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Active Search on Carcasses versus Pitfall Traps: a Comparison of Sampling Methods

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Abstract

The study of insect succession in cadavers and the classification of arthropods have mostly been done by placing a carcass in a cage, protected from vertebrate scavengers, which is then visited periodically. An alternative is to use specific traps. Few studies on carrion ecology and forensic entomology involving the carcasses of large vertebrates have employed pitfall traps. The aims of this study were to compare both sampling methods (active search on a carcass and pitfall trapping) for each coleopteran family, and to establish whether there is a discrepancy (underestimation and/or overestimation) in the presence of each family by either method. A great discrepancy was found for almost all families with some of them being more abundant in samples obtained through active search on carcasses and others in samples from traps, whereas two families did not show any bias towards a given sampling method. The fact that families may be underestimated or overestimated by the type of sampling technique highlights the importance of combining both methods, active search on carcasses and pitfall traps, in order to obtain more complete information on decomposition, carrion habitat and cadaveric families or species. Furthermore, a hypothesis advanced on the reasons for the underestimation by either sampling method showing biases towards certain families. Information about the sampling techniques indicating which would be more appropriate to detect or find a particular family is provided.

Introduction

The study of insect succession in carcasses and the classification of arthropods have mostly been done by placing a dead animal, which is protected within a cage from the action of vertebrate scavengers, and then visited periodically. This method is advantageous as it allows the detection of the major areas of colonization of the carcass (as evidenced by the presence of eggs, maggots, or puparia) and where the location of any insect activity on the ground or substrate near the body is the assessment of the distance from the body to remote insect activity sites, and provide data on insect behavior among other data (Byrd & Castner 2001). In general, observations and recording can provide valuable information to the overall death scene investigation and substantiating data for entomological evidence evaluation (Byrd & Castner 2001). Other advantages are that fast flying and fast crawling adult insects can be collected, including those that rest on nearby vegetation. Yet, living insects can be collected and reared to the adult stage and/or for the establishment of insect colonies in forensic labs to facilitate larval identifications or perform different types of studies (Byrd & Castner 2001). But, there are some disadvantages to this method as well. Only the fauna present at the moment of sampling is collected, and, thus, many species or families may be ignored. The ability and experience of the collector is an important factor and the combination of both factors could lead to an incomplete list of taxa, making comparisons with other studies difficult (Ordóñez *et al* 2008).

Another method for sampling cadaveric fauna makes use of specific traps, which are also used to a number of studies, such as phenology (Topping & Sunderland 1992), activity patterns (Ericson 1978, Den Boer 1981, Topping & Sunderland 1992), associations with habitats (Honêk 1988, Hanski & Niemelä 1990) and spatial distribution ranges (Barber 1931 Niemelä 1990, Giblin-Davis *et al* 1994), relative abundance of species (Desender & Maelfait 1986, Mommertz *et al* 1996), establishment of species (Niemelä *et al* 1994), and the effects of disturbances over biodiversity (Niemelä *et al* 1992, Pekár 2002, Mazía *et al* 2006). Moreover, pitfall traps are useful to obtain data on community structure (Hammond 1990, Jarosík 1992) and on pest monitoring programs (Obeng-Ofori 1993, Simmons *et al* 1998). Pitfall trapping is a passive, economical, and efficient method, easy to handle (Spence & Niemelä 1994) and transport, and quick to install (Lemieux & Lindgren 1999). Sampling is continuous, so the bias of techniques used on discrete samplings is avoided (Topping & Sunderland 1992), and allows the collection of large numbers of specimens simultaneously from different areas and/or with distinct trophic roles, with minimum effort, which is advantageous for statistical analyses (Spence & Niemelä 1994). It is not mainly dependent on the observer (Pekár 2002), thus contributing to the objectivity of the method and the establishment of reliable comparisons (Vennila & Rajagopal 1999). On the other hand, the efficiency of trapping depends on the activity and the density of the species (Curtis 1980). Therefore, some authors consider this method limited for the quantitative estimation of the absolute abundance or density of a population, or the comparison between communities (Greenslade 1964, Ahearn 1971, Mazía *et al* 2006). The information can also be biased, because species of large size can be overestimated (Spence & Niemelä 1994, Arneberg & Andersen 2003), or sexes can be biased in certain taxonomic groups (Topping & Sunderland 1992). However, despite the possibility of bias, the majority of the species are represented in frequencies that reflect their true relative abundance (Woodcock 2005).

Despite the advantages of using traps or their variations, few studies on carrion ecology and forensic entomology involving large carcasses of vertebrates have employed pitfall traps (Centeno *et al* 2002, Archer & Elgar 2003, Zanetti *et al* 2014). However, these are necessary to understand the carrion community and thus have a wider variety of insects available for forensic investigations.

The aims of this study were to compare both methods (active search on carcasses and pitfall trapping) for each coleopteran family, and to establish whether there is a discrepancy (underestimation and/or overestimation) in the presence of each family by either method.

Material and Methods

Four experiments, one per season, beginning in winter 2010 and finishing in spring 2011, were carried out in a field owned by the Universidad Nacional del Sur, Bahía Blanca (38°41′41″S, 62°15′10″W), Buenos Aires province, Argentina. The selected area can be described as semirural. Additional data on the vegetation, climate and study area are described in Zanetti *et al* (2014).

Three cages measuring 120 × 80 × 60 cm were built with wood and wire mesh to exclude vertebrate scavengers. In each experiment, we used three domestic pigs weighting 15 to 16 kg, which were killed by a stab to the heart 1 h before exposure, and kept inside a plastic bag until the beginning of the experiment. This procedure was approved by the Ethical Commission of the Universidad Nacional del Sur. Six pitfall traps were placed around each cage 50 cm away from the carcasses, two per each long side and one per each wide side. Another set of six pitfall traps with the same spatial pattern were placed 15 to 30 m away from the last pig carcass to serve as control. The pitfall traps were made from plastic containers of 500 mL and 8.5 cm diameter, each buried to the rim of the soil. They had a solution of 90% distilled water and 10% coolant. Cages were placed under direct sun 100 m from one another along a transect.

We followed the criterion established by Centeno *et al* (2002) to define the stages of decomposition. Carcasses were visited daily until the end of the experiment (more information is in Zanetti *et al* (2014)).

The data obtained was grouped according to the decomposition stage and the family of beetles. The accumulated capture of beetles in six traps and observations of beetles on, under, and inside a carcass per stage of decomposition and season were considered as sampling units (a total of 48 observations = 3 carcasses × 4 stages of decomposition × 4 seasons).

The variables analyzed were **T(fam)** = number of beetles of each family per sampling unit of the "trap method" per day; **C(fam)** = number of beetles of each family per sampling unit of the "carcass method" per day. Because sampling units for both methods were different, we established a transformation to compare their abundances, which consisted in dividing the variable for the maximum value (of abundance) registered for the family involved. This was applied to all samples. Because the minimum value observed was always "zero", the quotient defined above was equal to the standard per range (R): max-min.

$$\textit{Thus}: \mathbf{RT}(\mathbf{fam}) = \mathbf{T}(\mathbf{fam})/\mathbf{max}\{(\mathbf{Tfam})\}$$
$$\mathbf{R\,C}(\mathbf{fam}) = \mathbf{C}(\mathbf{fam})/\mathbf{max}\{\mathbf{C}(\mathbf{fam})\}$$

If each season of the year (represented by the sub-index "**i**"), and each stage of decomposition (sub-index "**j**") is considered, the observations of the traps and of the carcasses will be a combination "**ij**":

$\mathbf{RT}(\mathbf{fam})_{\mathbf{ij}} \mathit{and} \mathbf{RC}(\mathbf{fam})_{\mathbf{ij}}$, *respectively*.

Finally, we defined a measure of the discrepancy between both methods for each family:

$$\Delta TC(fam) = \sum_i \sum_j \left[\frac{RT(fam)_{ij} - RC(fam)_{ij}}{RT(fam)_{ij} + RC(fam)_{ij}} \right]$$

Therefore, if for a family in one observation "**ij**" traps detected the presence of individuals, but the observation of the carcass did not, the corresponding term adds a "**+1**" to the measure:

$$\left[\frac{RT(fam)_{ij} - 0}{RT(fam)_{ij} + 0} \right] = 1$$

The sum of all such terms, **A**(**fam**), represents the frequency with which "presences in traps and absences in carcass" was recorded. On the other hand, if for a family in an observation "**ij**" the traps did not detect the presence of individuals but the observation of the carcass did, the corresponding term adds a "**-1**" to the measure:

$$\left[\frac{0 - RC(fam)_{ij}}{0 + RC(fam)_{ij}} \right] = -1$$

The sum of all such terms, **B**(**fam**), represents the frequency with which "absences in traps and presences in carcass" was recorded. Finally, if for a family in an observation "**ij**" traps and carcasses detected the presence of individuals but with a difference in favor of the first group, the term adds a positive fraction. If this difference favors of the second group, the fraction is negative:

$$if \; RT(fam) > RC(fam) \Rightarrow \left[\frac{RT(fam)_{ij} - RC(fam)_{ij}}{RT(fam)_{ij} + RC(fam)_{ij}} \right] > 0$$

$$if \; RT(fam) < RC(fam) \Rightarrow \left[\frac{RT(fam)_{ij} - RC(fam)_{ij}}{RT(fam)_{ij} + RC(fam)_{ij}} \right] < 0$$

The sum of all the first terms of this kind was named **C**(**fam**), and the sum of the second terms **D**(**fam**). This allowed us to make a graphic representation of each term as follows (Fig 1): a diagram of boxes and arms for each family was built, with the center at "zero", and with the limits of the box at the height of **B**(**fam**) towards the negatives and **A**(**fam**) towards the positives. The diagram was completed with arms whose lengths were equal to the rest of the other negative terms: **D**(**fam**) towards the side, and the positives: **C**(**fam**), towards the other side. Thus, the total length of the diagram equals the known Canberra distance (Lance & Williams 1966). For a better interpretation, this measure can be set between "0" and "1" by dividing it per the number of observations without "double-zeros" (n^*) (Lance & Williams 1967). The discrepancy measure (ΔTC*) can also be set between −1 and 1 by dividing ΔTC by the Canberra distance as **ΔTC*** = **ΔTC**/**Canberra Distance**. Thus, a relative discrepancy (ΔTC*) was obtained, which can be interpreted as the bias of one of the methods (according to its sign) with respect to the total accumulated differences (Canberra distance between both).

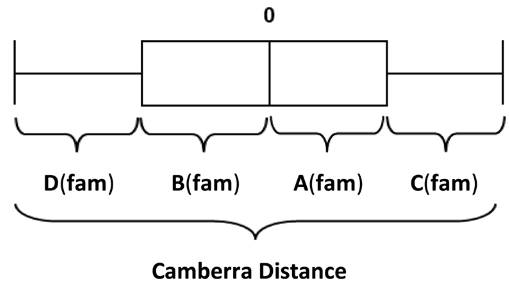

Fig 1 Graphic representation of the terms that form the Canberra distance. ***A(fam)*** frequency "presences in traps and absences in carcass," ***B(fam)*** frequency "absences in traps and presences in carcass," ***C(fam)*** presence of individuals detected by traps and carcasses but with a difference in favor of the traps, ***D(fam)*** similar to C(fam) but with a difference in favor of the carcasses.

Results

We observed for almost all the families recorded a great discrepancy between sampling methods (Table 1, Fig 2); even those with a low frequency of appearance, such as Trogidae and Nitidulidae, showed quite high relative values: 73 and 92%, respectively. The bias in favor of traps was considerable for Anthicidae, Tenebrionidae, and Carabidae (especially in spring, with 95% of relative bias). Dermestidae, Cleridae, Nitidulidae, and Trogidae could be positively related to the observation method in carcasses. We only observed a "slight" overestimation by traps for Scarabaeidae, whereas the other two families, Histeridae and Staphylinidae, did not exhibit a noticeable bias towards any sampling method.

Discussion

Pitfall traps are generally better for the study of invertebrates with distinct trophic roles and habitats, and active at ground level (Weeks & McIntyre 1997, Standen 2000, Prasifka *et al* 2007). Some authors consider them good tools for the study of walking and crawling arthropods, especially those that are active at night above the ground, but less efficient for the capture of flying arthropods (Mesibov *et al* 1995, Ward *et al* 2001, Hansen & New 2005). This could

Table 1 Bias (ΔTC) and discrepancy (Canberra D.) between the trap and cadaver methods for each family.

Families	ΔTC	Canberra D.	n^*	Canberra D.*	ΔTC*
Anthicidae	26.61	27.92	32	0.872	0.953
Carabidae	14.99	33.01	39	0.846	0.454
Cleridae	−16.41	27.48	36	0.763	−0.597
Dermestidae	−20.93	25.40	43	0.591	−0.824
Histeridae	0.37	23.22	46	0.505	0.016
Nitidulidae	−14.25	20.20	22	0.918	−0.705
Scarabaeidae	7.55	25.53	37	0.690	0.296
Staphylinidae	−4.05	23.61	48	0.492	−0.172
Tenebrionidae	17.73	27.57	41	0.673	0.643
Trogidae	−7.26	16.14	22	0.734	−0.450

n^* number of observations without double-zeros, *Canberra D.** distance of Canberra demarcated between 0 and 1 (relative discrepancy), *ΔTC** bias demarcated between −1 and 1 (relative bias).

explain, at least in part, our observations on Tebrionidae and Carabidae, which were mainly captured in pitfall traps. These families include apterous or brachypterous specimens. Moreover, several authors suggested pitfall traps as the best method to sample individuals of these families (Wallin 1986 Perfecto *et al* 1986, Riddick & Mills 1995). In the case of Scarabaeidae, although many species are adapted to flight, several are copronecrophages and sapronecrophages, also adapted to roll their resources along large distances from the main source, forming small balls with part of their food, and then burying them to serve as food for larvae (Halffter & Mathews 1966, Hanski & Cambefort 1991). This habit may have favored a "slight" bias of these beetles towards pitfall traps. Favila & Halffter (1997) suggested that the best method for assessing the abundance of scarab beetles is using pitfall traps baited with feces, fruit, or decomposing meat. Anthicidae are good fliers, but there is not much information about this family, so their overestimation in pitfall traps could be explained because they are opportunistic beetles that can feed on small insects, pollen, or small dead arthropods (Chandler 1994); as a consequence, the arthropods captured by traps would be easy prey for anthicids.

On the other hand, Dermestidae, Trogidae and, Nitidulidae could be more abundant in carcasses because they are good fliers, capable of detecting carrion over long distances (Colvin *et al* 2006). Once they had located the necessary substrate for feeding and reproducing, these beetles may have remained on or near the carcass, and so they did not fall into the traps. Dermestids and Trogids are considered members of the necrophagous fauna, capable of reproducing in carrion (Smith 1986, Schoenly *et al* 1991, Mayer & Vasconcelos 2013). The family Nitidulidae comprises omnivorous beetles, with adults that can feed on living prey and carrion (Smith 1986, Sánchez Piñero 1997). Cleridae, which are good fliers as well, were found mainly on carcasses, and were represented by *Necrobia rufipes* (De Geer). This species is also omnivorous, as it preys on dipteran and beetle larvae at the same time it feeds on carrion (Reed 1958, Ashman 1963, Gredilha *et al* 2005). According to Gredilha & Lima (2007), the life cycle of this species is dependent on the life cycle of their prey, so their occurrence on carrion is correlated with the abundance of their common prey (Kočárek 2003).

Rove beetles and clown beetles were sampled equally by both methods. Several species of these families are necrophiles preying on adults, pupae, and larvae of Diptera and other insects (Smith 1986, Goff & Catts 1990, Tantawi *et al* 1996, Sánchez Piñero 1997, Byrd & Castner 2001), or parasitize pupae of Diptera (Mise *et al* 2010). Moreover, they lay eggs on or near carcasses). Therefore, having a feeding source and a place to reproduce could favor their observation on carcasses. On the other hand, dipteran larvae are capable of moving a few meters away from carrion when

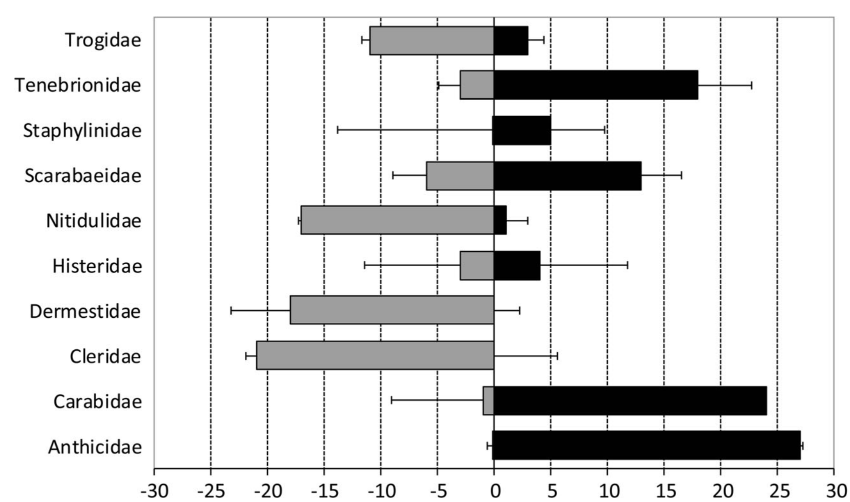

Fig 2 Bias (asymmetry of the arm box) and discrepancy (total length of the figure) in the abundance of each family of cadaveric beetles collected by each method. Underestimation of the traps (overestimation of the carcasses): negative area. Underestimation of carcasses (overestimation of traps): positive area.

their food source is consumed or in order to pupate and to avoid cannibalism, parasitism, or drying, which can interfere with their survival or the completion of their life cycle (Levot *et al* 1979, Von Zuben *et al* 2001, Gómez 2005). In the present study, many larvae of clown and rove beetles fell into pitfall traps with other insects. These beetles may have been hunting their prey or have been attracted to insects already captured in traps, and, thus, they may have shared the same fate as their prey.

According to the concept of activity-abundance, the capture rate of invertebrate species is proportional to the interaction between abundance and activity (Tretzel 1954, Heydemann 1957, Thiele 1977). Therefore, species with low mobility, but highly abundant, may be underestimated by traps in comparison with those that are less abundant, but more active (Woodcock 2005). This may explain our results, considering that species mainly observed on carcasses do not need to move away as the carcass provides all the resources they need, while those which are more active were captured mainly in traps.

Furthermore, the behavior of each species can influence the rate of capture in traps (for example: probing, skirting, and spontaneous retreat) (Den Boer 1981, Halsall & Wratten 1988, Topping 1993). Woodcock (2005) suggested that each sampling technique is biased by the behavior of each species. This would influence the frequency with which species come into contact with a trap. Therefore, it is important to have information on the behavior of different families and species exploiting carrion as a resource, which can be infrequent in literature (Halsall & Wratten 1988). As a conclusion, selecting one or another sampling technique will depend on the family to be studied or the type of study to be performed. In the case of forensic entomology, the application of both techniques covers well the chance to assess the true diversity of beetles involved.

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Compliance with Ethical Standards

Ethics Approval This procedure was approved by the Ethical Commission of the Universidad Nacional del Sur.

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