Bioacoustics or pitfall traps: comparison of a modern and traditional method to estimate Ensifera richness

The order Orthoptera occurs in a variety of terrestrial ecosystems and serves as an important primary consumer and prey base for bats[1], birds and spiders[2]. Orthopteran communities are affected by vegetation structure and composition and respond strongly to changes in plant community composition. Hence, they are suitable for monitoring and conservation studies[3,4]. A variety of sampling methods such as sweep netting, pitfall traps and malaise traps or light traps are employed for estimation of Orthoptera diversity and their abundance from a localized or over a large area[5,6]. Studies on transect count, quadrat sampling and sweep netting have been carried out mainly in grasshoppers and in a few cases in katydids and field crickets for comparing efficiency of sampling methods in estimating orthopteran diversity patterns[7,8]. One of the most commonly employed techniques for collecting surface-active invertebrates is pitfall trapping[6]. However, pitfall traps for Orthoptera species estimation have given mixed results due to changes in vegetation structure[8]. Species-specific calls of ensiferan insects serve as a reliable method for species identification as each species has a unique frequency and temporal pattern. Bioacoustics monitoring is widely used for Orthoptera species richness estimation[9,10]. Though studies on effectiveness of acoustic methods with respect to traditional methods have been carried out in birds and amphibians, studies are lacking in acoustically active insect groups[11–13]. Also, distribution and abundance pattern of Orthoptera at an urban ecosystem level has received little attention in India and worldwide[10,14–16].

The aim of the present study is to (1) estimate species richness of Ensifera (Infraorder: Gryllidea) in Delhi and suburban region using bioacoustics method; (2) Compare the bioacoustics method and pitfall trapping in estimating species richness of Ensifera (Infraorder: Gryllidea), and (3) quantify diversity of other invertebrates captured in the pitfall traps.

The acoustic sampling of ensiferans was carried out at four locations in Delhi region (28.6139°N, 77.2090°E). Site one (S1) was a predominantly undisturbed grassland area, site two (S2) was an urban residential area, site three (S3) had scrubby vegetation characteristics of an arid region with *Prosopis* sp. and *Acacia* sp. as dominant tree species and site four (S4) had regularly maintained landscaped gardens with seasonal flowering species managed by civic authorities. The acoustic and pitfall sampling were carried out from January to April during 2013 and 2014 respectively. The temperatures ranged from 10°C in January to 38°C in April, and relative humidity ranged from 50% to 65% during the sampling months.

Call of the ensiferan males was recorded in the field in the evening between 6:00 and 9:00 pm. Individual calling insects were first tracked by ear and located. Recordings were made by holding a digital recorder (TASCAM DR-08, TEAC, America Inc., USA, 44.1 kHz, 16 bit, .wav format) at a distance of 25 cm from the calling animal. As the calling orthopterans are active only in the evening, the acoustic sampling was restricted to 3 h per evening for a total of 42 days. Only two individuals of a call type were preserved in 70% alcohol for taxonomic identification and the rest were released back in the place where they were captured. Song analysis was performed using the signal processing software RAVEN Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY, USA) and Spectra Plus 5 (Pioneer Hill Software, Poulsbo, WA, USA) for temporal and spectral analysis.

Pitfall traps were laid in the same locations where bioacoustics sampling was carried out previously. Cylindrical plastic pots of diameter 17 cm and depth 16 cm were used as pitfall to catch orthopteran and other insects. Each pit was covered with a funnel to make sure that the captured insect does not jump out. A non-invasive method of pitfall trap was used to avoid unnecessary killing of invertebrate species. Pitfall traps without any chemical preservative have been demonstrated as a viable alternative to trap Orthoptera species previously[17]. Each trap was monitored every alternate day (spanning a period of 42 days) to check for captured insects. Captured invertebrates were photographed for taxonomic placement, and invertebrates trapped in the pitfalls were counted and released back in the field but away from the pit. In case of Orthoptera, two individuals per species were preserved. A total of 50 pits were laid for data collection. However, over a period of time 27 pits were stolen and hence, sampling had to be abandoned for these sites. Hence a comparison between the two methodologies, i.e. acoustic and pitfall sampling is limited to a period of four months.

Orthopteran specimens were identified up to the genus level using keys in Chopard[18]. Other invertebrates were classified till order level using entomology keys[19]. A standardized abundance of each taxon was estimated by dividing the number of individuals in each taxon by the sampling effort (number of pits) to correct for unequal sampling effort in

Table 1. Distribution of Ensifera species recorded using acoustic sampling

Ensifera species	S1	S2	S3	S4	Total no. of individuals
1. Superfamily: Grylloidea					
Family: Gryllidae					
Gryllodes sp.	1	1	3	3	8
Gryllus bimaculatus	–	–	–	1	1
Gryllus X	1	1	2	2	6
Gryllus Y	–	–	–	1	1
Family: Trigonidiidae					
Dianemobius sp.	1	1	3	1	6
2. Superfamily: Gryllotalpoidea					
Family: Gryllotalpidae					
Gryllotalpa sp.	1	1	1	2	5
Total individuals	4	4	9	10	27

four sites owing to loss of pitfall traps. The standardized abundances of various taxa were compared across four sites using a non-parametric test.

A total of six ensiferan species were recorded using acoustic sampling. Five call types belonged to superfamily Grylloidea; four to family Gryllidae and one to Trigonidiidae, *Dianemobius* sp. (n = 5). One call type belonged to superfamily Gryllotalpoidea (*Gryllotalpa*, n = 5). Of the species belonging to family Gryllidae, two species could be identified up to the genus level, i.e. *Gryllodes* sp. (n = 8) and *Gryllus bimaculatus* (n = 1). Two species belonging to the same genus *Gryllus* have been referred as *X* (n = 6) and *Y* (n = 1). The spectrogram of calls of ensiferan species and their call characteristics are provided in the Supplementary Material (Figure S1 and Table S1). Four ensiferan species (except *Gryllus* sp. Y and *Gryllus bimaculatus)* were recorded from all sites (Table 1 and supplementary Figure S2).

A total of 7012 individuals of various invertebrate species were recorded from 23 sampling pits. Individuals belonging to two phyla, namely Arthropoda and Molluscs were captured from the pitfall method. The most dominating order of insects was Coleoptera with 2302 individuals, followed by order Hymenoptera with 2250 individuals belonging to a single family, i.e. Formicidae. Other orders of insects were Hemiptera with 218 individuals, Blattodea with 420 individuals, and Isoptera with 93 individuals (Figure 1). Only two Orthoptera species with a total of 133 individuals were found from the 23 pitfalls. One genus belonged to the family Gryllidae (*Gryllodes* sp.) in the suborder Ensifera and the other belonged to suborder Caelifera.

All four sites were roughly equal in abundance for various groups of invertebrates in the pitfall traps (Kruskal wallis χ^2 (3, N = 18) = 2.22, P = 0.53), except for class Chilipoda and order Isopoda in class Insecta that were only found from S3 and grasshoppers in S1 (Table 2 and supplementary Figure S3). Interestingly, S3 with the highest diversity of invertebrates in the pitfalls did not trap any ensiferan species, providing support to the disparity between the two sampling methods. Highest catch in pitfalls on S3 with higher sampling efforts but no orthopteran species also indicates that the results are not a consequence of unequal sampling effort or loss of pitfalls in the area.

In the present study only one species belonging to the family Gryllidae was observed from the pitfall sampling method among a total of 7012 individuals, which is in contrast to the bioacoustics sampling carried out in the same region. This disparity between the two methods could be ascribed to the sampling procedure involved in each of them. The pitfalls were placed in a particular habitat frequented by ensiferan species and probability of collection depended on the abundance of invertebrates in the region and thus worked as a passive method of capturing crickets. However, in the bioacoustics method, a calling cricket was actively searched and located through sound. This increases the chances of finding even a single rare individual in the habitat[17]. Also the acoustic sampling did not yield data on calling density or abundance of calling species. Abundance of the six species recorded acoustically at the four sites could be estimated non-invasively using psychoacoustic sampling by a trained listener, or by active playback from pitfall traps and by mark recapture methods.

Another reason for the difference in the estimation of orthopteran species diversity in the two methods could be due to microhabitat preference of ensiferans. Previous studies in India have

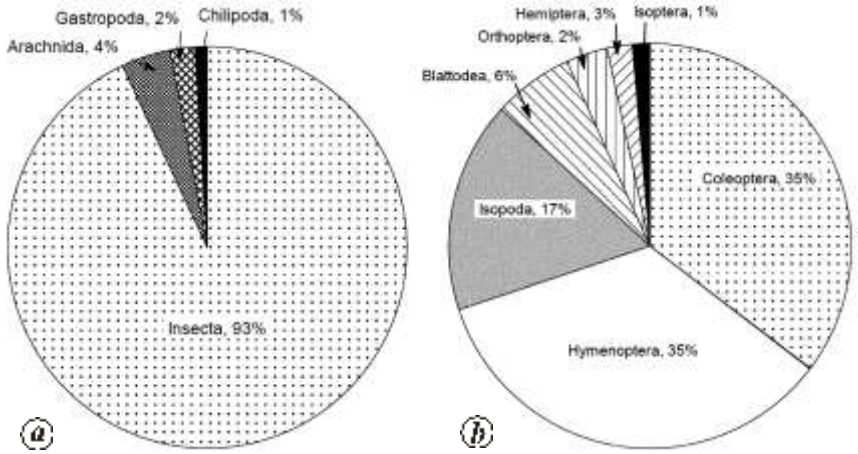

Figure 1. Pie chart showing (***a***) distribution of invertebrates found during pitfall sampling and (***b***) percentage of individuals captured in pitfalls belonging to orders in class Insecta.

Table 2. Taxonomic classification and abundance of invertebrates captured in 23 pitfalls across four sites in Delhi

Invertebrates	S1 (3 pits)	S2 (3 pits)	S3 (11 pits)	S4 (6 pits)	Total no. of individuals
1. Phylum – Arthropoda					
(i) Class – Insecta					
(a) Order – Orthoptera	63	20	0	0	133
(b) Order – Coleoptera	132	250	1370	550	2302
(c) Order – Hymenoptera	200	300	1000	750	2250
(d) Order – Hemiptera	58	20	60	80	218
(e) Order – Blattodea	0	60	310	50	420
(f) Order – Isoptera	0	63	0	30	93
(g) Order – Isopoda	0	0	1100	0	1100
(ii) Class – Arachnida					
(a) Order – Araneae	0	3	5	305	313
(iii) Class – Chilipoda	0	0	28	0	28
2. Phylum – Mollusca					
(i) Class – Gastropoda	0	0	125	30	155
Total individuals	443	716	3998	1855	7012
Simpson's index (1-*D*)	0.75	0.78	0.86	0.78	–

demonstrated that crickets and katydids show preference for microhabitat[10,20]. Ensifera species in this study were found calling from cracks, crevices, and under the rocks and burrows in the ground. Such microhabitats were not covered during random pitfall sampling. A larger number of randomly placed pitfall traps in varied microhabitats could probably remove this microhabitat-specific bias.

The present study also estimated diversity of invertebrates other than Orthoptera collected in the pitfalls located in and around Delhi region. The overall invertebrate diversity was comparable across sites, except S3, which showed higher diversity due to the presence of specific taxa.

The two sampling methods compared and discussed here varied in the mode of operation and each method provided unique data on Orthoptera. The study indicated use of multiple sampling techniques for species inventory and monitoring in a region[5,6]. It can be further strengthened with long-term sampling, larger sample sizes and sex segregation for population level assessment.

1. Raghuram, H., Deb, R., Nandi, D. and Balakrishnan, R., *Proc. R. Soc. B*, 2015, **282**(1798), 20142319.
2. Belovsky, G. E. and Slade, J. B., *Oikos*, 1993, **68**(2), 193–201.
3. Batáry, P., Orci, K. M., Báldi, A., Kleijn, D., Kisbenedek, T. and Erdős, S., *Basic Appl. Ecol*., 2007, **8**(3), 280–290.
4. Stoner, K. J. and Joern, A., *Ecol. Appl.*, 2004, **14**(5), 1306–1320.
5. Gardiner, T. and Hill, J., *J. Orthoptera Res*., 2006, **15**(1), 45–51.
6. Samways, M., McGeoch, M. A. and New, T. R., *Insect Conservation: A Handbook of Approaches and Methods*, Oxford University Press, UK, 2009.
7. Gardiner, T., Hill, J. and Chesmore, D., *J. Insect Conserv*., 2005, **9**(3), 151–173.
8. Schirmel, J., Buchholz, S. and Fartmann, T., *J. Insect Conserv*., 2010, **14**(3), 289–296.
9. Sueur, J. and Aubin, T., *Syst. Entomol*., 2003, **28**(4), 481–492.
10. Diwakar, S. and Balakrishnan, R., *Bioacoustics*, 2007, **16**(2), 113–135.
11. Acevedo, M. A. and Villanueva-Rivera, L. J., *Wildl. Soc. Bull*., 2006, **34**(1), 211–214.
12. Celis-Murillo, A., Deppe, J. L. and Ward, M. P., *J. Field Ornithol*., 2012, **83**(2), 166–179.
13. Klingbeil, B. T. and Willig, M. R., *PeerJ*., 2015, **3**, e973.
14. Tandon, S. K. and Hazra, A. K., *Faunal Diversity in India* (*Order: Orthoptera*), ENVIS Center, Zoological Survey of India, Kolkata, 1998, pp. 183–188.
15. McIntyre, N. E., *Ann. Entomol. Soc. Am*., 2000, **93**(4), 825–835.
16. Penone, C., Le Viol, I., Pellissier, V., Julien, J. F., Bas, Y. and Kerbiriou, C., *Conser. Biol*., 2013, **27**(5), 979–987.
17. Divya, B. U., Metrani, S. and Balakrishnan, R., *J. Bombay Nat. Hist. Soc*., 2005, **102**(1), 38–43.
18. Chopard, L., *Fauna of India and the Adjacent Countries: Grylloidea*, Baptist Mission Press, Calcutta, 1969.
19. Brues, C. T. and Melander, A. L., *Classification of Insects: A Key to the Known Families of Insects and other Terrestrial Arthropods*, Harvard University Press, USA, 1932.
20. Jain, M. and Balakrishnan, R., *Insect Conserv. Divers*., 2011, **4**(2), 152–155.

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