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A comparison of three sampling techniques used to estimate the population density and assemblage diversity of Orthoptera

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Abstract

We undertook a comparative study in 2004 at Writtle College, Chelmsford, Essex, UK, using box quadrat, open quadrat and transect sampling techniques, to ascertain the significance of emigration and immigration of individuals from survey plots during sampling and the implications for abundance estimation and subsequent calculation of assemblage diversity. Both open quadrat and transect techniques consistently produced underestimates of total Orthoptera density, *Chorthippus* nymph density and *Chorthippus parallelus* adult density, when compared to box quadrat sampling, although the differences between techniques were not statistically significant. We suggest that these underestimates of density using the former techniques were due to individuals escaping from the observer during sampling, whereas individuals jumped onto the high sides of the box quadrats.

Both open quadrat and transect monitoring tended to miss the tettigoniids *Metrioptera roeselii* and *Conocephalus discolor*, leading to underestimates of species richness when compared to box quadrat sampling. We suggest that if surveyors wish to ascertain bushcricket abundance or species richness at a study site, methods that constrain movement of individuals, such as box quadrats, should be used.

Key words

Orthoptera, quadrat, transect, sampling, abundance, diversity

Introduction

Ecologists have frequently quantified the density of populations of Orthoptera in grassland ecosystems, often as indicators of the effects of changes in land management on species conservation (for instance, van Wingerden *et al.* 1992, Wettstein & Schmid 1999, Bolger *et al.* 2000, Gardiner *et al.* 2002). Many of these studies have attempted to make objective measures of population size in the area of interest using mark, release, recapture techniques (MRR: Richards & Waloff 1954, Southwood 1978, Evans *et al.* 1983, Greenwood 1996) or biocenometers (Isern-Vallverdu *et al.* 1993). These methods produce data on the total number of individuals per unit area and allow other ecological indicators, such as insect biomass, to be measured (Isern-Vallverdu *et al.* 1993). However, problems with these methods are the high labor requirement and slow rates of sampling.

Constraints on the rate of sampling often dictate that ecological studies are not conducted using the most precise and accurate methods (New 1998). Therefore, to estimate population size, a strategy of subsampling is necessary to ensure adequate coverage of the survey site. In the majority of UK grassland systems, the sampling methods used to estimate the abundance of Orthoptera have included box quadrats with high sides to prevent individuals

migrating away from survey plots (Grayson & Hassall 1985, Cherill & Brown 1990), open quadrats, which lacked physical barriers to control emigration of individuals (Clarke 1948, Richards & Waloff 1954, Gardiner & Pye 2001, Gardiner *et al.* 2002, Gardiner *et al.* 2003), and transects (Gardiner *et al.* 2005b). Transects are a commonly used method of recording insect abundance, particularly for butterflies (Pollard & Yates 1993) and grasshoppers (Wettstein & Schmid 1999). This technique involves a set route walked by an observer at a slow strolling pace, recording any individuals of the target species sighted within agreed parameters (*e.g.*, in a 0.5-m strip in front of the recorder). These three relatively quick and inexpensive sampling techniques require grasshoppers to be flushed out of the sward and then identified visually (Gardiner *et al.* 2005a). In most studies, flushing of grasshoppers is conducted by brushing the vegetation with a pole, which causes the grasshoppers present in the survey plots to jump upon disturbance, allowing visual identification by an observer. The methods are much affected by the locomotion of the grasshoppers upon disturbance and by the skill of the observer in identifying jumping or flying species. Early data by Clarke (1948) demonstrated that the movement of *Chorthippus parallelus* could lead to an underestimation of population size, as the majority of grasshoppers jumped when disturbed, but then burrowed down into the vegetation upon landing. If observation of the species was not achieved quickly after the initial sighting, the grasshopper might be overlooked, leading to an underestimation of abundance. In addition, such visual surveys are feasible only at very low population densities.

Estimations of grasshopper density might also be affected by the sampling behavior of the surveyor. For example, is a stationary observer peering into a constrained survey area (*e.g.*, a box quadrat) likely to see more grasshoppers than one who is walking a transect, where grasshoppers on the periphery of the observer's vision could escape undetected?

Open quadrat and transect methods can also suffer from the emigration and immigration of individuals from and to plots during sampling, leading to incorrect estimations of orthopteran abundance at a survey site. Box quadrats seem to provide a solution to these migration errors, with the high sides (>50-cm height) preventing the majority of resident individuals from escaping during quadrat positioning or from entering the plot subsequently. Use of box quadrat sampling also allows orthopterans to be more easily captured, weighed and classified (instar, sex, species: Grayson & Hassall 1985, Cigliano *et al.* 2002) than in open quadrat and transect sampling. However, it is important to note that all sampling methods provide

only an estimation of the number (density of individuals m^{-2}) of Orthoptera present and not the actual population size (Gardiner *et al.* 2005a). It is almost impossible to quantify total population size at a survey site, especially where contagious (or clustered) distributions of Orthoptera and tall vegetation are present. For example, in tall vegetation, Orthoptera may remain hidden deep in the foliage, whilst in heterogeneous swards, clusters of Orthoptera may be missed by random sampling methods.

Our objective was to examine and compare population density estimates obtained using box quadrat, open quadrat and transect sampling techniques. The analysis aimed to ascertain the significance of emigration and immigration of individuals from survey plots during sampling and the implications for abundance estimation and subsequent calculation of assemblage diversity.

Methods

Study site for comparison of methods.—The study site was located on an area of pasture (O.S. grid reference TL664067; lat $51^{\circ} 44' 05''\text{N}$, long $0^{\circ} 24' 33''\text{E}$) on the Writtle College Estate, Chelmsford, Essex, UK. The vegetation was dominated by *Lolium perenne*, interspersed with small patches of bare earth. The overall grasshopper density (of all species combined) at the study site in previous years was 0.48 adults m^{-2} , with *Chorthippus albomarginatus* and *C. parallelus* the most abundant species; *Chorthippus brunneus* was comparatively rare (Gardiner *et al.* 2002).

Small populations of *Chorthippus* grasshoppers are known to occur in *L. perenne* grasslands in the surrounding area. For example, assemblage densities at nearby sites ranged from 0.01 adults m^{-2} to 0.13 adults m^{-2} (Gardiner *et al.* 2002), suggesting that the study site was representative of the local grasshopper populations.

The experiment was conducted in an area of homogeneous vegetation where the sward height was uniformly <20 cm, thus ensuring that Orthoptera could be readily flushed and visually identified. Areas of grassland with tall, dense vegetation (>50 cm in height) were avoided, as there was the possibility that grasshoppers may have remained hidden deep in the foliage, thus avoiding detection.

The approximate size of the Writtle College Estate is 210 ha, mainly comprised of mixed farmland and horticultural areas, with some designated conservation sites. A high proportion of the arable area (amounting to 45% of the total) has been cropped with winter cereals (wheat and barley) for many years. The estate extends over many different soil types, but most originate from glacial boulder clay and have variable pH (5.8 to 8.1) and high moisture content in winter (Neate 1979). Southeast Essex has a relatively dry, temperate climate, characterized by an average air temperature of 10°C (normalized range -5 to 32°C) and annual rainfall of approximately 550 mm.

Sampling methods for Orthoptera density.—Ten surveys were conducted at the study site starting on 25 May 2004, with repeat surveys occurring at approximately 10-d intervals (weather permitting) throughout the summer, and with the last survey date being 30 August. On each survey occasion, 3 sampling methods (box quadrat, open quadrat and transect) were used to search 40 m^2 by the observer. The observer had approximately 5 y experience of estimating grasshopper densities in UK grasslands and had extensive experience of using all three methods.

It was possible to accurately determine adult grasshoppers (Orthoptera: Acrididae) and bushcrickets (Orthoptera: Tettigoniidae)

to species on sight. However, grasshopper nymphs are difficult to place to species visually (Richards & Waloff 1954). In preliminary surveys of the experimental areas on the Writtle College Estate in previous years (1999 to 2001), only three *Chorthippus* species were identified: therefore nymphs on the plots could be confidently assumed to belong to this genus and any nymphal individuals flushed were recorded as *Chorthippus* spp. nymphs.

Box quadrat design and method of counting Orthoptera.—Box quadrats, with high sides (>50 cm), trap Orthoptera within the boundaries of the quadrat and “a chase” after any escaping individuals is therefore not required (Cherill & Brown 1990, Ausden 1996). The method reduces the effect of migration from a site during a survey. For this reason data collected using box quadrats were compared against other sampling techniques where Orthoptera can more easily escape from the surveyor. The box quadrat used in this study had an area of 1 m^2 ($1 \times 1\text{-m}$) with sides of 0.6 m height, constructed from wood (Grayson & Hassall 1985). The quadrat was dropped over the vegetation from a height of 0.5 m, trapping grasshoppers and bushcrickets inside and allowing visual identification and counts *in situ*. Orthoptera were occasionally observed to escape from the plots as the quadrat was dropped onto the vegetation. The location of box quadrat placement was determined by random coordinates selected before the surveys commenced. The x and y coordinates (to the nearest m) of a predetermined corner of the quadrat were taken from a random number table.

Within the quadrat, Orthoptera were flushed from the sward using a pole (diameter: 50 mm, length: 1 m) and quite often jumped onto the wooden quadrat sides where they could be easily identified. On each of the survey occasions, 40 box quadrats were searched for Orthoptera (total area searched 40 m^2). A thorough search period, to ensure complete coverage of the vegetation within a quadrat, required approximately 30 s, although the authors acknowledge that in higher density populations (*e.g.*, $>2 \text{ m}^{-2}$) a longer search period (>60 s) may be required to record all visible individuals. A fixed ‘no-find’ period can be adopted, after which surveying of a plot is terminated [*e.g.*, 10 min as in Bridle *et al.* (2002)]. All surveys commenced in the late afternoon (1600 to 1800) when Orthoptera are less active and therefore easier to identify (Marshall & Haes 1988). Air temperatures just before surveys began varied from 19 to 25°C .

Open quadrat sampling for Orthoptera.—Open quadrat sampling does not control displacement of Orthoptera during a counting period and therefore it is possible that individuals can escape before identification has been confirmed or appear though not originally present. The technique used for the open quadrat study has been reported in detail previously by Gardiner *et al.* (2002). The observer marks out the corners of each $2 \times 2\text{-m}$ quadrat with poles, without disturbing the grasshoppers within by casting shadows. The insects are then flushed by brushing the vegetation with a pole (same dimensions as for box quadrat surveys) and flushing proceeds from one edge of the quadrat to the other, sweeping the vegetation in an arc of 180° . Only grasshoppers within the quadrat at the start of the sweep are recorded, with those leaping in from outside discounted. On each of the survey occasions, 10 randomly located quadrats (positions determined using a random number table as in the box quadrat sampling) were flushed for Orthoptera (total area searched 40 m^2). Again, as with the box quadrat sampling, it took the observer approximately 30 s to search and identify the Orthoptera in each quadrat. All surveys commenced in the late

afternoon (1600 to 1800) and air temperatures just before surveys, varied from 19 to 25°C.

Transect counts.—At the study site, eight transects of 10-m length were established on each of the survey occasions (total area searched 40 m²). Each transect was walked at a slow pace (2 km/h) and the number of Orthoptera individuals flushed in a 0.5-m strip in front of the observer counted (in an identical fashion to that reported by Isern-Vallverdu *et al.* 1993). Each transect walk took the observer approximately 18 s to complete and to identify and record the Orthoptera present. Transect surveys of Orthoptera assemblages by Wettstein & Schmid (1999) used a similar walking speed (1.2 km/h) to survey grasshoppers in a 1 × 20-m strip. All surveys commenced in the late afternoon (1600 to 1800) and air temperatures as surveys commenced varied from 19 to 25°C.

Sweep sampling to determine species composition and developmental stage on each survey occasion.—Sweep-net sampling was undertaken at the study site on each survey occasion to determine the relative abundance of the developmental stages of Orthoptera. The method used was similar to that reported in other studies (Evans *et al.* 1983, Karpakakunjaram *et al.* 2002, O'Neill *et al.* 2002): a 30-cm diameter net was used to sweep the vegetation once back and forth in a 180° arc (one sample) at a height of 5 cm, the arc covering approximately 3 m. The number of adults and nymphs of grasshoppers and bushcrickets in 20 such sweep samples was recorded on every sampling occasion (10 survey dates) using the key of Capinera & Sechrist (1999) as a general guide to developmental stage. As the key may not be exactly applicable to UK Orthoptera, where there was doubt re the developmental stage of any individual, it was released without classification. Sweep netting was conducted in the late afternoon (1600 to 1800, air temperature 19 to 25°C) on every survey occasion.

Statistical analysis.—All three methods gave a direct measurement of the number of Orthoptera per unit area on the sampled quadrats or transects (Duffey *et al.* 1974), and the data were compared as the number of individuals m⁻². For each survey occasion, the total counts of all species combined, *Chorthippus* spp. nymphs and *C. parallelus* adults for each 2 × 2-m open quadrat, 10-m transect section and box quadrat, were reported as a total density m⁻² for each sampling unit. These data were analysed using Friedman's two-way ANOVA (Heath 1995). The number of species per survey obtained using each sampling technique was also compared using Friedman's two-way ANOVA statistic, with sampling technique and survey occasion as the factors.

Assemblage diversity estimates were calculated using Version 3.02 Species Diversity and Richness software (Pisces Conservation Ltd, IRC House, The Square, Pennington, Lymington, Hampshire) from data collated from each of the three methods. The Shannon-Wiener Diversity Index (H', Kent & Coker 1992) was calculated for each survey occasion for all methods, using the total number of individuals recorded for each Orthoptera species. The median assemblage diversity for the whole season using each sampling technique was compared using Friedman's two-way ANOVA.

To ascertain whether data collected using the open quadrat and transect methods demonstrated similar trends to that produced using the box quadrats, we used Spearman's rank correlation (Heath 1995). Data included in the correlation analysis were the total number of Orthoptera m⁻², density of *Chorthippus* spp. nymphs, density of *C. parallelus* adults (other species were not abundant

Table 1. Number of Orthoptera species recorded using each sampling technique; species absent from open quadrat and transect recording but observed in box quadrat sampling.

Survey date	Life stage	Sampling technique			Species absent	
		Box quadrat	Open quadrat	Transect	Open quadrat	Transect
25/05	1 st instar	3	2	3	Cd	-
04/06	1 st instar	2	2	1	-	Mr
11/06	2 nd instar	1	1	1	-	-
21/06	3 rd instar	3	3	2	-	Cd
01/07	3 rd instar	3	1	1	Cd, Mr	Cd, Mr
13/07	4 th instar	4	3	2	Cd	Cd, Mr
26/07	Adult	3	3	2	-	Mr
10/08	Adult	2	2	2	-	-
20/08	Adult	4	4	2	Cd	Cb, Cd
30/08	Adult	3	3	3	Cb	Cb
Median		3	3	2		
Mean		3	2	2		

Key: Cb: *Chorthippus brunneus*; Cd: *Conocephalus discolor*; Mr: *Metrioptera roeselii*

enough for analysis), species richness and assemblage diversity. All statistical analyses were performed using SPSS Version 10 (SPSS 1999) according to the methods of Mead *et al.* (1993).

Results

Comparison of the three sampling methods

Assemblage composition.—Five Orthoptera species were recorded at the study site. The most abundant species was *C. parallelus*, with the proportion of the total number of individuals being >60% and about the same for all three sampling methods (Fig. 1). *Metrioptera roeselii* and *Conocephalus discolor* however, were recorded at a greater incidence in box quadrats (combined proportion: 25%) compared to the open quadrat (combined proportion: 13%) or transect methods (combined proportion: 6%). The relative abundance of *C. albomarginatus* was similar for box quadrat and open quadrat sampling techniques (12% and 13% of total individuals respectively). However, this species formed a much higher proportion of the total Orthoptera number in the transect counts (23% of total individuals). *C. brunneus* was recorded using the box and open quadrat techniques, but not by transect counting.

Species richness.—Species richness estimates are presented in Table 1. The median species richness per survey for box and open quadrat techniques were identical (three species); however, for the transect sampling method the median species richness was two species per survey. The method of sampling the grassland area had a significant impact on species richness estimates (Friedman's ANOVA: 8.27, d.f. 2, $p < 0.05$).

The tettigoniids, *M. roeselii* and *C. discolor*, discovered when using box quadrat counting, apparently often went unobserved when using open quadrat and transect survey techniques. Consequently, estimates of species richness were lower with open quadrat and transect sampling on several occasions, notably when most Orthoptera were in the late instar (3rd, 4th) stage of development. *C. brunneus* (a rare species at the site, Fig. 1) was frequently unobserved using the transect and open quadrat methods, despite being recorded during the box quadrat sampling.

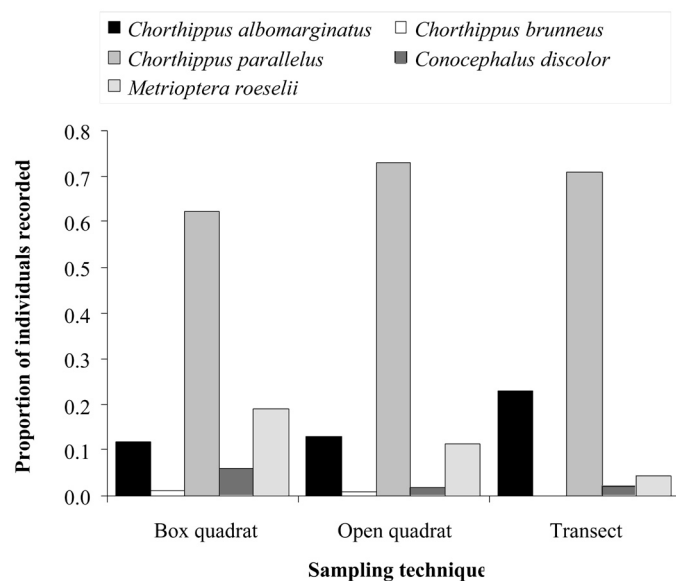


Fig. 1. Proportion for each Orthoptera species of the total number of individuals recorded for each sampling technique.

Assemblage diversity.—Assemblage diversity estimates were not significantly different among the three sampling techniques (Table 2; Friedman's ANOVA statistic: 3.26, d.f. 2, $p > 0.05$). However, diversity estimates obtained using open quadrat and transect sampling had considerably greater variability than estimates derived using the box quadrat technique.

Orthoptera abundance.—The highest densities were reported on 21 June by all three methods (Fig. 2). Early (25 May–04 June) and late (26 July–20 August) in the monitoring season, both open quadrat and transect techniques underestimated the abundance of Orthoptera when compared to the box quadrat counts. The median Orthoptera densities m^{-2} for the whole monitoring season, produced using box quadrat (0.88 individuals m^{-2}), open quadrat (0.69 individuals m^{-2}) and transect (0.57 m^{-2}) sampling methods, were not significantly different (Friedman's ANOVA statistic: 2.60, d.f. 2, $p > 0.05$).

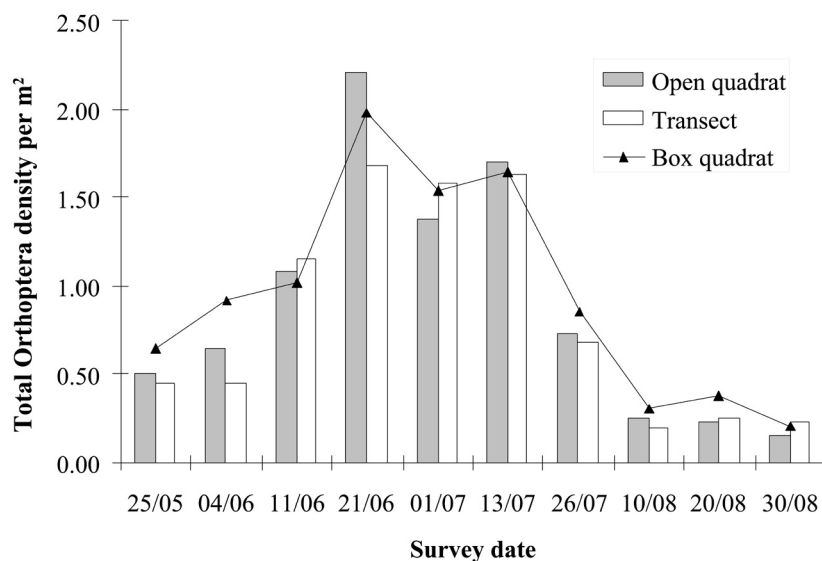


Fig. 2. Density estimates m^{-2} of Orthoptera (all species and life stages combined) using each sampling technique.

Table 2. Assemblage diversity (H') estimates using each sampling technique.

Survey date	Sampling technique			Difference of H' estimates from uncontrolled techniques to box quadrat data	
	Box quadrat	Open quadrat	Transect	Open quadrat	Transect
25/05	0.72	0.50	0.56	-0.22	-0.16
04/06	0.18	0.43	0.00	0.25	-0.18
11/06	0.00	0.00	0.00	0.00	0.00
21/06	0.59	0.12	0.13	-0.47	-0.46
01/07	0.20	0.00	0.00	-0.20	-0.20
13/07	0.35	0.00	0.27	-0.35	-0.08
26/07	0.72	0.21	0.53	-0.51	-0.19
10/08	0.58	0.65	0.56	0.07	-0.02
20/08	0.85	1.15	0.67	0.30	-0.18
30/08	0.94	1.01	0.97	0.07	-0.04
Median	0.58	0.32	0.40	-0.10	-0.17
Mean	0.51	0.41	0.37	-0.11	-0.14

Abundance of nymphs.—Only nymphs and *C. parallelus* adults were in sufficient abundance at the study site to warrant individual analysis. The trends for *Chorthippus* spp. nymphal abundance were similar for all three sampling methods, with a peak density on 21 June observed for all three techniques (Fig. 3). A considerable underestimation (when compared to the box quadrat) of nymphal abundance using both open quadrat and transect techniques was observed on 4 June; contrastingly, an overestimation of abundance occurred using the open quadrat compared to the other techniques on 21 June when densities were at their highest. However, the median nymphal densities m^{-2} for the whole monitoring season, using box quadrat (0.50 individuals m^{-2}), open quadrat (0.48 individuals m^{-2}) and transect (0.41 individuals m^{-2}) sampling methods, were not significantly different (Friedman's ANOVA statistic: 0.06, d.f. 2, $p > 0.05$).

Abundance of *Chorthippus parallelus* adults.—Akin to *Chorthippus* spp. nymphal abundance, *C. parallelus* adult density followed a similar trend for all three sampling methods, although peak density was determined on 13 July for all three techniques (Fig. 3). A large underestimate of adult abundance using the transect technique oc-

Table 3. Spearman's rank correlations (r_s values displayed) between assemblage attributes, recorded from box quadrat sampling and open quadrat and transect techniques.

Assemblage attribute	Box quadrat vs open quadrat	Box quadrat vs transect
Total Orthoptera density	0.98**	0.97**
<i>Chorthippus</i> spp. nymph density	0.93**	0.93**
<i>Chorthippus parallelus</i> adult density	0.98**	1.00**
Assemblage diversity	0.73*	0.90**
Species richness	0.73*	0.45

* significant correlation at $p < 0.05$

** significant correlation at $p < 0.01$

curred on 13 July. However, the median *C. parallelus* adult density m^{-2} for the whole monitoring season, produced using box quadrat (0.10 individuals m^{-2}), open quadrat (0.09 individuals m^{-2}) and transect (0.10 individuals m^{-2}) sampling methods, was not significantly affected by sampling technique (Friedman's ANOVA statistic: 3.44, d.f. 2, $p > 0.05$).

Correlation of open quadrat and transect data with box quadrat estimates.—Highly significant correlations ($p < 0.01$) between the abundance estimates of total Orthoptera (all species combined), *Chorthippus* spp. nymphs and *C. parallelus* adults using open quadrat and transect techniques, and estimates from box quadrat sampling were observed (Table 3). Estimates of assemblage diversity were also significantly correlated between box quadrat sampling and open quadrat ($p < 0.05$) and transect sampling ($p < 0.01$). Although species richness was significantly correlated between box and open quadrat sampling ($p < 0.05$), no such relationship was detected for the transect technique, suggesting that patterns of species richness using this method did not follow the overall trend produced by the box quadrat surveys.

Discussion

Both open quadrat and transect sampling methods produced statistically similar estimates of the density of Orthoptera m^{-2} (Fig. 2), and of *Chorthippus* spp. nymphs and *C. parallelus* adults (Fig. 3), throughout the season (May–August), when compared to box quadrat estimates. Density estimates using open quadrat and transect techniques also seemed to follow the seasonal patterns of abundance displayed from the box quadrat counts (Figs 2, 3; Table 3). However, on 4 June, there was a substantial underestimate of the abundance of *Chorthippus* spp. nymphs using the open quadrat and transect sampling methods (Fig. 3).

One explanation for the underestimation of density using open quadrat and transect flushing techniques could be emigration. During sampling, grasshoppers were observed escaping from the survey area before identification (of developmental stage/species) could be completed; therefore these individuals were not counted. However, with box quadrat counting, in approximately 50% of box quadrats, individuals attempting to escape jumped onto the quadrat sides thus allowing their easy identification. It may be very easy for individuals on the periphery of the observer's vision to escape without being counted in a high-density population and some confusion (and overestimation of numbers due to counting the same individuals twice) can be caused when multiple individuals are flushed at the same time, especially if there is a need to confirm species identifica-

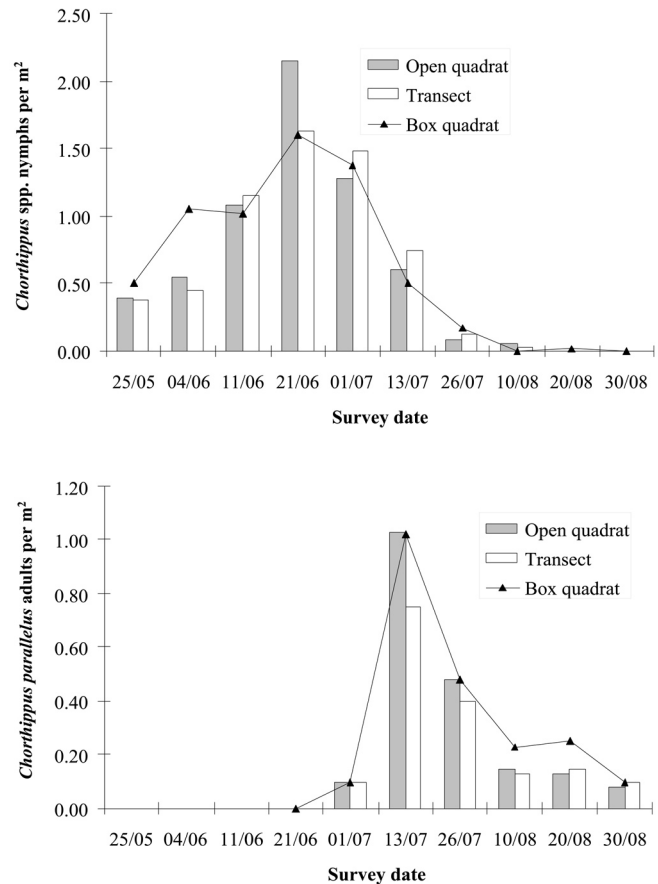


Fig. 3. Density estimates m^{-2} of *Chorthippus* spp. nymphs (top) and *Chorthippus parallelus* adults (bottom) using each sampling technique.

tion.

The data reported in this paper suggest that migration of individuals during sampling had no significant effect on the density estimates of nymphal and adult Orthoptera at the study site. A comparison between the species richness and assemblage diversity using each sampling technique was also undertaken (Tables 1 and 2). *M. roeselii* and *C. discolor* were not recorded on several occasions using the open quadrat and transect methods, whereas they were identified within the box quadrats. Overlooking these two species led to an underestimate of species richness on many occasions which affected subsequent assemblage diversity calculations. It may be better to use sweep-sampling techniques, which have been thoroughly tested in the field, to determine the species richness of Orthoptera assemblages, particularly in grasslands (sward height < 50 cm) with moderate or high densities of grasshoppers (> 2 adults m^{-2} ; Gardiner *et al.* 2005a).

The two bushcricket species at the study site may have emigrated from the survey plots before identification could be confirmed. Indeed, *M. roeselii* and *C. discolor* were observed to jump onto the box quadrat sides on numerous occasions, allowing easy identification; with the other methods where emigration was not controlled, they may well have escaped unnoticed, particularly *C. discolor*, as it is a very small elusive bushcricket that often adopts a cryptic posture on the vegetation (Marshall & Haes 1988). *C. discolor* individuals often flatten their bodies against grass stems and extend their hind limbs when approached by an observer. This cryptic behavior is often

accompanied by movements of the individual around a grass stem so as to keep the perch between themselves and the observer (G. Morris pers. comm.). Macropterous forms of *M. roeselii* (f. *diluta*) and *C. discolor* are highly mobile and able to escape readily from the observer before identification has been confirmed. The behavior of the observer differed between the sampling techniques and may also have led to bushcrickets being overlooked, particularly when transect counting, because the observer is constantly on the move (with only short stops to record sightings) and unable to spend time searching the vegetation in any detail. However, during open and box quadrat counts the observer was able to inspect the vegetation with more care due to a stationary position; therefore individuals on the periphery of the survey areas may not have escaped detection as easily as with transect counting. Both *M. roeselii* and *C. discolor* can however be detected by their stridulation (Marshall & Haes 1988), and it may be possible to record their presence and abundance using bioacoustic techniques (Gardiner *et al.* 2005a).

Over the course of the season, assemblage diversity determined using open quadrat and transect methods was not significantly less than the box quadrat diversity index, indicating that both methods may be used to ascertain assemblage diversity in a study of UK grasslands with some confidence, especially where relative differences are of interest. Where the abundance and species richness/assemblage diversity of bushcrickets is the main focus of the study, more precise methods such as box quadrats or sweep sampling should be used. However, the low densities of elusive species such as *C. discolor* and *M. roeselii* would mean that a high number of box quadrats would need to be placed in grasslands to accurately determine abundance.

This study provides data on the abundance of Orthoptera throughout the season in a UK grassland, which may be useful in determining the optimum survey dates (although this will depend on weather conditions in different years) to maximize detection rates. It would seem that immature grasshoppers are in highest numbers in June or early July (peak on 21 June) and surveys for nymphs should be undertaken at this time. *C. parallelus* adults were in highest abundance on 13 and 26 July and declined rapidly after the latter date; therefore there may be a narrow time window for surveying adults of this species in *L. perenne* grasslands.

Caution must be exercised in relating the results from this study of assemblages of improved *L. perenne* grassland to other sites. For example, densities of Orthoptera were relatively low in this study (densities mostly <2 individuals m⁻²) when compared to acid grassland where densities can exceed 4 adults m⁻² (Gardiner *et al.* 2002). The accuracy of open quadrat and transect techniques may be severely reduced in such high-density populations due to migration of individuals during counting (Gardiner *et al.* 2005a). Further research is needed into the efficiency of open quadrat and transect methods at a range of sites with differing vegetation types and Orthoptera densities.

In conclusion, open quadrat and transect methods may provide fairly accurate and quick estimates of nymphal and adult Orthoptera abundance in UK grasslands, providing that the same surveyor undertakes the monitoring. Species richness and assemblage diversity may be underestimated using both methods in comparison to box quadrat counts, although the differences may be small. Therefore, when viewing data collected using these techniques, it is important to state the limitations of the sampling. When the objective of the study is to determine bushcricket presence, box quadrat counts or sweep sampling are more suitable, reflecting the tendency of both *C. discolor* and *M. roeselii* to be overlooked by open quadrat and transect counting.

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