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Estimating predation rates from molecular gut content analysis

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Abstract

Several methods have been published to estimate per capita predation rates from molecular gut content analysis relying on intuitive understanding of predation, but none have been formally derived. We provide a theoretical framework for estimating predation rates to identify an accurate method and lay bare its assumptions. Per capita predation can be estimated by multiplying the prey decay rate and the prey quantity in the predators. This assumes that variation in per capita predation rate is approximately normally distributed, prey decay occurs exponentially, and predation is in steady state. We described several ways to estimate steady state predation, including using only qualitative presence-absence data to estimate the decay rate and in addition, we provided a method for estimating per capita predation rate when predation is not in steady state. We used previously published data on aphid consumption by a ladybird beetle in a feeding trial to calculate the predation rate and compare published methods with this theoretically derived method. The estimated predation rate (3.29 ± 0.27 aphids/h) using our derived method was not significantly different from the actual predation rate, 3.11 aphids/h. In contrast, previously published methods were less accurate, underestimating the predation rate (0.33 ± 0.02 to 1.66 ± 0.8 aphids/h) or overestimating it (3.64 ± 0.30 aphids/h). In summary, we provide methods to estimate predation rates even when variation in predation rates is not exactly normally distributed and not in steady state and demonstrate that the prey decay rate, and not the prey detection period, is required.

KEYWORDS
food webs, generalist predators, molecular diet analysis, natural enemies, predation rate

1 | INTRODUCTION

Estimation of predation rates of predators in the field has been a central goal of biological control and an important objective of community ecology for over a century. Many methods have been used including analysis of the physical remains in a predator gut and direct observation of predation, both first reported by Summerhayes and Elton (1923) and later using video recordings (e.g., Frank et al., 2007), emergence cages (Varley & Gradwell, 1960) and prey baits through sentinel or artificial prey (e.g., Andow & Risch, 1985; Roslin et al., 2017). However, each of these methods have serious limitations. Gut dissections can identify only prey with body parts that are slow to digest and can rarely resolve taxonomic identity below the family level. Direct observation and video recordings provide observations over a limited time period and spatial extent. Emergence cages are also limited in time and space and are effective only for parasitoids. Sentinel prey may be artificially presented, and artificial prey may not be accepted in the same way as real prey, introducing potential unknown biases in estimated predation rates.

Molecular gut content analysis has promised to revolutionize the estimation of per capita predation rates of predators in the field. Ever since the pathbreaking work of Dempster (1960) with the precipitin test, a serological test that precipitates an antigen of the prey out of solution, molecular ecologists have been trying to quantify the predation rate on the detected prey. Dempster (1960) was prescient in understanding that the predation rate depended on more than the detection of prey in the predator and that it also depended on the amount of prey consumed by an individual predator and the length of time the prey remained detectable after consumption (D_{Demp}). As the precipitin test provided only a qualitative presence-absence of prey, Dempster (1960) used ancillary experiments to estimate these other values, but as we show later, Dempster's (1960) concept of D_{Demp} was imprecise.

Subsequent workers sought to generalize Dempster's quantification method in two different ways. One way was to allow for varied predation rates (either higher or lower than one prey per day) and to allow for detection periods different from 1 day (Kuperstein, 1979; Rothschild, 1966). These values were still determined from ancillary experiments, and in particular, the detection period (D_R), was estimated from the longest time that a prey was detected experimentally. While this was an improvement over Dempster's formulation, D_R remained imprecisely defined. A second approach was to assume that successful attacks on prey by a predator were distributed as a Poisson distribution (Lister et al., 1987; Nakamura & Nakamura, 1977). However, subsequent research has indicated that this method poorly estimated per capita predation rates (e.g., Naranjo & Hagler, 2001; Sopp et al., 1992), probably because prey is not equally likely to be successfully attacked, violating the key assumption of the Poisson distribution.

A major advance in molecular prey detection was the use of quantitative enzyme-linked immunosorbent assays (ELISA), which allowed direct measurements of the amount of a prey antigen present in the gut of a predator (Crook & Sunderland, 1984; Fichter & Stephen, 1981; Hance & Rossignol, 1983; Lövei et al., 1985). With the use of quantitative ELISA, researchers could directly estimate the biomass of prey consumed by predators. Sunderland et al. (1987) suggested that the per capita predation rate could be estimated by multiplying the frequency of positive prey detections in the predators by the quantity of prey antigen measured in the positives and dividing by D_{max}, an estimated maximum detectability period. Sopp et al. (1992) improved this method by dividing the Sunderland et al. (1987) method by a constant that depended on the decay of prey antigen in the predator. The constant was defined as the average amount of prey left in a random predator at the end of a detection period. In their work, Sopp et al. (1992) found that a constant equal to 0.2 provided excellent empirical estimates of predation rates.

Lister et al. (1987) proposed a forgotten method for estimating per capita predation rates. They studied predation on an Antarctic collembolan using quantitative electrophoresis. They estimated the decay rate of prey enzyme in the predator and estimated the predation rate by multiplying together the frequency of predators with positive prey detection, the biomass of prey in the positive predators and the decay rate. Unfortunately, they did not provide any justification or derivation of this method, and it has not been used to estimate predation rates in any publication since it was proposed 35 years ago.

Greenstone and Hunt (1993) introduced the concept of detectability half-life when using qualitative prey detection methods, such as conventional PCR. The detectability half-life is the time to when only 50% of the predators test positive for prey. They advocated the use of detectability half-life as opposed to D_{max} (Sunderland et al., 1987) for the cases in which prey DNA detectability decays exponentially during digestion. As we discuss below, the "detectability half-life" is not a half-life, but an estimate of the average time to when the prey quantity drops below the limit of detection (LOD), which we call t_{LOD}.

Quantitative PCR (qPCR) has also been used to quantify prey in predators (Deagle & Tollit, 2007; Durbin et al., 2008; Lundgren et al., 2009; Lundgren & Fergen, 2011; Weber & Lundgren, 2009; Zhang et al., 2007). An index of predation rate was developed using Cq values (quantification cycle, e.g., Weber & Lundgren, 2009). Other quantification methods include radial immunodiffusion (McIver, 1981) and species-specific alkaloids (Hautier et al., 2008). None of these methods, however, have been used to quantify per capita predation rates to date.

Here, for the first time, we provide a theoretical derivation of per capita predation rates by modelling the prey quantity in a predator. We lay bare the three unstated assumptions implicit in all of the published work to date and relax one of these assumptions to estimate temporally changing per capita predation rates. We also show two approaches to estimate the parameters to calculate per capita predation rate. Finally, we provide an empirical example and show that the theoretically derived predation rate is the most accurate predation rate.

2 | MATERIALS AND METHODS

2.1 | Definitions

Because previous work on the estimation of predation rates relied on an intuitive understanding of the components of predation and how they can be combined to estimate a predation rate, we provide definitions of terms to eliminate vagueness. These definitions are summarized in Box 1. Each of these definitions is for one species (or stage) of predator and for any number of prey. We designate the prey with the subscript i. If we wanted to make the definitions for multiple species (or stages) of predators, we would add the subscript, j, for the different predators. Parameter values with a circumflex are estimated values.

We define the following:

p_i = average per capita predation rate on prey i.

BOX 1   Definitions of parameters.

i = prey i.

p_i = per capita predation rate on prey i.

d_{if} = dilution factor at which Q_{i0} is reduced to the LOD.

d_i = decay rate of prey i in the predator.

D_{Demp} = the amount of time a prey meal remains detectable in the predator (Dempster, 1960).

D_{max} = maximum period over which prey remains could be detected in any individual of a given predator species (Sunderland et al., 1987).

D_R = maximum period over which prey remains detectable in a predator (Rothschild, 1966).

f_i = probability that an individual predator is detected with prey i.

H_{i50} = analyte decay half-life of prey i.

k_i = average amount of prey i left in a predator at the end of the detection period (Sopp et al., 1992).

LOD_i = limit of detection of the quantification method for prey i.

n_i = average number of prey i consumed by a predator.

q_i = average quantity of prey i in a predator given that prey i is detected in the predator.

Q_i = average quantity of prey i in a predator.

Q_{i0} = quantity of prey i in a predator immediately after consumption of a known quantity of prey, calibration constant.

t_{i50} = time to when 50% of predators test positive for prey i.

t_{iLOD} = average time of detection of prey i.

$\delta_i(t)$ = time-varying rate of decay of prey i in the predator.

$\varphi_i(t)$ = time-varying per capita rate of predation on prey i.

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f_i = probability that an individual predator is detected with prey i. This can be estimated from the proportion of tested predators that test positive for prey i.

q_i = average quantity of prey i measured in a predator, given that prey i was detected in the predator. This is estimated only from the predators that tested positive for prey i.

Q_i = average quantity of prey i in a predator for all individual predators, including those that did not test positive for prey i.

Q_{i0} = average quantity of prey i in a predator immediately after feeding on a specified number or amount of prey i.

n_i = average number of prey i consumed by a predator.

$\varphi_i(t)$ = time-varying rate of per capita predation on prey i.

$\delta_i(t)$ = time-varying rate of decay of prey i in the predator (digestion plus excretion).

d_i = first order rate of decay of prey i in the predator, equal to exponential decay.

H_{i50} = decay half-life, time it takes for half of the prey quantity in the predator to decay, equal to analyte half-life and equal to $1/d$.

LOD_i = limit of detection of prey i for a specific quantification method expressed in the units of detection of the quantification method.

d_{if} = dilution factor, the dilution necessary to reduce Q_{i0} to the LOD_i. Can be determined without knowing Q_{i0} or the LOD_i.

t_{iLOD} = average time of detection from consumption of a given amount of prey i until the prey quantity in the predator is equal to the LOD_i and can no longer be detected. This value will depend on the size of the prey meal, the prey species, and the predator species. This value is different from D_{max}, as described in the text.

t_{i50} = the time when 50% of predators test positive for prey i. This is the definition for the detectability half-life proposed by Greenstone and Hunt (1993).

D_{Demp} = the amount of time a prey meal remains detectable in the predator as defined by Dempster (1960). This value will depend on the size of the prey meal, the prey species, and the predator species. This definition is vague because it is not clear how many or what proportion of predators must test positive to determine this time period.

$D_R = D_{max}$ = maximum period over which prey remains detectable in a predator as defined by Rothschild (1966), and the maximum period over which prey remains could be detected in any individual of a given predator species as defined by Sunderland et al. (1987). This value will depend on the size of the prey meal, the prey species, and the predator species. This definition is clearer than D_{Demp} in that only one predator must test positive to determine the time period. However, it depends on determining the extreme value of the detection times, and will depend on the distribution of detection times and the number of predators tested. No study to date has used the distribution of detection times and number of predators tested to estimate D_{max}.

2.2 | Development of theory to lay bare assumptions

A formal derivation of per capita predation enables identification of an accurate method for estimating predation rates and lays bare the assumptions involved. This then allows extensions of the method when these assumptions are relaxed.

The quantity of prey i in a predator, Q_i, is related to the balance between the time-varying rate of predation on the prey ($\varphi_i(t)$) and the time-varying rate of prey decay in the predator from digestion and excretion ($\delta_i(t)$). Specifically, the change in Q_i in some small time interval is:

$$\Delta Q_i = \varphi_i(t)\Delta t - \delta_i(t)\Delta t, \tag{1}$$

so, in general:

$$\frac{dQ_i}{dt} = \varphi_i(t) - \delta_i(t). \tag{2}$$

We simplify equation 2 with two assumptions. First, we assume that $\varphi_i(t)$ can be modelled using the average predation rate over individual predators and during the time period of the study, which we designate p_i. This is different from assuming that the predation rate is the same for all predators and constant over time, which is

clearly unrealistic. However, it assumes that variation among individual predators and over time can be treated as a random process with approximately normally distributed variation. For such a process, the average of the process is typically equal to the deterministic process using the average of the random variable, which in our case is the average predation rate.

Second, we assume that decay follows a first order decay process, which means that the prey quantity decays at a constant rate in the predator. This is analogous to radioactive decay, which means that over time prey quantity declines exponentially. We designate this rate as d_i. Lister (1984) and Lister et al. (1987) observed exponential decay using quantitative electrophoresis of the springtail *Cryptopygus antarcticus* (Willem) (Collembola: Isotomidae) in the predaceous antarctic mite, *Gamasellus racovitzai* (Trouessart) (Mesostigmata: Ologamasidae), and Lövei et al. (1985) using quantitative ELISA found exponential decay of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) in larvae of *Poecilus cupreus* (L.) (Coleoptera: Carabidae) in four of four experiments. Sopp and Sunderland (1989) used quantitative ELISA to detect the aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae), in the guts of predators in the Linyphiidae, Carabidae and Staphylinidae and observed exponential decay in 35 of 36 comparisons. Paula et al. (2015) used read mapping of shotgun sequenced DNA fragments in the gut contents of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and found that decay of pea aphid DNA *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) in the predator gut was exponential. Additional work using the same method with two coccinellid predators on an aphid and a chrysopid prey also exhibited exponential decay in 12 of 12 cases (Paula et al., 2022). Thus, while it is by no means assured in every case, the assumption of first order decay is well supported by empirical results.

With these two assumptions, that variation in predation rate is approximately normal and decay of prey is exponential, the rate of change of Q_i is

$$\frac{dQ_i}{dt} = p_i - d_i Q_i(t). \tag{3}$$

In the field, the average prey content in the population of predators may be in a steady state with the rate of consumption balancing the rate of decay. The steady state assumption is that, on average, the rate that prey i are consumed equals the rate that they are eliminated from the predator. Under this condition,

$$\frac{dQ_i}{dt} = 0, \tag{4}$$

and consequently the per capita predation rate is estimated by

$$\widehat{p}_i = \widehat{d}_i \widehat{Q}_i, \tag{5}$$

where the estimated per capita predation rate, $\widehat{p}_i$ is equal to the product of the estimated decay rate, $\widehat{d}_i$, and the estimated quantity of prey in a predator, $\widehat{Q}_i$. The decay rate of prey in the predator gut, d_i,

is essential to estimate the predation rate. As we will show, the use of the detectability period (D_{max} or t_{LOD}) in the estimation of predation rate will underestimate the per capita predation rate and the trophic interaction strength.

As the decay rate, d_i, is equal to the inverse of the decay half-life, H_{i50}, an equivalent estimator of per capita predation rate at steady state is

$$\widehat{p}_i = \frac{\widehat{Q}_i}{\widehat{H_{i50}}}. \tag{6}$$

The decay half-life is different from the detectability half-life that was developed by Greenstone and Hunt (1993). The decay half-life is the time it takes for half of the analyte to decay (i.e., half the prey quantity in the predator), while the detectability half-life is the time it takes for the frequency of detection of prey in predators to decline by half.

2.3 | Estimation of Q_i, the quantity of prey i in a predator

The quantity of prey i in a predator, Q_i, typically has been estimated in two parts, the frequency of positive detections, f_i, and the amount (biomass or number) of prey in the positive predators, q_i. Then $Q_i = f_i q_i$ is the average amount of prey i detected in a predator. This two-part method may slightly underestimate Q_i because the detection methods have a non-zero limit of detection (LOD_i) and the quantities below the LOD_i are set to 0. The two-part method is necessary for quantifications relying on ELISA and qPCR because these do not provide comparable estimates for 0 values. For example, a negative detection in qPCR has no Cq or n_0 (relative initial template concentration), and therefore cannot be readily averaged with samples that have a Cq and n_0. ELISA values are estimated on a log scale for absorbance so 0 values would be $-\infty$, which cannot be averaged with finite values.

An alternative to the two-part method is to estimate Q_i directly for a population of predators. This can be done by pooling a sample of individual predators, making sure that each individual predator contributes an equal biomass on average to the pooled sample. Then the estimate of the prey in the pooled sample is an estimate of Q_i for that pool of predators. This means that predation rates can be estimated from pooled samples of predators, which can greatly reduce the cost of the molecular analysis. For example, for methods using mapping of unassembled shotgun reads, pooling reduces the cost of library construction tremendously (Paula et al., 2022).

It is essential to calibrate Q_i either to prey biomass or prey number whether Q_i is estimated directly or in two parts. One way to calibrate Q_i is to estimate the quantity of prey in the predator immediately after it consumed a measured biomass of prey. This quantity, designated Q_{i0} (the measured amount of a known amount of prey i in a predator at time 0 after consumption), can be used to convert Q_i from the measurement units of the detection

method into prey biomass. Lister et al. (1987) used quantitative electrophoresis to estimate prey quantity in a predator in this manner. They estimated the electrophoresis units for the prey quantity detected in a predator immediately after it consumed a known biomass of prey to convert the electrophoresis units into prey biomass. The use of Q_{i0} can be incorporated into equation 5 as $\hat{p}_i = \frac{\hat{d}_i \hat{Q}_i}{Q_{i0}}$. Alternatively, as done by Sopp and Sunderland (1989), a calibration curve can be constructed to convert absorbance values obtained from quantitative ELISA to prey biomass. Calibration curves can be used in conjunction with other molecular methods, including quantitative electrophoresis (Lister et al., 1987), quantitative ELISA (Sopp & Sunderland, 1989), quantitative PCR (Deagle & Tollit, 2007) and mapping unassembled shotgun reads (Paula et al., 2022), to convert prey quantity measured into prey biomass consumed.

2.4 | Estimation of d_i, the decay rate of prey i in a predator

Typically, d_i (or H_{i50}) is estimated in an independent laboratory feeding trial experiment, in which predators consume a known quantity of a known prey, and at different times after consumption, the quantity of prey remaining in the predator is measured. This was done by Lister et al. (1987) using quantitative electrophoresis, Lövei et al. (1985) and Sopp and Sunderland (1989) using quantitative ELISA and Paula et al. (2015, 2022) using mapping unassembled shotgun reads (a method called Lazaro) and the reader is referred to these studies for detailed experimental methodologies.

Decay rates can also be estimated from qualitative methods that provide only presence-absence of prey in individual predators assuming that prey quantity declines exponentially in the predator. Under this assumption,

$$Q_i(t) = Q_{i0}\exp(-d_i t). \tag{7}$$

All qualitative methods have an LOD_i below which prey i are not detectable. $Q_i(t)$ will reach the LOD_i at some time t_{iLOD} based on equation 7,

$Q_i(t_{iLOD}) = LOD_i$, and therefore

$$LOD_i = Q_{i0}\exp(-d_i t_{iLOD}). \tag{8}$$

Consequently,

$$\hat{d}_i = \frac{1}{t_{iLOD}}\ln\left(\frac{Q_{i0}}{LOD_i}\right) \tag{9}$$

The decay rate can be estimated from the time that Q_i equals the LOD_i starting from Q_{i0}, which is defined as the t_{iLOD}, and the natural logarithm of the ratio of the initial prey quantity to the LOD_i. Technically, t_{iLOD} is different from D_{max} (see definitions in Box 1). Both

are measures of the time that a prey is detectable, but D_{max} is the maximum time and t_{iLOD} is the average time. Consequently, t_{iLOD} is easier and more accurately measured than D_{max}.

The natural logarithm of the ratio of the initial prey concentration to the LOD_i, $\ln(Q_{i0}/LOD_i)$ can be determined by running a dilution series with predators that had consumed Q_{i0} of prey. One would have predators consume Q_{i0} of prey, extract the DNA, and set up a dilution series with the extracted DNA. The natural logarithm of the dilution factor, d_{if}, at which Q_{i0} is no longer detectable, that is, $Q_{i0}/d_{if} = LOD_i$, is the required estimate. The dilution factor can be estimated without estimating either Q_{i0} or the LOD_i.

The t_{iLOD} also can be estimated from qualitative data without having to estimate the LOD_i. Theoretically, for prey quantities higher than the LOD_i, prey should always be detectable and when the quantity drops just below the LOD_i, prey will not be detectable. Consequently, detection should follow a step function (Figure 1a), not exponential decay function, with prey being detectable up to the time, t_{iLOD}, when the prey quantity drops below the LOD_i and becomes undetectable. Theoretically, t_{iLOD} could be estimated from the time the step function drops, which could be identified by change point analysis (Fong, Di, et al., 2017; Fong, Huang, et al., 2017). However, in reality, there will be variation in the precision and accuracy of the detection method, in the amount
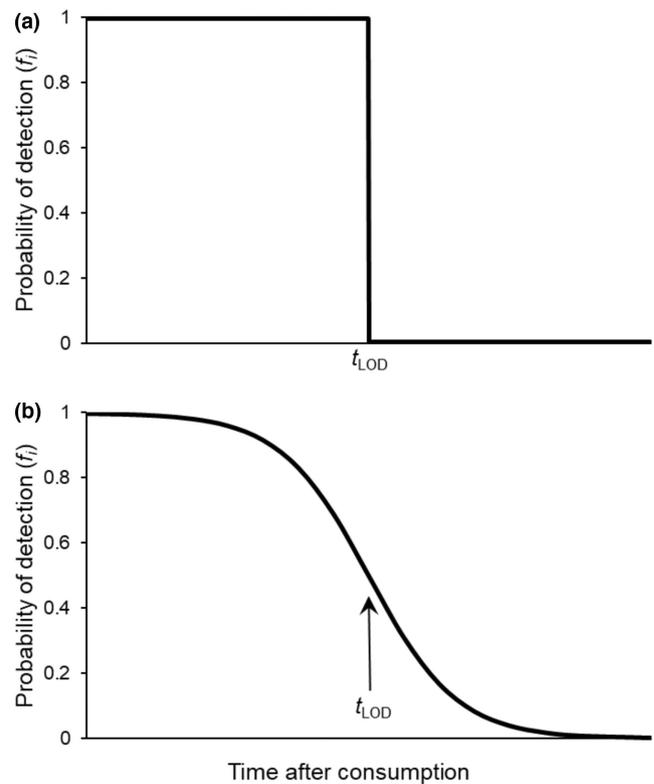
FIGURE 1 Decay curves for presence-absence methods of detection of prey i. (a) Theoretical curve with complete detection when prey quantity is above the limit of detection (LOD) and no detection when it is below the LOD. (b) Realistic decay curve with variation in detectability. t_{LOD} is when the probability of detection is 0.5.

of prey consumed, and in the decay rates among individual predators. Assuming that this variation is approximately normal, the detection curve will be sigmoidal (Figure 1b). In this case, the frequency of predators testing positive for the prey will follow the sigmoidal function:

$$f_i(t) = 1 - \frac{1}{1 + \exp\left(-a_i\left(t - t_{iLOD}\right)\right)}, \qquad (10)$$

where a_i governs how fast the sigmoidal function declines. This is a nonlinear equation with two unknowns (a_i and t_{iLOD}), which can be estimated using one of many available software platforms, including R, or using logistic regression or probit analysis. Substituting t_{iLOD} for t in equation 10, $f_i(t_{iLOD}) = 0.5$, so t_{iLOD} is the time when $f_i(t) = 0.5$, which is exactly the time when only 50% of the predators will test positive for the prey. This time, which we can call t_{i50} is exactly the "detectability half-life" that was previously proposed (Chen et al., 2000; Greenstone et al., 2010; Greenstone & Hunt, 1993). In other words, $t_{i50} = t_{iLOD}$ and is estimated from the time when the prey can be detected in 50% of the predators.

Equation 10 can be used when the molecular method provides presence-absence of the prey. Such methods include the precipitin test, qualitative ELISA and other qualitative immunoblot methods, and conventional nonquantitative PCR. As an example, we reanalysed data by Chen et al. (2000) in their Figure 7, which shows the decay of detection of an aphid in two different predators by conventional PCR (presence-absence of prey). We fit both equations 7 (exponential detection) and 10 (sigmoidal detection) to their data (Figure 2, Table 1) using the function NonlinearModelFit in Mathematica. For *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae) (Figure 2a), sigmoid detection (AICc = −0.57) fit significantly better than exponential detection (AICc = 8.04), which means the probability that exponential detection was as good as sigmoid detection was equal to 0.014. For *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae) (Figure 2b), sigmoid detection (AICc = −6.00) fit significantly better than exponential detection (AICc = 1.18) and the probability that exponential detection was as good as sigmoid detection was equal to 0.028. These decay curves estimate t_{iLOD} of the aphid, which was 9.21 h in *H. convergens* and 4.24 h in *C. plorabunda* (Table 1). The decay curves in Greenstone et al. (2010, their Figure 1) also appear sigmoidal and would also provide estimates of t_{iLOD}. These theoretical considerations show that the decay rate can be estimated from qualitative detection data as follows:

$$\hat{d}_i = \frac{1}{t_{i50}} \ln\left(d_{if}\right). \qquad (11)$$

2.5 | Nonsteady state estimation of predation rate

If Q_i is measured at two different times, $Q_i(t_1)$ and $Q_i(t_2)$ and $Q_i(t_1) \neq Q_i(t_2)$, then the steady state assumption is incorrect. If predation had been in steady state, $Q_i(t_1) = Q_i(t_2)$. If it is not in steady state,
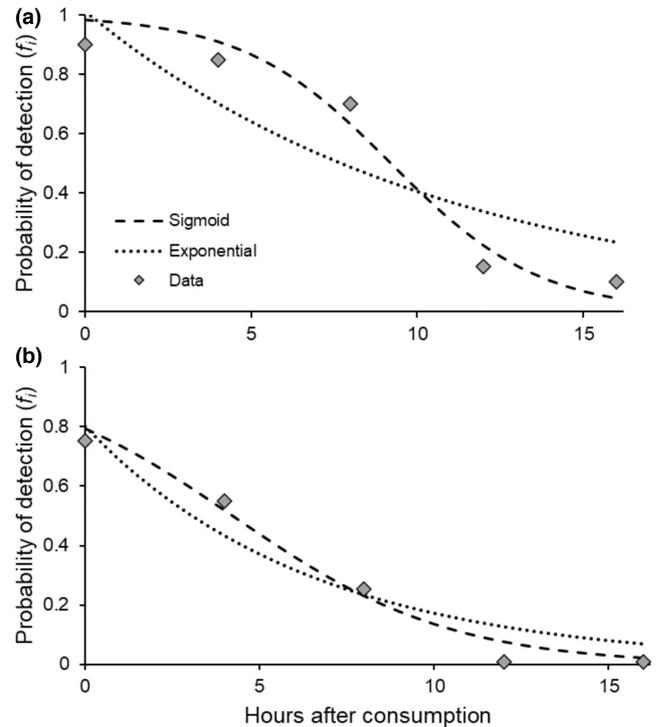
FIGURE 2 Reanalysis of data in Figure 7 of Chen et al. (2000), showing decay of detection of an aphid in two predators, (a) *Hippodamia convergens* and (b) *Chrysoperla plorabunda*, with a sigmoidal decay curve and an exponential decay curve.

then it is possible to estimate a nonsteady state value for the predation rate at the first time predation was measured. In this case, the nonsteady state per capita predation rate at time t_1 is:

$$\hat{p}_i(t_1) = \hat{c} + \hat{d}_i \hat{Q}_i(t_1), \qquad (12)$$

where $\hat{c}$ adjusts the steady state estimate for the increasing or decreasing predation rates and

$$\hat{c} = \frac{\hat{Q}_i(t_2) - \hat{Q}_i(t_1)}{t_2 - t_1}. \qquad (13)$$

The per capita predation rate at time t_2 can be estimated only if one makes the steady state assumption or if one has an additional measure of predation at a later time t_3.

2.6 | Comparison with previously published methods

All of the previously published methods assumed that predation on the prey is in a steady state, that is, equation 4 is assumed to be true. As can be seen from equation 12, the only way to know if predation is not in steady state and either increasing or decreasing, is to measure predation at different times, which none of the previously proposed methods have done. Thus, we compared previously published measures of predation rates (Dempster, 1960; Lister et al., 1987; Nakamura & Nakamura, 1977; Rothschild, 1966; Sopp et al., 1992;

TABLE 1 Parameter estimates, *t*-values and *p*-values for sigmoid and exponential curves fitted to data in Figure 7 of Chen et al. (2000) for *Hippodamia convergens* and *Chrysoperla plorabunda* feeding on one aphid of *Rhopalosiphon maidis*.

		Hippodamia convergens			*Chrysoperla plorabunda*		
	Parameter	Estimate (SEM)	t_3	*p*-value	Estimate (SEM)	t_3	*p*-value
Sigmoid	a_i	0.446 (0.115)	3.897	.0299	0.319 (0.045)	7.032	.0059
	t_{iLOD}	9.21 (0.65)	14.101	7.73E−04	4.24 (0.46)	9.269	.0027
Exponential	Q_{i0}	1.01 (0.19)	5.271	.0133	0.800 (0.102)	7.856	.0043
	d_i	0.091 (0.036)	2.543	.0844	0.154 (0.038)	4.018	.0277

TABLE 2 Summary of previously published methods for estimating per capita predation rate on prey *i* (A–G) compared to the steady state methods proposed in this manuscript (H–J).

Method	Per capita predation rate (p_i)	Decay or detection time (d_i or D)	Prey quantity in predator (Q_i)	Estimated predation rate (aphids/h) (SEM)	*z*-score	*p*-value
A. Dempster (1960)	f_i	$D_{Demp} = 1$		NA	NA	NA
B. Rothschild (1966)	$(f_i n_i)/D_R$	D_R		NA	NA	NA
C. Nakamura and Nakamura (1977)	$\ln(1-f_i)/D_R$	D_R	$\ln(1-f_i)$	NA	NA	NA
D. Sunderland et al. (1987)	$(Q_i)/(Q_{i0}D_{max})$	t_{iLOD}	Q_i	0.33 (0.02)	−164.7	.0000
E. Sopp et al. (1992) $k = 0.2$	$Q_i/(Q_{i0}kD_{max})$	t_{iLOD}	Q_i	1.66 (0.08)	−17.19	.0000
F. Sopp et al. (1992) exponential	$(d_i Q_i)/(Q_{i0}(1-(LOD_i/Q_{i0})))$		Q_i	3.64 (0.30)	1.796	.0726
G. Lister et al. (1987)	$d_i Q_i/Q_{i0}$	d_i	Q_i	3.29 (0.27)	0.677	.4984
H. Equation 5	$d_i Q_i/Q_{i0}$	d_i	Q_i	3.29 (0.27)	0.677	.4984
I. Equations 5 and 13, alternative	$\ln(Q_{i0}/LOD_i)Q_i/(Q_{i0}t_{i50})$	$\ln(Q_{i0}/LOD_i)/t_{i50}$	Q_i	2.81 (0.24)	−1.261	.2073
J. Equation 6	$Q_i/(Q_{i0}H_{i50})$	$1/H_{i50}$	Q_i	3.31 (0.27)	0.709	.4783

Note: Symbols are defined in Box 1. We have added a calibration constant (Q_{i0}) for all of the methods using Q_i. It is also possible to calibrate Q_i with a calibration curve. Derivation of Method F is shown in the Appendix S1. The *z*-score and *p*-value test if the estimated predation rate is equal to the actual predation rate (3.11 aphids/h).

Sunderland et al., 1987), which we have designated Methods A–G, with the steady state estimates based on our theoretical results, which we have designated Methods H–J, based on equations 5, 6 and/or 9 (Table 2).

We used previously published data (Paula et al., 2022) to illustrate how predation rate can be estimated, and compared these estimates with the actual predation rate. Paula et al. (2022) conducted a feeding trial giving a predaceous *H. convergens*, 1, 3 or 6 apterous *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) prey and sacrificing the predators 3, 6 or 9 h after they had consumed the prey. The guts of ten replicate predators for each of the three food quantities and three times of sacrifice (9 treatments, 90 individuals total) were dissected and pooled, their DNA was extracted and sequenced without barcode amplification, and the number of reads of *M. persicae* associated with each treatment was counted. We eliminated the two treatments that had no *M. persicae* reads detected, which were 1 *M. persicae* consumed and the predator sacrificed at either 6 or 9 h, leaving seven treatments. We mathematically combined the seven treatments into a single artificial population with equal representation of each of the seven treatments to estimate the per capita predation rate by this artificial population. In addition, three sets

of 10 *H. convergens* were fed one *M. persicae* aptera and sacrificed immediately after feeding to calibrate the observed read numbers. These beetles were handled the same as the treatment beetles with three samples of 10 beetles each. Their DNA was extracted and sequenced and the number of reads counted. Read numbers were normalized for all samples and treatments to equal DNA quantities and equal sequencing depth. We estimated the decay rate and the average time of detection (t_{iLOD}) of *M. persicae* reads in *H. convergens*. All samples and treatments were sequenced in the same lane on an Illumina HiSeq2500 (250 nt paired-end). Additional methodological details can be found in Paula et al. (2022).

Depending on the method for estimating predation rate, we substituted Q_i for $f_i q_i$ as our experimental methods allowed direct estimation of Q_i and we did not estimate f_i and q_i separately. Q_i was estimated from the natural logarithm of the number of prey reads, which was linearly related to the time since consumption and the natural logarithm of the number of prey consumed (Paula et al., 2022). We used the experimental treatments to estimate the decay rate, *d*, of *M. persicae* reads in *H. convergens* and the decay half-life, H_{i50}. These values can be found in Paula et al. (2022) and are repeated here for convenience: $d_i = -0.9604\,\text{h}^{-1}$ (± 0.1243, SEM)

and $H_{i50} = 1.041$ h (± 0.137, SEM). We used t_{iLOD} instead of D_{max} for Methods D and E (Sopp et al., 1992; Sunderland et al., 1987; Table 2). Because $t_{iLOD} < D_{max}$, as will be seen below, the use of t_{iLOD} give results that are not as severely underestimated as results that would use D_{max}. Standard errors for the estimated predation rates were calculated by Monte Carlo simulation with 10,000 replicates.

3 | RESULTS

We calculated the actual predation rate in the experimental treatments because we knew the number of aphids consumed per capita in each of the treatments. There were three treatments with six aphids consumed per capita in a 9 h period, three treatments with three aphids consumed per capita in a 9 h period and one treatment with one aphid consumed per capita in a 9 h period. Together, the treatments consumed 28 aphids per capita in a 9 h period, for an actual per capita predation rate of $29/9 = 3.11$ aphids/h.

We calculated a calibration constant to convert the number of reads into the number of prey and this calibration constant, Q_{i0}, was estimated to be 7.162 (± 0.066, SEM), the logarithm of the average number of prey reads in the three calibration samples (predator sacrificed immediately after consuming 1 *M. persicae* aptera). The LOD$_i$ was $\ln(2)$ reads, as a positive detection required detection by both the forward and reverse reads of a pair of paired-end reads. The average detection period, t_{iLOD}, varied with the number of aphids consumed.

We could not estimate a predation rate using Methods A–C (Dempster, 1960; Nakamura & Nakamura, 1977; Rothschild, 1966, Table 2) because we did not estimate the frequency of predators with positive prey detections (f_i). However, these Methods err by dividing by a detection period (D_{Demp}, or D_R) rather than a decay half-life, and consequently will underestimate the predation rate as the detection period is greater than the decay half-life. For example, in our previously published experiments (Paula et al., 2015, 2022), the detection period was 4–12× longer than the decay half-life. The Sunderland et al. (1987) method for estimating predation rate (Method D, Table 2), used a similar formulation as Methods A–C (Dempster, 1960; Nakamura & Nakamura, 1977; Rothschild, 1966), by dividing by D_{max} (in Table 2 we used t_{LOD}), and provided an illustration of this underestimation problem. As predicted, Method D (Sunderland et al., 1987) estimated the predation rate to be 0.33 ± 0.02 aphids/h, which severely underestimated the true predation rate (Table 2).

Sopp et al. (1992) must have realized that dividing by the detection period severely underestimated predation rates, because they introduced a correction factor, which we have called k, to reduce the influence of the detection period. They allowed for general functional forms for prey decay in a predator but did not develop this idea. When they estimated the predation rates for seven predators feeding on the aphid, *Sitobion avenae*, they found that $k = 0.2$ resulted in the best estimation of predation rate for the predators (Sopp et al., 1992; Method E). This method (Table 2) using a constant

$k = 0.2$, significantly underestimated the true predation rate in our example, with the per capita predation rate estimated to be 1.66 ± 0.08 aphids/h. For the data used here, $k = 0.106$ would have provided an accurate estimate of predation rate.

Sopp et al. (1992) also provided numerical examples for the value of k for different values of exponential decay, which ranged from 0.14 to 0.39. In the Appendix S1, we derived a general formula for the "correction factor," k, for any value of exponential decay. The general result is $k_i = d_i/(1-(LOD_i/Q_{i0}))$. What this means is that the "correction factor" converts the maximum detection period (D_{max}) into an adjusted decay rate (d_i) with the adjustment equal to $(1-(LOD_i/Q_{i0}))$. When $Q_{i0} \gg LOD_i$, $(1-(LOD_i/Q_{i0})) \rightarrow 1$ and $k_i \rightarrow d_i$, that is, the general equation for the method for exponential decay (Sopp et al., 1992: Method F in Table 2) is an approximation of our equation 5. This approximation will be good in cases where the LOD$_i$ is much smaller than Q_{i0}. However, Method F (Sopp et al., 1992 exponential; Table 2) overestimated predation (3.64 ± 0.30 aphids/h; p-value $= .0726$). This overestimation occurred because Q_i, Q_{i0} and LOD$_i$ were measured on a logarithmic scale, and on this scale the LOD$_i$ was not much smaller than Q_{i0}. Specifically, $(1-(LOD_i/Q_{i0})) = 0.903$, which inflated the predation rate by about 10%.

One of the two methods proposed by Lister et al. (1987; Method G in Table 2) is identical to our newly derived Method H (based on equation 5). Although Lister et al. (1987) provided an accurate method to estimate predation rate, they did not provide a rationale for, nor a derivation of the method. Consequently, it has been overlooked by subsequent researchers and has never been used since they first proposed it. However, despite these failings, we acknowledge that they were the first to propose an accurate method to estimate predation rates for molecular gut content data.

Our Methods H–J provide mathematically equivalent equations for estimating per capita predation rates but differ in the parameters used to estimate predation rates. Method H relied on equation 5, which used the parameters d_i and Q_i. Method I used equation 9 to estimate the decay rate instead of an experiment and used estimates of LOD$_i$, Q_{i0} and t_{iLOD}. As t_{iLOD} depends on the amount of prey initially consumed, the use of Method I may be limited unless t_{iLOD} can be shown to be independent of the amount of prey initially consumed. It would also be possible to estimate the predation rate using the parameters in equation 11 instead of equation 9. Method J (based on equation 6) used the decay half-life, H_{i50}, instead of the decay rate. Additional research is needed to determine how the variance of the estimated predation rate differs, if at all, between Methods H and J.

The estimated predation rate from Methods G–J (Lister et al., 1987 and our new methods; Table 2) were not significantly different from the true predation rate (3.29 ± 0.27 aphids/h). Method I (based on equation 9), although theoretically equivalent to these other methods generated a different value of the predation rate (2.81 ± 0.24 aphids/h), because it relied on estimates of the LOD$_i$, Q_{i0}, and t_{iLOD}, which may not be as accurately estimated as the parameters in equations 5 and 6 (Methods H and J; Table 2). The estimated value of predation was not significantly different for Method

I from the true predation rate (p-value = .2073), indicating that the theory was correct.

4 | DISCUSSION

We demonstrated that estimation of per capita predation rates from molecular data requires estimating the average quantity of prey i in the predator (Q_i) and the decay rate of prey in the predator (d_i), and not the detection period (D_{Demp}, D_R, D_{max} or t_{iLOD}). This result rests on the three assumptions: that variation in per capita predation rates is approximately normally distributed, decay of prey remains in a predator is exponential, and predation is in a steady state. The normality assumption is the one most likely to be violated, although the Central Limit Theorem suggests that it is likely to be a good approximation for any more complex distribution. Formal treatment of normal and non-normal stochastic variation remains for future analysis using stochastic differential equations. While virtually all empirical examples of prey decay in a predator are consistent with exponential decay, we know of no theoretical treatment of the prey digestion process to indicate that exponential decay should be the general case. Despite this uncertainty, exponential decay is the first order approximation of any more complex decay process, which means that it will be a reasonable approximation for any more complex decay process. Thus, the methods developed here should provide reasonable estimates of predation rate even if the first two assumptions are violated. All previous methods for estimating per capita predation rates implicitly assumed that predation was in a steady state. When predation is estimated at only one time point, it is not possible to determine if it is in a steady state, so previous estimates of predation have all assumed steady state.

We have built on previous methods by developing an equation for estimating per capita predation rates when they are not in steady state. This requires estimating predation at two time points and using the difference between them to determine if predation is increasing or decreasing and to estimate by how much (equations 12 and 13).

We showed, using an empirical example that the methods we developed here provide accurate estimates of the predation rate of an artificial population of predators and that previously proposed methods were less accurate. Overall, none of the previously published methods (Methods A–F, Table 2; Dempster, 1960; Nakamura & Nakamura, 1977; Rothschild, 1966; Sopp et al., 1992; Sunderland et al., 1987) provided an accurate estimate of predation rate except for Method G, which was one of the methods proposed by Lister et al. (1987). All of these methods recognized that some combination of the probability of detection, the quantity of prey consumed, and the time prey were detectable were important to estimate the predation rate, and while the methods were intuitively reasonable, the bias can be substantial, particularly in those that relied on the maximum detection period.

An important issue that remains to be resolved is the applicability of laboratory measures of decay rates to the field. This would be a difficult experiment to conduct unless predators were caged in the field, but caging might introduce biases due to alterations in microclimate and predator behaviour. Alternatively, instead of trying to determine the effect of any environmental condition on decay rates, a first step could be to focus on the effect of temperature. As metabolic rates in arthropods vary with ambient temperature, d_i is expected to be temperature dependent. The limited data available suggest that prey decay rates are lower in arthropods when temperatures are <15°C, but when temperatures are >15°C decay rates are less affected by temperature. McIver (1981) found significantly longer prey detection times (slower decay rates) when the temperature was below 15°C than above 20°C, and Sopp and Sunderland (1989) found slower decay rates at ≤13°C than at 16°C in seven of 10 species. We found no significant difference in decay rates at 20°C, 25°C and 30°C for *Cycloneda sanguinea* larvae feeding on *Myzus persicae* (unpublished data). These results suggest that the effect of temperature on decay is highly nonlinear, which may create challenges in extreme environments and for extrapolation under climate change.

In an experiment mimicking diurnal field temperature fluctuations, Sopp and Sunderland (1989) found that fluctuating temperatures had the same effect on the decay rate as the constant average temperature in the three predator species studied. Lövei et al. (1988) found that the presence of alternative food in the gut did not significantly affect the decay rate. Based on these results, Sopp and Sunderland (1989) suggested that simple laboratory experiments may be adequate to provide ecologically realistic data on decay rates. Clearly, however, more experimentation is needed before this suggestion can be accepted with confidence.

In summary, our results provide a pathway to estimate predation rates in field populations, rather than indices of predation (e.g., Ragsdale et al., 1981; Weber & Lundgren, 2009) or the previously used approximations discussed above. These predation rates can be incorporated into models enabling ecologists and biological control practitioners to project the potential direct effects of the predator on its prey, thereby deepening understanding of predator communities and food webs and improving methods for selecting or prioritizing biological control agents. In addition, our clarification of the theoretical basis for the estimation of predation rates should stimulate additional theoretical work generalizing the results we have derived.

AUTHOR CONTRIBUTIONS

David A. Andow and Débora Pires Paula designed and performed the research and jointly finalized the manuscript. David A. Andow analysed the data and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data used in this manuscript have been previously made publicly available associated with Paula, D. P., Timbó, R. V., Togawa, R. C., Vogler, A. P., & Andow, D. A. (2023). Quantitative prey species detection in predator guts across multiple trophic levels by mapping unassembled shotgun reads. *Molecular Ecology Resources*, 23(1), 64–80, doi.org/10.1111/1755-0998.13690.

BENEFIT-SHARING STATEMENT

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.