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NOTE

Use of wet, air-dried, or oven-dried bulk mass to quantify insect numbers: an assessment using *Chilothorax distinctus* (Müller) (Coleoptera: Scarabaeidae)

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Abstract

We examined the use of bulk mass to predict the number of individuals in samples of the dung beetle *Chilothorax distinctus* (Müller) (Coleoptera: Scarabaeidae: Aphodiinae: Aphodiini). We first developed linear regression equations to characterise the relationship between the number of beetles in a sample and sample wet mass, air-dried mass, or oven-dried mass. We then applied these equations to samples containing unknown numbers of beetles to obtain a predicted number. The predicted number was subsequently compared to the number obtained by counting each beetle by hand. Wet mass was as suitable as air-dried or oven-dried mass to estimate beetle numbers and was quicker to obtain. The predicted number of beetles in individual samples based on wet mass deviated from the actual number by 0.6–19.9%. For results combined across samples, the discrepancy was 2.2%. We conclude that quantifying *C. distinctus* by bulk wet mass rather than by hand count provides a reasonable alternative that accelerates the pace of sample processing while providing substantial cost savings. These results add to the small body of literature assessing the accuracy of bulk insect mass as a predictor for the actual number of individuals in large samples of conspecifics.

Introduction

Dung-baited pitfall traps are commonly used in surveys to characterise the diversity and seasonal activity of dung beetles (Coleoptera: Scarabaeidae) (Howden and Nealis 1975; Floate and Gill 1998; Brousseau *et al.* 2010; Viegas *et al.* 2014; Rentz and Price 2016). Insect collections from these traps are often dominated by large numbers of conspecifics, most of which are recovered during restricted periods. In southern Alberta, Canada, surveys recovered 157 000 (39% *Onthophagus nuchicornis* (Linnaeus); Coleoptera: Scarabaeidae, 35% *Melinopterus prodromus*, (Brahm); Coleoptera: Scarabaeidae) (Floate and Gill 1998), 126 000 (54% *O. nuchicornis*, 29% *Chilothorax distinctus* (Müller); Coleoptera: Scarabaeidae) (Kadiri *et al.* 2014), and 107 000 (90% *C. distinctus*) (Bezanson 2019) beetles, respectively. Samples collected from May through mid-June and from August through mid-September were dominated by *O. nuchicornis* (Floate 1998; Kadiri *et al.* 2014). Samples collected in April and October were dominated by *Melinopterus prodromus* (Floate 1998). Although present from March to May, almost all *C. distinctus* were recovered from late September onwards (Floate 1998; Kadiri *et al.* 2014;

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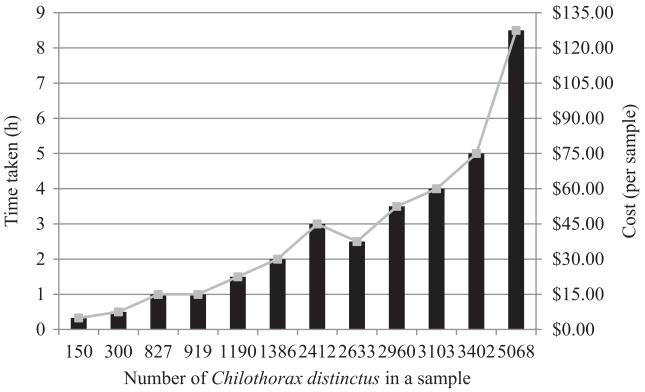

Fig. 1. Estimated cost of processing samples containing different numbers of the dung beetle *Chilothorax distinctus*. Estimates are based on the amount of time required for students to process that sample and the Alberta minimum wage in 2018 of CAD $15/hour.

Bezanson 2019). Bertone *et al.* (2005) recovered 86 000 beetles during a survey in North Carolina, United States of America, of which 64% were *Onthophagus taurus* (Schreber) that mainly were captured in May or from July to mid-September. Fiene *et al.* (2011) recovered an estimated 230 000 beetles during a survey in Arkansas, United States of America; (98% were *Labarrus pseudolividus* Balthasar; Coleoptera: Scarabaeidae) that were recovered mainly during one-month periods starting in mid-June or in late July.

Processing the samples recovered in pitfall traps is often the most time-consuming and costly aspect of such studies. The sample is normally first rinsed in water to remove dirt and residues of the solution used in the trap to kill and (or) preserve captured insects. Components of this solution commonly contain water, ethanol, ethylene glycol, polypropylene glycol, and (or) formalin (Brown and Matthews 2016). A cursory examination follows during which bits of vegetation and taxa clearly not of interest are discarded (*e.g.*, Orthoptera, Hymenoptera, Lepidoptera, Opiliones, Oligochaeta). A more detailed examination of the sample is done using a dissecting microscope to further discard taxa not of interest and then to sort and count the number of individuals for each dung beetle species. To illustrate the cost of this process, consider the following. Bezanson (2019) recovered 94 samples of insects from dung-baited pitfall traps emptied and rebaited weekly in southern Alberta from 23 August to 23 November 2017. The samples contained 80 871 dung beetles of which 99% were *C. distinctus* (one sample of 5068, 12 samples of 2000–3557, 19 samples of 1000–1915, 39 samples of 100–970). Per-sample processing costs (CAD) in 2018 were estimated to range from about $3 (approximately 150 beetles) to $127 (approximately 5000 beetles) (Fig. 1). More beetles would have been recovered were it not for two separate snowfall events during the trapping period.

To reduce the time and cost of processing dung beetle samples, we examined bulk sample mass as a surrogate to individually counting beetles. For this purpose, we used samples of *C. distinctus* recovered during a pitfall trapping study at the Purple Springs Grazing Reserve, Alberta, Canada (49.827°N, 111.895°W) in 2017 (Bezanson 2019) and stored in 70% ethanol. The pitfall trapping study was undertaken to compare dung beetle assemblages at different locations in southern Alberta. More specifically, we used subsets of these samples to develop linear regression equations to determine whether wet, air-dried, or oven-dried mass best predicted the number of *C. distinctus* in a sample. Measurements of wet mass were quickest to obtain, but estimates were expected to be confounded by variation in moisture across samples of different sizes, for example, samples of 100 versus 3000 beetles. Air-drying samples were expected to reduce this confounding factor, but potentially might require several days for the mass to stabilise. Estimates using oven-dried samples were expected to be both faster to obtain and more accurate than use of air-dried samples, but require access to a drying oven that might not be available to the researcher.

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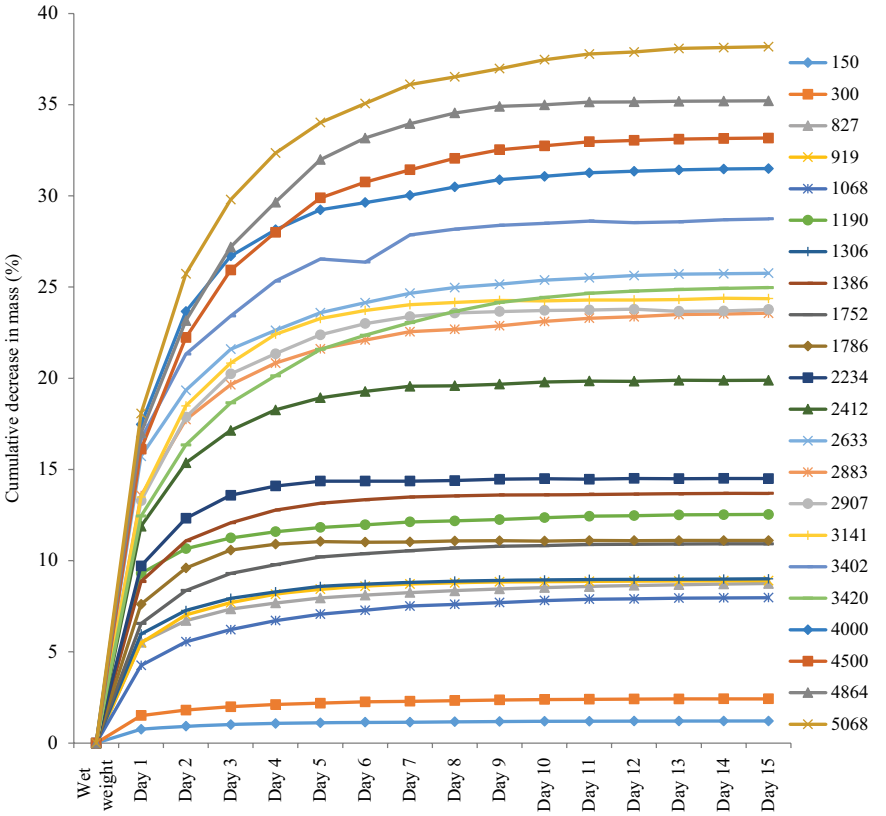

Fig. 2. Daily change in cumulative mass for different sized samples of *Chilothorax distinctus* held at room temperature (approximately 21 °C).

Each sample of beetles was first examined under a dissecting microscope to remove debris and taxa other than *C. distinctus*; the number of *C. distinctus* was then recorded using a hand counter. For measurements of wet mass, beetles in a sample ($n = 40$ samples) were removed from ethanol, placed on paper towel for one to three minutes to absorb excess ethanol draining from the collective mass of beetles, and then weighed. For measurements of air-dried mass, samples ($n = 22$ samples) were left in a shallow plastic dish at room temperature (approximately 21 °C) and weighed daily until no further decrease in mass was detected. Depending upon the number of beetles, this process required up to two weeks (Fig. 2). For measurements of oven-dried mass, samples ($n = 9$ samples) were placed in a drying oven (approximately 57 °C) and weighed daily until no further decrease in mass was detected. For oven-dried samples, 24 hours was sufficient for complete dehydration (Fig. 3). Mass was measured to the nearest 0.1 mg using an A&D ER-182A electronic balance (Carmet Scientific, Santa Rosa, California, United States of America).

Sample mass was then plotted against the number of *C. distinctus* in the sample to develop linear regression equations for which the y-intercept was set to 0, that is, samples without beetles should have no mass. Based on observed changes in daily mass over time (Figs. 2–3), we used mass measurements for samples that were air-dried and oven-dried for six and three days, respectively. The resultant equations to determine beetle number (y) from mass (x) were as follows: wet mass, $y = 99.25x$, $R^2 = 0.9812$; air-dried mass, $y = 361.54x$, $R^2 = 0.9565$; and oven-dried mass, $y = 480.03x$, $R^2 = 0.9708$ (Fig. 4).

Visual assessments of the plotted data identified some samples that appeared to be outliers (Fig. 4). Thus, beetles in 31 of the 40 samples used to develop the wet mass equation were

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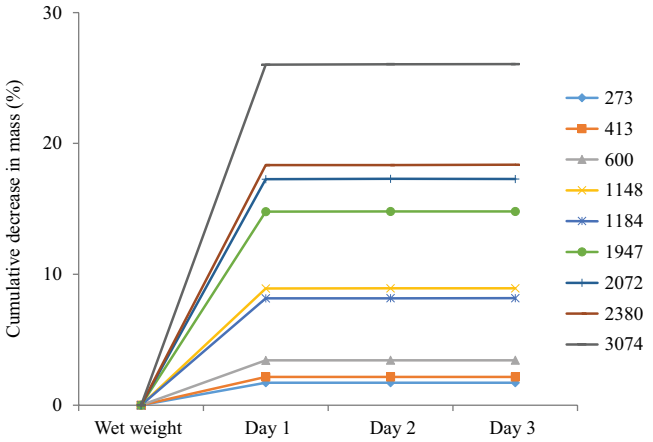

Fig. 3. Daily change in cumulative mass for different sized samples of *Chilothorax distinctus* held in an oven (approximately 57 °C).

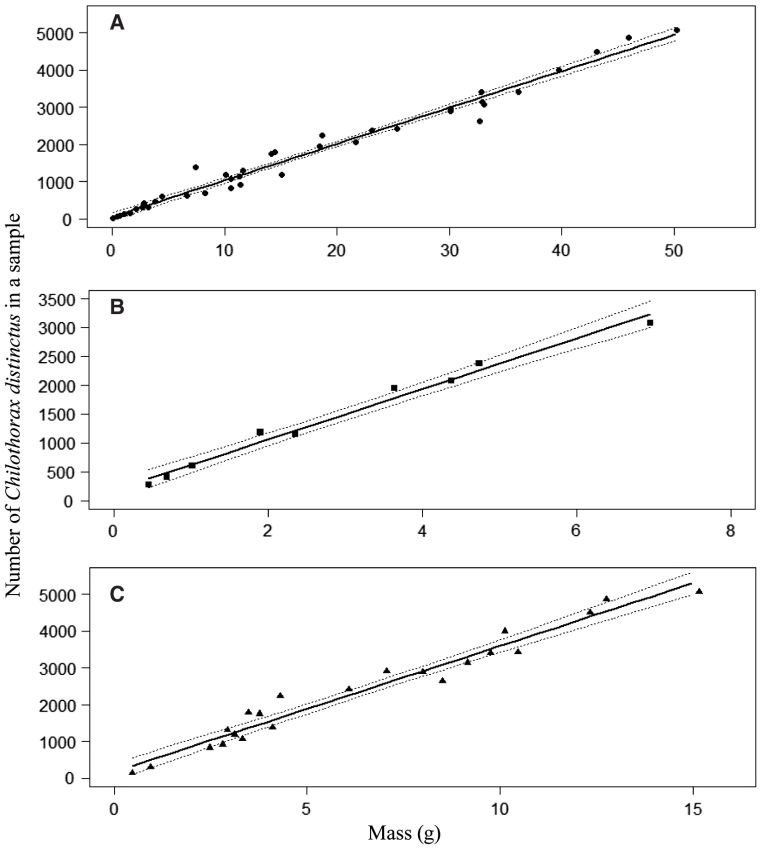

Fig. 4. Scatter plots showing the linear regression relationship between the number of *Chilothorax distinctus* in a sample and sample: **A**, wet mass ($y = 99.25x$, $R^2 = 0.9812$); **B**, air-dried mass ($y = 361.54x$, $R^2 = 0.9565$); **C**, oven-dried mass ($y = 480.03x$, $R^2 = 0.9708$). The thick black line represents the linear regression with the dotted black lines denoting the 95% confidence intervals.

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Table 1. Percentage discrepancy between the actual number of *Chilothorax distinctus* beetles in a sample (actual) versus the predicted number based on sample wet mass, air-dried mass, or oven-dried mass. Bold font denotes results for combined samples.

Actual number	Wet mass (g)	Predicted number	Discrepancy (%)	Dry mass (g)	Predicted number	Discrepancy (%)
Wet samples				Air-dried samples (six days)		
200	2.0631	205	2.5	0.4675	169	−15.5
589	6.2515	620	5.3	1.3328	482	−18.2
1138	12.451	1236	8.6	2.9755	1076	−5.4
1500	14.3685	1426	−4.9	3.2806	1186	−20.9
3427		**3487**	**1.8**		**2913**	**−15.0**
				Oven-dried samples (three days)		
216	1.748	173	19.9	0.3075	148	−31.5
500	4.9277	489	−2.2	1.0587	508	1.6
1600	15.2973	1518	−5.1	3.3453	1606	0.4
2292	21.6235	2146	−6.4	4.714	2263	−1.3
2781	27.8345	2763	−0.6	6.0185	2889	3.9
7389		**7089**	**−4.0**		**7413**	**0.3**
10 816		**10 576**	**2.2**			

Predicted numbers are estimated using linear regression equations reported in Figure 4. A negative value identifies an underestimate of the actual number.

recounted again by hand. Ten of these samples had counts that differed from the original counts with discrepancies of from four to 280 individuals; five cases each in which the recounts had more and less beetles than originally counted. These discrepancies highlight that hand counts of beetles, especially for samples with large numbers of individuals, are still prone to measurement error.

We then tested the utility of the equations using them to predict numbers of *C. distinctus* in samples based on their wet, air-dried, or oven-dried mass. We obtained wet mass measurements for a set of four samples that were then air-dried, and for a second set of five samples that were then oven-dried. The number of beetles in each sample was then counted by hand. Discrepancies between the predicted versus the actual number of beetles in individual samples ranged from 0.4 to −31.5% (Table 1).

The large discrepancies observed for some samples suggest that estimates obtained using bulk mass are too inaccurate to be of value. However, some of these discrepancies may reflect errors in the count of the actual number of beetles (see previous paragraph) rather than a failure of the bulk mass method as a predictive tool. Unfortunately, we did not think to retain the samples for recounting and could not directly test this hypothesis. Thus, we tested this hypothesis indirectly. For example, the calculated average mass of individual beetles (*i.e.*, bulk mass/actual number) ranged from 2.1–2.2 mg for four of the five oven-dried samples (Table 1). The calculated average mass for individual beetles in the fifth sample (216 beetles) was 1.4 mg. Assuming an average mass of 2.1 mg as was the case in the four other samples, the actual number of beetles in this sample should have been 146 beetles, rather than 216 as was recorded. For this reason, we attributed the unusually high discrepancy of −31.5% to a counting error. If this result is removed from consideration, the percentage discrepancy for oven-dried samples ranges from −1.3 to 3.9%. A counting error also may have contributed to the discrepancy of −20.9 for the air-dried sample of 1500 beetles. The calculated average mass for individual beetles in this sample was 2.2 versus 2.4 mg (range of 2.3–2.6) for the three other air-dried samples. Assuming an average mass of

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2.4 mg, the actual number of beetles in this sample may have been closer to 1361, reducing the discrepancy to −12.9%.

Regardless, assessing the utility of bulk mass to estimate the number of beetles in individual samples may be moot. If the objective requires statistical analyses, it may be desirable not to combine samples from individual pitfall traps for a given date, for example, comparisons of dung type or habitat (Fincher *et al.* 1970; Howden and Nealis 1975; Holter *et al.* 1993; Spector and Ayzama 2003; Tiberg and Floate 2011). However, if the objective does not require statistical analyses, such samples probably can be combined without loss of critical information, for example, reports of seasonal activity (Floate and Gill 1998; Bertone *et al.* 2005; Fiene *et al.* 2011; Rounds and Floate 2012). Thus, even though use of bulk mass may overestimate or underestimate the true number of beetles in individual samples, these errors will tend to cancel each other out when individual samples are combined. For example, the discrepancy between the predicted versus actual number of beetles in individual wet mass samples ($n = 9$) ranged from −0.6 to 19.9%, but was 2.2% when these samples are combined (Table 1). When samples for air-dried mass ($n = 4$) are combined, the percentage discrepancy was −15.0% rather than a range of −5.4 to −20.9%. Similarly, the percentage discrepancy was 0.3% for the combined samples for oven-dried mass ($n = 5$), versus a range of −31.5 to 3.9% for the individual samples.

Although the linear regression equations used here were developed for *C. distinctus* (length: 4.0–5.7 mm, width: 1.2–2.8 mm), they may have broader application. The subfamily Aphodiinae is comprised of nearly 2000 species worldwide, many of which are similar in size to *C. distinctus* (Gordon and Skelley 2007). These include the common species *Calamosternus granarius* (Linnaeus) (length: 3.4–6.0 mm, width: 1.5–2.8 mm), *Otophorus haemorrhoidalis* (Linnaeus) (length: 4.1–5.4 mm, width: 2.1–2.6 mm), and *Labarrus pseudolividus* (Balthasar) (length: 3.5–5.8 mm, width: 1.6–2.5 mm) (Gordon and Skelley 2007). Pending validation, the equations developed for *C. distinctus* may be equally applicable for these species. For beetles of other sizes, different sets of equations would of course be required.

We are aware of only two studies that have assessed the use of mass as a surrogate to individually counting insects by hand. Stark and Vargas (1990) compared three methods (grid, volume, oven-dry mass) to quantify numbers of *Dacus cucurbitae* Coquillett, and *D. dorsalis* Hendel (Diptera: Tephritidae) recovered in pheromone traps. They concluded that oven-dry mass was both quicker and more accurate than the other two methods. For honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), Atkins (1986) quantified numbers recovered in traps either by hand count, volume, or weight, but found no difference in the accuracy of counts across methods. To process the approximately 230 000 *L. pseudolividus* recovered in their study, Fiene *et al.* (2011) adopted a two-pronged approach. Beetles in samples of 6000 individuals or less were hand-counted, whereas the number of individuals in larger samples was estimated on the basis of bulk oven-dried mass.

The current study adds to this small body of literature and provides further proof of concept to the general approach – whether it be applied to dung beetles or other taxa. We found that wet mass was generally as suitable as either air-dried or oven-dried mass to estimate beetle numbers and was much quicker to obtain. We further found that estimates based on wet mass were reasonably accurate, that is, a 2.2% discrepancy between actual and predicted numbers in the current study when samples were combined (Table 1). We also note that counting errors are more likely to occur due to tiredness and boredom, when processing large samples containing several thousand beetles. On this basis, we conclude that quantifying *C. distinctus* by bulk wet mass rather than by hand count provides a reasonable alternative that accelerates the pace of sample processing with substantial cost savings (Fig. 1).

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