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Comparative efficiency of different collection methods of carabid beetles in Eastern Visayas, Philippines

Myra A Abayon*

College of Arts and Sciences, Leyte Normal University, Tacloban City, Philippines

Key words: Pitfall, Handpicking, Vinegar, Mixture of ketchup and vinegar, Fermented fish with vinegar, Ground meat, Carabid beetle

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Abstract

Two different methods (pitfall traps and hand picking) were used to sample carabid beetles in six forests of Eastern Visayas, namely: Lake Danao, Mt. Nacolod, Kuapnit Balinsasayao, Asug Forest, City Forest, and Closed Canopy on January to June of 2019. The total number of individuals caught using the two methods was 7844 carabids belonging to 25 genera and 49 species. Among these, 3,326 individuals, representing 41 species, were caught in pitfall traps, while the remaining 4,518 individuals, representing 49 species, were caught using handpicking or ground searching. Using Kruskal-Wallis test, indicated a no significant difference in the abundance and species richness per method of collection, i.e. handpicking vs. pitfall trapping (p-value = 0.2178). The study used four different baits in the pitfall trapping, namely: vinegar, mixture of ketchup and vinegar, fermented fish with vinegar, and ground meat. The use of fermented fish with vinegar has never been used before by any author and is a new bait method discovered by the researcher during the study. A total of 3,326 carabid individuals belonging to 40 species were collected. Fermented fish with vinegar garnered the highest number of indivuals at 1,894 (56.94%) of the total catch. Likewise, fermented fish with vinegar showed the highest diversity (5.168), high equitability, highest dominance (2.984) and richness (40) compared to other baits used in the pitfall trapping. The use of Kruskal-Wallis test indicates significant difference in the abundance and species richness per bait method in pitfall trapping.

* **Corresponding Author:** Myra A Abayon ✉ myra.abayon@lnu.edu.ph

Introduction

The traditional technique of collecting ground-dwelling invertebrates like carabid beetles is by the use of pitfall traps (Southwood and Henderson, 2000). It has been commonly used in the sampling of carabid beetles in inventories of biodiversity (Niemela *et al.*, 1994; Davies, 2000; Nyundo, 2002), population and community ecology (Greenslade, 1968; Refseth, 1980; Niemela, 1988; Niemela *et al.*, 1989), as well as in research on sampling techniques (Clark *et al.*, 1995; Bremen and Terlutter, 1994). The reason for the wide use of pitfall traps in invertebrate sampling is their simplicity of setting and using, and their low cost in terms of manpower.

Despite their usefulness, there are many issues with interpreting pitfall trap information as they rarely represent the real abundance of the target organisms being sampled. This is because there are various factors that affect the effectiveness of the traps, including the materials from which the trap is produced, the size and shape of the trap, the layout, the bait or preservative used in the target organism's trap and characteristics such as size and foraging behavior (Adis 1979). Pitfall traps have continued to be used despite these deficiencies. The crisis in biodiversity in tropical habitats makes it a swift cheap collection technique required for inventorying species.

Studies investigating carabid diversity rely mostly on pitfall trapping as the standard sampling method (Rainio & Niemela¨ 2003). These traps are easy to operate (Greenslade & Greenslade, 1971) and are regarded as a highly effective and cheap means to survey arthropods dwelling on the soil surface. They are, hence, seen as a powerful tool to gain standardized quantitative samples of ground arthropod assemblages, in general, and carabid beetles, in particular (Thiele, 1977; Southwood, 1978; Luff, 1996). In pitfall traps, the effective capture rates depend both on activity patterns and population densities of the species captured (Mitchell, 1963), so that pitfall trapping results do not necessarily reflect the prevailing density of species in a habitat. Furthermore, size, shape and material of pitfalls, and the liquid used in the collecting jars, as well as the type of cover, also affect the sampling results to some extend (Briggs, 1961; Luff, 1975; Baars, 1979; Peka´r, 2002). These initial constraints, combined with habitat-related differences in the activity patterns of carabid species, render it somewhat difficult to use and compare pitfall trapping results of different studies investigating carabid species composition (Niemela¨ *et al.*, 1990; Spence & Niemela¨, 1994) and to evaluate their overall indicative value (Duelli, 1997; Duelli & Obrist, 1998).

Another relatively cheap and robust sampling method used to record a wide range of primarily nocturnal arthropods like carabid beetles is handpicking or ground searching. In the past, few studies reported on the use of handpicking sampling to assess the diversity of ground beetles, although these beetles do arrive at such devices in significant numbers, as our results clearly indicate. Methods such as sifting, hand picking, light trapping or net sweeping can also be used to study carabid beetle assemblages (Freude *et al.*, 1965; White, 1983). Some of these techniques seem appropriate if ground beedes of the given habitats do not show locomotor activity on the surface. For this reason, this study compares the efficiency of pitfall trapping and hand picking. Most previous studies on the efficiency of different metthods were conducted in temperate climate regions of Europe and North America (Andersen, 1995; Prasifka *et al.*, 2007). For the tropical region like Eastern Visayas, to date, there have been no studies which deal with the efficiency of pitfall traps and other methods of collecting ground beetle assemblages. The six selected forests with different habitats were used to determine the efficiency of these methods. Knowledge on the detectability of ground beetle species in different habitats and the most efficient methods for collecting are important for studying the significance of this animal group in landscape planning and biological conservation research approaches. Carabid beetles are used increasingly for nature conservation strategies. Prerequisites for nature conservation strategies are methods for obtaining comprehensive knowledge on the existing fauna. This is crucial for identifying changes and threats.

In many general entomological texts, bait trapping for insects is addressed, ranging from techniques such as sugaring and pheromone trapping to the use of natural organic baits, such as carrion and dung (Oldroy, 1958; Ford, 1973; Southwood, 1978; Harde, 1981). The use of rotting fruit or vegetable baits to study natural coleopteran assemblages appears relatively scarce in tropical forests, although field surveys of beetle assemblages at fallen fruit and comparisons of different fruit baits to survey particular beetle families have been carried out elsewhere (Williams *et al.*, 1994; Paarmann *et al.*, 2002). Investigations into host usage and habitat preferences of fungivorous beetles appear more prevalent (Thunes and Willassen, 1997; Komenen, 2003). But no studies have been conducted yet, using fermented fish with vinegar. The positive result showing high abundance of carabid beetles attracted to this kind of bait is new discovery and contribution of this study. By adopting 'unorthodox' collecting techniques such as bait trapping with fermented fish and vinegar, is possible to find new species not previously recorded by conventional insect survey techniques.

The aim of this study was to compare catches of carabid beetles derived from both pitfall trapping and handpicking, with regard to diversity and species composition, in order to evaluate how effective both techniques are in the recording of species ensembles of carabids in the agricultural landscape. The central hypothesis is that pitfall traps, as the established method of carabid recording, will be superior with regards to both the number of species caught and the diversity of species over handpicking. Specifically, the study aimed to compare the effectiveness of trapping methods namely: hand picking (ground searching) and pitfall trapping from the different habitat types; and to compare the efficiency of different baits used in pitfall trapping in terms of species composition and richness.

Materials and methods

Site selection

The study was conducted in selected forests of Eastern Visayas: (1) Lake Danao National Park of Ormoc City, Leyte; (2) Kuapnit Balinsasayao National Forest of Abuyog and Baybay City, Leyte; (3) City Forest and Marble Park in Calbayog City, Samar; (4) Asug Forest Reserve, Biliran; (5) Mount Nacolod Forest in Silago, Southern Leyte; and (6) Borongan-Llorente Closed Canopy Forest in Borongan, Eastern Samar. These forests were chosen based on (1) slope position (incline extending from ≥8-18% can be utilized for regular and lasting yield generation), (2) cultivated area is located near the forest, (3) portions of the forest have been formed by slash-and-burn practices, and (4) contains areas under current cultivation. These forests are either declared as protected areas or proposed protected areas by the Department of Environment and Natural Resource (DENR) of Eastern Visayas.

Survey methods

Carabid beetles were collected using pitfall trapping and manual collection and/or searching the ground. The pitfall traps were 500-ml plastic containers (11.4cm long x 11.4cm wide x 8cm high) which were half-filled with bait substance and were buried in the ground so that the top of the trap was at the soil surface. One hundred traps were placed in every habitat type at each forest with a total of 200 pitfalls traps placed in every study site. The traps were arranged in square grids with 20 m between adjacent traps to avoid trap interactions (e.g., the "digging in" effect [Hoekman *et al.*, 2017]). After a 2-wk comparison of baits, the researcher, decided to use vinegar, vinegar with catsup, fermented fish with vinegar, and ground meat as attractants in the traps. Pitfall traps captured carabid beetles using these bait materials, while previously used baits did not effectively capture any carabid beetles. A 13 x 13cm piece of metal was secured over each trap as a shield from rain, litter, and disturbance by animals. Traps were emptied and refilled twice weekly at which time carabids were collected and returned to the laboratory for sorting and identification.

Meanwhile, hand picking/searching on the ground (GS) was conducted by actively searching for the beetles on the ground, in leaf litter, under logs and other substrates, under tree bark, and in rotting deadwood. Sixty man-hours were spent in active searching for each visit at each site, occurring primarily between 2000 and 2300 h, as most carabid beetles are nocturnal. Collections were made 4 times a month over 6 months (i.e., January-June 2019) for each site. A 0.5-cm mesh screen was used to sift dry leaf litter for carabid beetles. Moist leaf litter was scooped onto white clothes and a pair of forceps was used to collect beetles. Likewise, resting and running beetles were sampled by manual searching under logs, stones and tree bark. Collecting took place both during the day and at night. All specimens were transferred into a killing agent preservative (9.0:0.5:0.5 parts of 70% ethyl alcohol, table vinegar, ethyl acetate (3:1) as per Hoekman *et al.* (2017).

Identification

Carabids were identified to the species level when possible using the works of Thiele (1977), Lindroth (1949), Scholtz (2005), Luff (1987), Kirschenhofer (2008), and Trautner *et al.* (1987). All identifications were confirmed by Dr. Bernard Lassalle (French Entomological Society, France) and Dr. Rainer Schnell (University of Duisburg Essen, Germany). Representative specimens are stored in the Biology Laboratory, Leyte Normal University, Philippines.

Data Analysis

Different indices measure different aspects of the partition of abundance between species. Species evenness usually has been defined as the ratio of observed diversity to maximum diversity, the latter being said to occur when the species in a collection are equally abundant (Margalef, 1958; Patten, 1962; Pielou, 1966). Simpson's index, likewise, is another measurement which is sensitive to the abundance only of the more plentiful species in a sample, and can therefore be regarded as a measure of "dominance concentration" (Whittaker, 1965). Diversity measurements were determined using Shannon index, an information statistic index which assumes that all species are represented in a sample following random sampling. This index estimates the affinity of different populations belonging to a community and, through the species composition, the similarity of the habitats (Popescu and Zamfirescu, 2004). Besides Pielou's evenness index, Simpson's dominance index, and Shannon index of diversity, other measurments of diversity of carabid beetles for each forest were also consulted, namely: Simpson's diversity, Menhinick index and Margalef's index (Simpson 1949). And before the data were statistically analyzed, the data were first checked for normality, i.e. to guide on the appropriate tests to be employed. Since data obtained is not normally distributed, i.e. data on richness by method per habitat type, thus nonparametric Mann-Whitney U test which is equivalent to the independent two-sample t-test was used. This is an appropriate analysis to compare differences that come from same population when the dependent variable is ordinal (Leech, Barrett & Morgan, 2005). Furthermore, Kruskal-Wallis test was used to determine if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. PAST (Version 3.10) RStudio and softwares were used for calculation and plotting (Hammer *et al.* 2001).

Results and discussion

Overall, 7844 carabid individuals belonging to 25 genera and 41 species were caught in the different study sites. Among these, 3,326 individuals, representing 41 species, were caught in pitfall traps, while the remaining 4,518 individuals, representing 49 species, were caught using handpicking or ground searching. Four species were solely caught by handpicking, namely Cicindela sp., Pheropsophus nigerrimus, a new species of Pheropsophus sp., and Trigonotoma goeltenbothi but none for pitfall trap. There were 41 species recorded for both methods (Table 1). A clear tendency for higher numbers of individuals caught by handpicking was consistent for all habitat types and forest sites investigated. The dominance structure of the carabid assemblages was also very different in relation to the method used. In handpicking, the most common species were Pheropsophus hassenteufeli, Tricondyla aptera punctipennis, Tricondyla conicicollis, Tricondyla ovicollis, and Pheropsophus lumawigi, which accounted for 35% of the total catch. In pitfall trap, the dominant species were Pheropsophus hassenteufeli, Pheropsophus azouleyi, Tricondyla aptera punctipennis, Tricondyla ovicollis, and Tricondyla conicicollis accounting for 24% of the total catch.

Table 1. Total number of carabid beetles caught by handpicking and pitfall trapping.

Species	Method of Collecting		Total
	Hand-picking	Pit-fall	
1. Brachinus leytensis (new)	108	79	187
2.Trigonotoma goeltenbothi	1	0	1
3. Prothyma heteromallicollis	39	26	65
4. Pheropsophus uliweberi (new)	68	99	167
5. Pheropsophus sp. *(new)*	2	0	2
6. Pheropsophus hassenteufeli	765	486	1251
7. Pheropsophus nigerrimus	1	0	1
8. Pheropsophus azouleyi	206	419	625
9. Pheropsophus fumigatus	1	1	2
10. Pheropsophus lumawigi	300	196	496
11. Neocollyris filicornis	54	40	94
12. Therates fasciatus quadrimaculatus	92	61	153
13. Lesticus samarensis	69	89	158
14. Pseudozaena orientalis opaca	119	61	180
15. Orthogonius luzonicus	47	30	77
16. Chlaenius sp. 1	84	72	156
17. Chlaenius sp. 2	40	27	67
18. Chlaenius sp. 3	45	27	72
19. Gnathaphanus impressipennis	51	35	86
20. Tricondyla aptera punctipennis	781	368	1149
21. Tricondyla ovicollis	371	280	651
22. Tricondyla conicicollis	462	257	719
23. Haplochlaenius femoratus philippinus	93	102	195
24. Oodes sp.	20	15	35
25. Trichotichnis sp.	19	16	35
26. Lebia Poecilothais sp.	28	14	42
27. Pentagonica ruficollis	43	36	79
28. Catascopus elegans	20	17	37
29. Catascopus aequatus	15	4	19
30. Therates fasciatus pseudolatreillei	50	36	86
31. Pentagonica sp.	39	25	64
32. Drypta lineola philippinensis	89	82	171
33. Dicranoncus philippinensis	93	82	175
34. Dolichoctis gilvipes	22	46	68
35. Paratachys leytensis	17	10	27
36. Tachys sp. 1	82	50	132
37. Tachys sp. 2	3	1	4
38. Thopeutica sp.	68	54	122
39. Prothyma sp.	44	38	82
40. Cicindela sp. 1	50	40	90
41. Cicindela sp. 2	4	0	4
42. Unidentified sp. 1	0	2	2
43. Unidentified sp. 2	1	0	1
44. Unidentified sp. 3	2	1	3
45. Unidentified sp. 4	1	1	2
46. Unidentified sp. 5	3	1	4
47. Unidentified sp. 6	2	0	2
48. Unidentified sp. 7	2	0	2
49. Unidentified sp. 8	1	0	1
50. Unidentified sp. 9	1	0	1
Total number of individuals	4518	3326	7844

Data on richness by method per habitat type is not normally distributed, i.e. Shapiro-Wilk normality test p-value < 0.05, thus nonparametric Mann-Whitney U test which is equivalent to the independent two-sample t-test was used. The Kruskal-Wallis test, indicated a no significant difference in the abundance and species richness per method of collection, i.e. handpicking vs. pitfall trapping (p-value = 0.2178). There was no significant difference in the number of carabid beetles caught using handpicking and pitfall traps in all study sites.

In this study, four different baits were used in the pitfall trap, namely: vinegar, mixture of ketchup and vinegar, feremented fish with vinegar, and ground meat. The use of fermented fish with vinegar has never been used before by any author and is a new bait method discovered by the researcher during the study. A total of 3,326 carabid individuals belong to 40 species were collected. Fermented fish with vinegar garnered the highest number of indivuals at 1,894 (56.94%) of the total catch. With this bait, Pheropsophus hassenteufeli and Pheropsophus azouleyi were the most dominant species with 356 and 219 individuals, respectively. Second most effective bait used in pitfall trap was ketchup with vinegar accounting for 826 catch individuals with Pheropsophus hassenteufeli and Tricondyla aptera punctipennis as the top most species. Comparatively lower in terms of catches is the use of pure vinegar which accounted for 410 individuals of the total catch and had Pheropsophus azouleyi with 75 individuals as the most dominant species. The least efficient bait method was the ground pork with 198 indivuals out of the total catch and *Tricondyla conicicollis* was its dominant species. Fermented fish with vinegar showed the highest diversity (5168), high equitability, highest dominance (2.984) and richness (40) compared to other baits used in the pitfall trapping. The use of Kruskal-Wallis test indicates significant difference in the abundance and species richness per bait method in pitfall trapping, (p-value < 0.05).

Mann-Whitney U test pairwise comparison among different baits in the pitfall traps indicate that fermented fish + vinegar is significantly different from the other, followed by meat, ketchup + vinegar, and the least is vinegar. For the difference of abundance in terms of habitat and method of collection, Mann-Whitney U test between handpicking in forest and agricultural land is significant, i.e. p-value = 0.02188 which indicates a significant difference in the mean abundance of richness between habitat types using handpicking. Furthermore, Mann-Whitney U test between pitfall traps in forest and agricultural land is not significant, i.e. p-value = 0.07, indicating that there is no significant difference in the mean abundance of richness between forest and agriculture using pitfall traps. Mann-Whitney U test between handpicking and pitfall trapping in the forest is not significant, i.e., p-value = 0.2516, indicating that mean abundance of richness is not significantly different whether collection is done by handpicking or pitfall trapping. The same result between handpicking and pitfall trapping using Mann-Whitney U test, in agriculture is not significant, i.e., p-value = 0.5675.

Discussion

Proper methodology is always a prerequisite for obtaining reliable results in scientific studies (Elphick, 2008). There are a number of ways of sampling carabid beetles, but pitfall trapping is by far the most widely used. This technique has been used in almost 90% of field studies about the ecology of carabid beetles published for last five years. Pitfall trapping is popular because it is an efficient, low cost means of sampling (Spence and Niemela, 1994). There was a significant difference in the number of individuals and the number of species collected by handpicking and pitfall trapping in all sites. In all cases, handpicking collected more individuals and more species of carabid beetles than pitfall traps. The reasons for the low number of specimens per sample in pitfall traps is not easy to explain. This kind of trap which is also called Barber trap is the traditional method for collecting carabid beetles. It is quick to set and easy to service, at low cost since they do not need constant attention. The most important methodological aspect of pitfall traps is that they are used to sample carabid beetles independent from the scientist or researcher. This is the main aspect which has to be questioned for the other method used, the handpicking. The success of hand picking strongly depends on the experience of the collector. In this study, the species that were caught only by pitfall traps could, with more intensive work, could also be caught by hand. Nevertheless, one severe disadvantage of pitfall traps which also was apparent in this study, was their extremely low capture rates. This potentially results in limitations in the statistical analysis, which has to be taken into account in the design of carabid studies using pitfall traps (Hayes 1970).

Nevertheless, using a variety of methods instead of sticking to only one method gives different impression of the carabid assemblages in a study area. Renner (1980) showed the difference between pitfall traps and some other catching methods. He indicated that with a combination of different methods, it can increase the catch by 25% more species compared to if only pitfall traps were used. This study, which utilized a combination of different methods resulted to a double number in species and catches. The result of this study is similar to the results of Nyundo and Yarro (2007) where handpicking garnered 609 catches over 360 for pitfall trap. In this study, diverse carabid beetles were higher and of advantages with handpicking over pitfall trap having baits. This is in contrast with the study of Anika *et al.* (2007) who collected and studied carabid beetles using pitfall trap and found out that a high diversity of carabid beetles is found in pitfall traps than in handpicking and sifting. These traps were successful with the presence of baits. But baits may be worthwhile in studies of a single, dominant species or in areas where the diversity of carabid beetles is low. When collecting carabids, the use of alcoholic preservatives in pitfall traps does not appear to raise problems of attraction (Darlington 1943). But for many other groups this is not the case. In this study, the use of fermented fish with vinegar showed the highest carabid number in all forests sampled over other baits like vinegar, ketchup and meat.

Furthermore, Carabidae, for instance, were notably infrequent in pitfall traps in both areas in comparison with cool temperate climates where ants are less numerous. Both Van der and Drift (1963) have attributed the scarcity of ground-living Carabidae in the tropics to the abundance of ants. They also concluded for a probable reason that ants limit the frequency of other large cursorial arthropods, either by direct predation or interference or by preempting their niches. In the majority of the attracted species the response to preservatives will be for feeding or oviposition and natural attractants are likely to be alcoholic fermentation products of plant tissues containing rapidly decomposed polysaccharides., sap and so on, are taken in numbers. In comparison among the four baits, fermented fish captured the most number of individuals captured. In the six forest ecosystems, the number of individuals captured by fermented fish was relatively higher than those of other baits. Forty species (40) were captured with fermented fish, thirty (30) for vinegar with ketchup, twenty-five (25) for vinegar, and seventeen (17) for meat. These results supported by the strongest smell of fermented fish over other baits. Since, this study is the first to use fermented fish, no comparison from other studies can be used. So, more carabid collection using fermented fish is encouraged to validate the results. The high efficiency of fermented fish with vinegar in this study cannot be compared with any survey yet since this is the very first that such method was used. The discovery of such method was only made after series of observations that carabid beetles tend to go out at night when there is a strong smell that would lead them to the pitfall traps. More pitfall trapping using this bait is being encourage so as to validate its robustness and efficiency.

Conclusion

The result of this study indicates that the total catch of a particular species and species composition can be substantially affected by trapping methods as well as the baits used in pitfall trapping. Although pitfall trapping has no advantage over handpicking in this study, with respect to species richness and composition, pitfall trapping cannot be left out of sampling protocols because the method sampled some species that were missed by handpicking method. Since Carabidae receive a comparable importance in conservation biology, we should not only use pitfall traps as a tracking method, but must also take other methods into consideration. If the results of these alternative sampling methods are to be compared satisfactorily, standards have to be developed for them. Although, it still early to doubt whether comparable standardizations are possible for handpicking. However, the use of the method should be carried out, as it can at least prove the presence of some species for certain ecological groups or guilds. Studies and experiments simultaneously comparing the performance of traps and baits are greatly needed if sound conclusions are to be drawn about the suitability of traps and baits. In addition to its methodological benefits, simultaneous use of methods (like pitfall, handpicking and use of different baits may provide new information about carabid beetles assemblage.

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