer). Eppendorf tubes were filled with lysis buffer, 25 $\mu\text{L}$  of Proteinase K (20mg/mL) and 180 $\mu\text{L}$  of T1 solution (Qiagen kit), then mixed vigorously and spanned down. Lysate was incubated overnight at  $50^\circ\text{C}$ . DNA was extracted using the BioSprint 96 DNA Blood Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. 100 $\mu\text{L}$  of DNA extract was obtained per sample and stored at  $-20^\circ\text{C}$  until PCR amplification. Four negative controls per batch of 96 samples were included to check for DNA contamination (two at the lysis step and two at the extraction step).

The gut content of a total of 643 carabids was screened for the 5 prey taxa using a previously developed DNA-based Multiplex PCR assay and following the same PCR cycling conditions (Sacco–Martret de Prévaille et al. 2024). PCR products were displayed using the QIAxcel electrophoresis system (Qiagen) with the AL420 method.

# Impact of correction factors in the assessment of the relative predation intensity

## Predation intensity indexes

The predation intensity of a prey  $i$  by a predator  $j$ ,  $pi_{ij}$ , was calculated as the product of the abundance of the predator  $j$ ,  $A_j$ , by the detection rate of the prey  $i$  in the gut content of the predator  $j$ ,  $DR_{ij}$ , and by its voracity, *i.e.* the amount of prey  $i$  consumed by the predator  $j$  when provided *ad libitum*,  $V_{ij}$ .

$$\varvec{p} \varvec{i}_{\varvec{j}} = \varvec{A}_j \times \varvec{D}_{ij} \times \varvec{V}_{ij}$$

The corrected version of the predation intensity index for a prey  $i$  by a predator  $j$ ,  $cpi_{ij}$ , was defined as the ratio of the predation intensity of the prey  $i$  by the predator  $j$ ,  $pi_{ij}$ , by the detectability half-life of the prey  $i$  in the predator  $j$ ,  $HL_{ij}$ , a proxy of the digestion speed for each pair of predator and prey. Similarly to Greenstone et al. (2010), detectability half-life was calculated for each carabid species-prey pair, except that detectability half-life was not defined as the time at which the probability of detection is 0.5, but as the time at which the probability of detection at 0h is reduced by half.

$$\varvec{c} \varvec{p} \varvec{i}_{\varvec{j}} = \frac{\varvec{p} \varvec{i}_{\varvec{j}}}{\varvec{H} \varvec{L}_{ij}}$$

Note that the units of the two predation intensity indexes are different: mg for the original predation intensity index and  $\text{mg.h}^{-1}$  for the corrected version. Consequently, and to facilitate the comparison between the two, the uncorrected predation intensity index was rescaled by dividing it by the average detectability half-life across studied carabid communities and prey,  $HL_{ave}$ .

$$\varvec{p} \varvec{i}'_{\varvec{j}} = \frac{\varvec{p} \varvec{i}_{\varvec{j}}}{\varvec{H} \varvec{L}_{ave}}$$

The predation intensity on a prey  $i$  of a predator community in a field  $k$ ,  $PI_{ik}$ , and its corrected version,  $CPI_{ik}$ , were obtained as the sum of the predation intensity on prey  $i$  by all predators present  $j$ ,  $pi'_{ij}$ , respectively  $cpi_{ij}$ .

$$\varvec{P} \varvec{I}_{\varvec{k}} = \sum \varvec{p} \varvec{i}'_{\varvec{j}} \\ \varvec{C} \varvec{P} \varvec{I}_{\varvec{k}} = \sum \varvec{c} \varvec{p} \varvec{i}_{\varvec{j}}$$

## Application to an agroecological dataset

To illustrate to what extent incorporating correction factors can alter the conclusion on natural regulation in an agroecological study, we compared the uncorrected and corrected predation intensity of carabid communities from fields in different agricultural systems and over time. The analysed sample included 3209 carabids and 53 taxa collected one week per month in April, May and June 2019 in five conventional and five conservation fields in the Bretagne region, France. The complete study design of this experiment and description of the carabid communities can be found in Sacco–Martret de Prévile *et al.* (2022).

Detection rates for each prey DNA in the guts of each carabid species were obtained from another experiment on the carabid diet in winter wheat fields, for which the gut analysis was performed with the same multiplex of primers (Sacco–Martret de Prévile et al. 2024). The carabid diet was studied over three sampling sessions, so prey DNA detection rates are session-dependent.

To calculate the whole community predation intensity, a detection rate must be defined for each carabid species and prey type. This was achieved in three ways depending on the sample size for each carabid species considered: 1) when the sample size was greater than 10 individuals, the detection rates observed for this species were assigned to it, 2) when the sample size was

less than 10 individuals but greater than 10 individuals for the sample including all species belonging to the same genus, the species was assigned the detection rates of its genus, and 3) when both the sample size for the species and for its genus were less than 10, the species was assigned the detection rates of the whole community. Details of the number of individuals sampled per species at each session are given in Online Resource 4.

For each carabid species and prey type, voracity was approximated by a model based on carabid size presented in Online Resources 5 and 6.

## Statistical analysis

Statistical analyses were performed using R (R Core Team 2023) version 3.6.1.

The effects of digestion time, prey type, carabid size, carabid voracity and the interaction between prey type and digestion time on DNA detection rate were fitted using a generalised linear model with the “glm()” function from the “stats” package (R Core Team 2023), assuming a binomial distribution and a logit link. Dispersion of residuals was checked with the “simulateResiduals()” function from the “DHARMA” package (Hartig and Lohse 2022). The significance of the explanatory variables was checked with the “Anova()” function from the “car” package (Fox et al. 2019).

The effects of prey type, agricultural system, session and all their double interactions were fitted separately for uncorrected and corrected predation intensities using linear models with the “lmer()” function from the “lme4” package (Bates et al. 2015). Dispersion of residuals and significance of the explanatory variables were checked as above. Pairwise comparisons between the levels of each significant factor were carried out using “emmeans()” and “pairs()” functions from the “emmeans” package (Lenth et al. 2024).

## Results

### Factors of variation in DNA detectability

Digestion time interval, carabid size and carabid voracity all significantly affect the probability of DNA detection (Table 1). Increasing the digestion time interval by one hour decreases the DNA detection odds by 9% ( $P < 0.001$ , Figs. 1a and 1b). At the start of digestion (digestion time interval = 0h), the odds of DNA detection do not significantly differ between aphids and the other prey taxa. The slopes of the decrease in detection probability with digestion time were similar to that for aphids for all prey types except earthworms, for which DNA detectability decreases significantly more slowly ( $P < 0.05$ , Figs. 1a and 1b) despite a general non-significant effect of the interaction between digestion time and prey. Increasing carabid voracity and carabid size by one unit (respectively, 1mg and 1mm) significantly increases the DNA detectability odds by 7% ( $P < 0.01$ ) and 11% ( $P < 0.05$ ), respectively (Figs. 1a, 1c and 1d).

Table 1

Analysis of Deviance Table testing the significance of the effect of digestion time, prey type, and their interaction, carabid voracity and carabid size on the probability of prey DNA detection in carabids. LR Chisq stands for the likelihood ratio chi-square statistic, Df for the degree of freedom, and Pr(> Chisq) for p-values associated with the chi-square statistic.

DNA detectability			
<i>Predictors</i>	<i>LR Chisq</i>	<i>Df</i>	<i>Pr(&gt; Chisq)</i>
Digestion time (h)	160.445	1	< 0.001 ***
Prey	8.981	4	0.262
Carabid voracity (mg)	10.773	1	< 0.01 **
Carabid size (mm)	4.133	1	< 0.05 *
Digestion Time (h) * Prey	6.041	4	0.196
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$			

## Application to an agroecological study

Prey type and all interactions between session, prey type and agricultural system have significant effects on predation intensity, whether corrected for digestion speed or not (Table 2). The session variable, apart from its effects in interaction with the other variables, was only significant for the model with the corrected predation intensity values ( $P < 0.05$ , Table 2).



Table 2

Analysis of Deviance Table testing the significance of the effect of session, prey type, agricultural system and all their double interactions on uncorrected and corrected values of the predation intensity of carabid communities per field. Predictors in bold indicate a change in significance between models. Chisq stands for the chi-square statistic, Df for the degree of freedom, and Pr(> Chisq) for p-values associated with the chi-square statistic.

		Predation intensity		
<i>Index</i>	<i>Predictors</i>	<i>Chisq</i>	<i>Df</i>	<i>Pr(&gt; Chisq)</i>
Uncorrected predation intensity index	Intercept	0.014	1	0.907
	<b>Session</b>	<b>2.370</b>	<b>2</b>	<b>0.306</b>
	Prey type	32.912	4	< 0.001 ***
	Agricultural system	0.140	1	0.708
	Session: Prey type	25.022	8	< 0.01 **
	Prey: Agricultural system	15.498	4	< 0.01 **
	Session: Agricultural system	15.481	2	< 0.001 ***
Corrected predation intensity index	Intercept	0.028	1	0.867
	<b>Session</b>	<b>6.558</b>	<b>2</b>	<b>&lt; 0.05 *</b>
	Prey type	29.227	4	< 0.001 ***
	Agricultural system	0.844	1	0.358
	Session: Prey type	32.671	8	< 0.001 ***
	Prey: Agricultural system	11.248	4	< 0.05 *
	Session: Agricultural system	18.515	2	< 0.001 ***
		<i>*p &lt; 0.05   **p &lt; 0.01   ***p &lt; 0.001</i>		

Multiple pairwise comparisons changed significance with the use of corrected predation intensity values compared to uncorrected ones. As the aims of this study are not the ecological conclusions per se but to test whether they change when digestion speed is accounted for, three examples linked to one prey of agricultural importance, aphids, were selected to illustrate the change in ecological conclusions. The complete list of pairwise comparisons that changed in significance is presented in Online Resource 7.

Uncorrected predation intensity on aphids was significantly lower than on earthworms in April ( $P < 0.01$ ) and June ( $P < 0.001$ ) but those differences turned insignificant (respectively  $P = 0.088$  and  $P = 0.851$ ) when the predation intensity index was corrected by digestion speed (Fig. 2a). On the contrary, the corrected predation intensity on aphids became significantly higher than the one on slugs in June ( $P < 0.05$ ), when it was not beforehand ( $P = 0.589$ , Fig. 2a). The significance of the differences in carabid predation on aphids between agricultural systems also changed when digestion speed was incorporated into the predation intensity index (Fig. 1b): uncorrected predation intensity was not significantly different between conventional and conservation agricultural systems ( $P = 0.236$ ), but after correction, it turned significantly higher in conservation fields ( $P < 0.05$ ). Results on the predation dynamics on aphids also changed when considering digestion speed (Fig. 1c). The difference in predation between April and June was not significant with uncorrected values ( $P = 0.078$ ) but was with corrected ones ( $P < 0.001$ ).

## Discussion

This study confirmed that DNA detectability is dependent on multiple biological factors, including time since prey consumption, prey type, predator voracity and predator size. We showed that accounting for these sources of variation in DNA detectability led to changes in the estimation of the predation intensity of carabid communities on various prey types. The use of the detectability

half-life correcting factor mostly influenced the relative predation intensity assessed on some preys compared to others, its dynamics over time and agricultural management, highlighting the importance of considering these biological factors in ecological studies based on molecular analysis of gut contents.

As in previous studies and irrespective of predator and prey taxon, DNA detectability decreased with time since prey consumption (Berg et al. 2008; Greenstone et al. 2010; King et al. 2010; Davey et al. 2013; Waldner et al. 2013). For the first time, this assay was performed simultaneously on multiple prey with a single multiplex of prey-specific primers allowing us to test the impact of prey type on detection rate in a common experiment. Earthworm DNA was detected for longer time intervals after feeding than aphid DNA. Digestion rates of carabids on aphids and earthworms had never been tested in the same study beforehand. Previously reported values of aphid DNA detectability half-life ranged from 5 to 25 hours (Wallace 2004; Sheppard et al. 2005; Harper et al. 2005; Firlej et al. 2013) while the values for earthworms showed a wider range of variation, from 10 to 90 hours (Harper et al. 2005; King et al. 2010), suggesting, as does our study, different time frame to detect their DNA in the gut content of predators. Diagnostic multiplex PCR relies on several primers with different amplicon lengths, which could explain the difference in DNA detectability (Juen and Traugott 2005; Sint et al. 2011). However, the only significant difference in DNA detectability was between the two prey with the smaller amplicon length (earthworm: 85bp, aphid: 120bp, versus springtail: 205bp, spider: 260bp, slug: 360bp) and primer concentration was adjusted during the multiplex development so that detectability was similar for all prey types for the same DNA amount (Sacco–Martret de Prévile et al. 2024). This explanation is therefore disregarded suggesting that the differences are in fact due to differences in digestion speed.

DNA detectability increased with voracity. Indeed, a higher food intake by the predator induces a higher amount of prey DNA in its gut content and consequently a higher detectability, which had been previously verified (King et al. 2010; Waldner et al. 2013) but not in all cases (Hoogendoorn and Heimpel 2001; Juen and Traugott 2005). Carabid species size also had a significant effect on DNA detectability with DNA detection rate increasing with carabid size for a fixed voracity, which might be due to faster metabolic rates in smaller species. In their meta-analysis, Uiterwaal and DeLong (2020) modelled the DNA detectability half-life in spiders as a function of the logarithm of their mass but the model still required to additionally account for the spider's family. Our study is the first in which a DNA detectability assay is performed for multiple carabid species and a functional trait, species size, is used as the sole proxy for predator identity independently of any other taxonomical information. The dependence of voracity and DNA detectability on carabid size is an exciting result as it makes this single trait a relevant proxy for assessing the half-life DNA detectability in any carabid species for the five prey items tested. Therefore, the digestion speed correction factor for the predation intensity can be calculated without having to repeat a detectability assay for each carabid species. The use of this proxy is a time- and resource-saving alternative considering the effort necessary to set up a detectability assay (Greenstone et al. 2014). This also offers the possibility to calculate detectability half-lives for rare species, which together account for a significant proportion of the highly diverse carabid communities in agroecosystems.

Correcting the predation index to account for the half-life of detectability partially changed the ecological conclusions on the predation intensity of carabid communities on the studied prey. The changes in ecological conclusions are driven by two main forces: differences in prey digestibility and differences between carabid species in their digestion speed, the second being important when comparing carabid assemblages differing in their species composition. Taking into account specific detection half-lives, predation on aphids by carabids increased to the point that it was no longer statistically different from predation on earthworms in two of the three sessions. Aphid consumption and the associated natural regulation provided by carabids tend, therefore, to be underestimated compared to the disservices resulting from the consumption of beneficial organisms such as earthworms. The trade-off between pest and beneficiary alternative prey consumption is hence more balanced than expected in this case. The difference in predation intensity between the two pests turned significant in June, highlighting again how aphid predation in molecular gut content analysis is underestimated compared to other prey because they are rapidly digested. Differences in digestion speed between carabid species also had an important impact on ecological conclusions as the results of the comparison of aphid predation intensity across time and agricultural systems significantly changed when specific detectability half-lives were included in the index. The predation intensity on aphids calculated with specific detectability half-lives significantly increased between April and June, as expected given the increased aphid availability in cereal fields during this period (Sacco–Martret de Prévile et al. 2022), which was not the case when using the uncorrected index. A similar change of significance occurred when comparing aphid predation intensity in two agrosystems: in conservation fields, aphid predation

intensity turned significantly higher than in conventional ones with the corrected index. Since a unique prey was used in both cases, carabids' specific digestion speed was the sole factor at play to explain the change. This suggests that carabid communities from conservation fields and globally in June are composed of species that digest aphids faster than communities in conventional agroecosystems and earlier in the season, and if unaccounted for, differences in predation between modalities would be missed. Adjusting measures of the predation intensity for carabid communities is therefore essential for an honest assessment of their suitability as natural regulation agents, but also to properly assess the balance between the services and disservices they provide over time and agricultural management.

This study has then focused on the differences in DNA detectability between carabids and proposed a correcting factor based on a simple functional trait, their size. However, it was limited to five of their common prey animals. The opportunistic and generalist diet of carabids includes many more potential prey, including lepidopterans, dipterans, thrips or even plant material, such as weed seeds (Staudacher et al. 2018; Frei et al. 2019; Reich et al. 2020), that would need to be considered as well in detectability and predation intensity assays. Here again, the huge diversity of potential prey prevents an exhaustive analysis from being conducted. It would therefore be valuable to have symmetrically a proxy for prey digestibility based on simple prey traits or nutrient content.

Overall, our results revealed multiple factors of variation in DNA detectability, including prey type, predator voracity and predator size. This last trait proved to be a useful proxy for implementing a correcting factor for DNA detection rate, allowing the assessment of predation intensity, even for untested carabid species and at the entire carabid community level. This study also illustrated how conclusions in trophic ecology studies based on molecular gut content analysis can be partially misleading when factors influencing DNA detectability are neglected.

## **Declarations**

## **Competing interests**

The authors have no relevant financial or non-financial interests to disclose.

## **Ethics approval**

No international, national and institutional guidelines for the care and use of animals were needed and followed.

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## **Author Contribution**

EC, MP and ASMDP conceptualised and designed the study. EC and ASMDP collected the molecular dataset. ASMDP analysed the data and wrote the first version of the manuscript with the help of EC and MP. All authors contributed to the revision of the draft and gave final approval for publication.

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# Data Availability

The data that support the findings of this study are openly available in the Recherche Data Gouv Repository at <https://doi.org/10.57745/XXV1XQ> except for the data of the molecular gut content analysis in natural conditions that are available at <https://doi.org/10.57745/BBHYX>.

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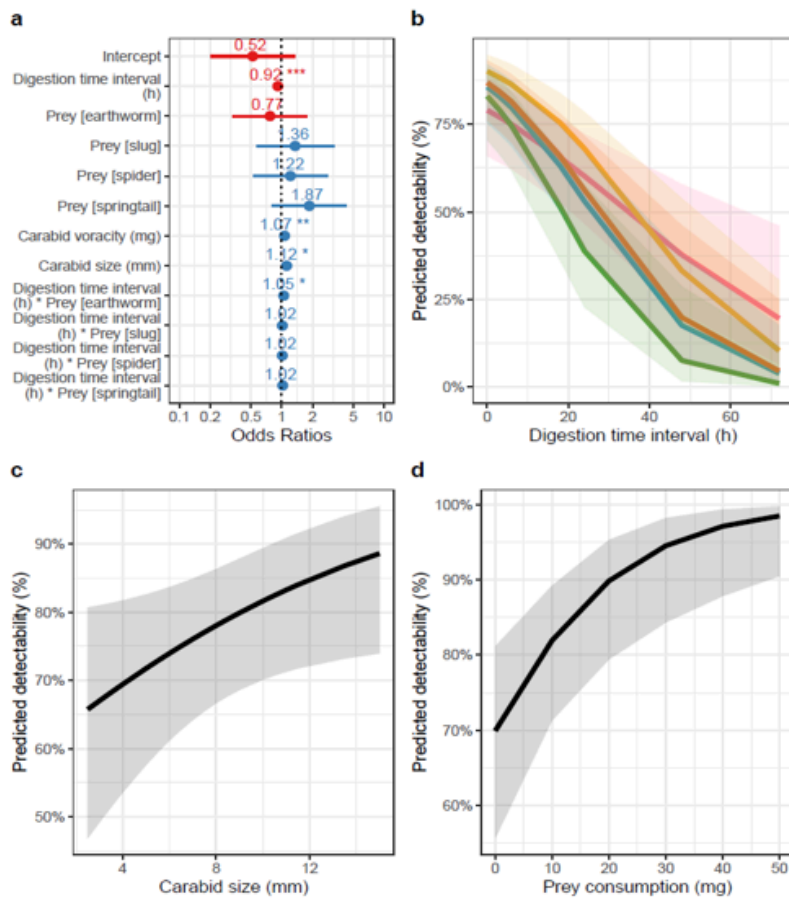
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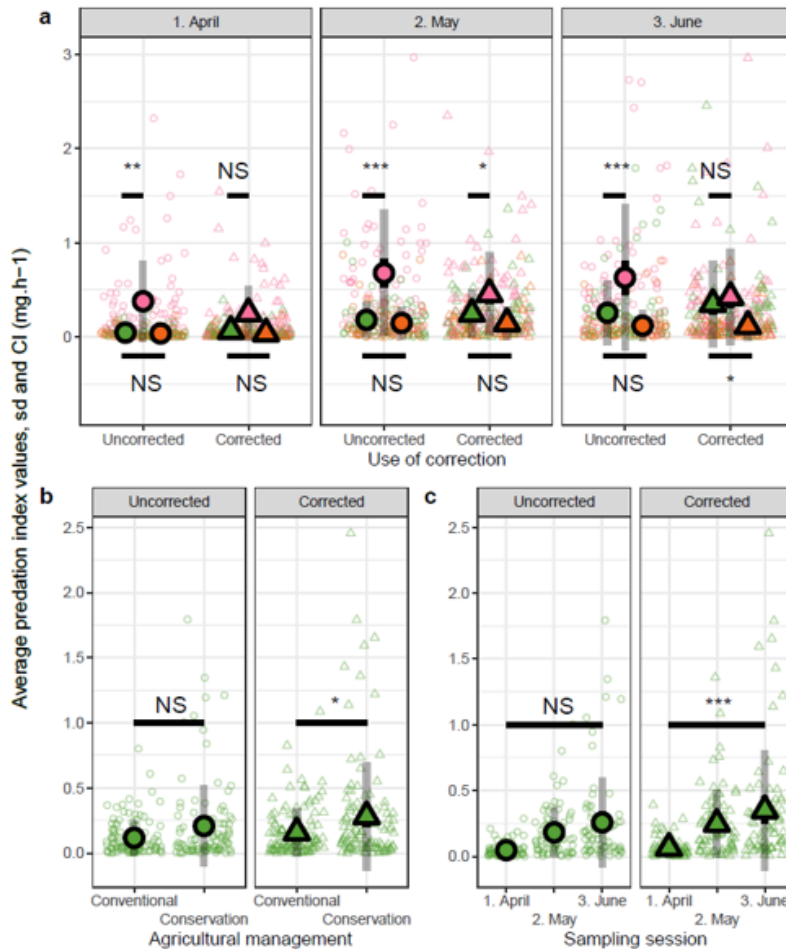
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## Figures



**Figure 1**

Estimates (odds ratio) of the DNA detectability model (a), with 95% confidence intervals (reference prey type: aphid). In this panel, red colour reflects a negative value and blue a positive one. Regression plots of the effects on DNA detectability of the digestion time for each prey type and a carabid species with the size and voracity similar to *Poecilus cupreus* (size: 11mm, prey consumption: 15mg, b), of carabid voracity for a digestion time fixed at 0h (c) and of carabid size with the digestion time fixed at 0h (d). Shaded areas represent 95% confidence intervals.



**Figure 2**

Uncorrected (circle) and corrected (triangle) aphids predation intensity of carabid communities (green, hollow) and their average (green, solid) with the standard deviation of the data (grey line range) and 95% confidence interval (black line range) represented in three situations: compared to earthworms (pink) and slugs (red) average predation intensity across sessions (a), in two different agricultural systems, conventional and conservation, (b), and across sessions (c). Changes in significance in pairwise comparisons are indicated with a horizontal black segment and the level of significance (NS: not significant, \*: p-value<0.05, \*\*: p-value<0.01, \*\*\*: p-value<0.001).

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